MEIOTIC AND BIOCHEMICAL STUDIES IN TWO GENERA *CUCUMIS* L. AND *MOMORDICA* L. OF FAMILY CUCURBITACEAE FROM NORTH INDIAN PLAINS.

A Thesis

Submitted in partial fulfillment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY

in

Botany

By

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DECLARATION

I hereby declare that the thesis entitled, "**Meiotic and biochemical studies in two genera *Cucumis* L. and *Momordica* L. of family Cucurbitaceae from North Indian Plains"** submitted for Ph.D. Botany, Degree to Department of Botany, Lovely Professional University is entirely original work and all ideas and references have been duly acknowledged. The research work has not been formed the basis for the award of any other degree.

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CERTIFICATE

This is to certify that **Rupinderpal Kaur** has completed the Ph.D. Botany titled **"Meiotic and biochemical studies in two genera *Cucumis* L. and *Momordica* L. of family Cucurbitaceae from North Indian Plains".** Under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study. No part of this thesis has ever been submitted for any other degree or diploma. The thesis is fit for the submission for the partial fulfillment of the condition for the award of degree of Ph.D. in Botany.

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ABSTRACT

Meiotic and biochemical studies in two genera *Cucumis* L. and *Momordica* L. of family Cucurbitaceae from North Indian Plains

Background:

The family Cucurbitaceae consists of fairly large species which are used as a fruit vegetables, and are considered to be rich in nutritive value and biochemical potential especially in case of ayurvedic preparation. The medical properties are due to presence of phytochemicals, and are, therefore, essential to be screened with respect to cytomorphological analysis, antimicrobial and antioxidant efficacy, genotoxic potential, proximate and mineral analysis for the present study on the basis of ethno medicinal uses two genera *Cucumis* L. and *Momordica* L. are selected namely: *Cucumis melo* L., *C. melo* var. *agrestis* (Morphotype I), *C. melo* var. *agrestis* (Morphotype II), *C. melo* var. *momordica* L., *Momordica charantia* L., *M balsamina* L., and *M. dioica* L. The study shall provide a platform for further analysis of the said species which can be a potential target for providing novel leads for various drug discoveries.

To cope with the above given evidences there is a scientific need to screen the cytological diversity of all species and phytochemcial studies in wild species *Cucumis* L. and *Momordica* L. from the North Indian Plains covering states of Punjab, Haryana, Rajasthan and Uttar Pradesh. Hence, the present study plant has been selected for the investigation with the following objectives which are outlined in a sequential manner as follows:

- Analysis of morphological and cytological diversity in different species of *Cucumis* L. and *Momordica* L. on poluplation basis from the North Indian Plains.
- To evaluate the phytochemical profile of different cyto-morphovariants of the wild members of *Cucumis* and *Momordica*.
- To compare bioactivity profile in different cyto-morphovariants of the wild members of *Cucumis* and *Momordica.*
- To analyze mineral elements and proximate composition in wild fruits of *Cucumis* and *Momordica*.

Based upon the literature survey and collected scientific information about *Cucumis* L. and *Momomordica* L. it is appropriate to hypothesize that phyto

constituents from both of these genera can be effective for bioactive potential in living beings.

Scope:

Medicinal plants, crude extract and their isolated fraction have shown a spectrum of biological activity which have been used as medicine in different tradition for various ailments as well as a dietary supplement. Applied scientific research in the field of ethanopharmacological finding is growing exceedingly around the world giving due respect to the huge source of herbals and their medicinal uses. Ancient text from various system of medicine have explored numerous plants having potential activities for different diseased conditions and there is an scientific need to approach using modern method with rationality to prove its importance thereby leading to find new leads in health care industry. The present work may help us to design and validate a standard pathway for analyzing herbal drugs for their effectiveness against genotoxicity, cytotoxicity, anti- microbial, antioxidant and mineral composition, proximate analysis. The present work can also guide the researchers for using various combinations of in vitro, behavioral, analytical and endpoints measurements for evaluation of herbal extracts. The success of the proposed hypothesis may create a basis for clinical studies. The present research may help to screen new herbal moieties for bioactive potential and nutritional value.

Results

The morphological characters and cytological study of studied plants may provide most valuable criteria for identification of plants at species level. The present study for cytological analysis revealed that populations of *Momordica charantia*, *M. dioica* and *M. balsamina* L. from various populations shows 2n=22, 2n=28, 2n=22 respectively. In *Cucumis* L. the 2n=24. In the genus *Cucumis* L. the *C. melo var. agrestis* two mophotypes were observed and out of these two, in Morphotype II, there is noticeable large size of fruit as compared to Morphotype I.

The preliminary phytochemical screening of studied plant fruits showed the presence of all screened compounds. The alkaloids are medicinally important to show bear analgesic, anti malarial, anti inflammatory and anti diarrheal properties, so these plants can be used as effective low cost phytomedicine against various diseases. The flavonoids have potential use for drugs against cancer and allergic diseases. The glycosides being detected, are used to cure heart diseases, so these plants may become

the cheapest source to develop cardio protective medicine in future. Saponins are abrotificient and antimicrobial in nature. Tannin is detected in studied plants, it possesses anti tumor and antimicrobial properties.

For genotoxicity evaluation the maximum value of mitotic index was recorded in aqueous extract of *Cucumis melo.* The maximum percentage of abnormal cells was recorded in methanol extract of *C. melo* var. *momordica.* The maximum number of cells with chromatin bridges, laggards was reported in methanol extract of *Cucumis melo* var. *agrestis* (morphotypes I and II).

For antibacterial assay, the maximum inhibitory zone was reported against *Staphylococcus aureus* in *Cucumis melo* L. and against *Bacillus coagulans* in *Cucumis melo var. agrestis* (morphotype II) extract in methanol extract.

In antioxidant activity, the yield of extraction of aqueous and methanol extract was found to maximum in case of *Momordica charantia* and IC_{50} of methanol extract of *Cucumis melo* value for DPPH scavenging assay comes under category of very strong antioxidant.

The present study has thrown a light on the mineral contents and concluded that the selected plants bears medicinal properties and in addition to this they are the cheap source of getting large amount of minerals. It is observed that the fruit has maximum percentage of proximate and minerals and lower value of anti nutritional factors. Moisture content was maximum in *Cucumis melo* var. *agrestis*. (Morphotype II). Ash content was found to be significantly high in *Momordica charantia.* Crude fiber was maximum in case of *Cucumis melo*. crude fat content was maximum in case of *Momordica charantia* L. Protein content was found to be maximum in case of *M. dioica*. Carbohydrates was maximum in *Cucumis melo* var. *agretsis* (Morphotype I). The energy efficiency was found to be maximum in case of *Momordica dioica*. Calcium content was maximum in *M. dioica*. Zinc content was found to be maximum in *Cucumis melo* var. *agrestis* (morphotype I). Out of all anti nutritional components alkaloids was maximum in case of *Cucumis melo* var. *agrestis* (Morphotype II), oxalate was maximum in case of *C. melo* var. *momordica* L. Saponin being maximum in case of *M. charantia* L.

By observing HPLC analysis it was found that amount of β-sitosterol compound in were observed maximum in *Cucumis melo* L. Amount of rutin compound were observed maximum in *Momordica dioica* L. Amount of Quercetin

compound in DWE were observed maximum in *Cucumis melo* L. Amount of cinnamic acid compound in DWE were observed maximum in *Momordica balsamina* L.

Lastly, the studied plants are rich in minerals, bears antimicrobial potential, phytochemical compounds and having antioxidant properties. The plants are wild in occurrence easily available. These plants may be studied pharmacologically to generate low cost generic medicine for human healthcare.

Conclusion

The comparative analysis in wild and cultivated cucurbits reveals significant difference in the morphology and cytology. Such studies are beneficial for solve taxonomic enigma(s). Genotoxic studies reveal significant results, which needs to be studies in animals models. Further, these cucurbits also differ in bacterial inhibition, scavenging activity, secondary metabolites and nutrient composition.

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Those who love hard work and love it because it is hard,
Those who try and fail and keep on trying,
Those who suffer the loss of months of hard labour and start all over again, those who cannot be discouraged no matter whatever happens,
Are invited to undertake "RESEARCH"

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Date : **(Rupinderpal Kaur)**

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INTRODUCTION

From the dawn of civilization, plants are constituent of human society to combat dreadful diseases (Biswas *et al.*, 2002). The history of medicinal plants is connected with the history of civilization. Plants have been utilized as medicines from the dawn of civilization (Ruffa *et al.*, 2002). Medicinal plants are among the most important "Non Timber Forest Produce". As per an estimate out of nearly 2, 97,000 species of plants present worldwide, about 52,885 species bear medicinal value (Kala *et al.*, 2006). The WHO has listed about 21,000 plants, used for medicinal purposes all over the world. Among these 2,500 species are found in India, of which 150 species are commercially used on a large scale (Seth and Sharma, 2004). The WHO estimates that approximately 80% of the world's population relies on traditional medicine for the primary health care system (Owolabi *et al*., 2007). The plants are repositories of secondary metabolites that provide an immense source of important chemicals having diverse biological properties (Lico *et al.*, 2012). Over 7,000 species of higher angiosperms are reportedly used for their medicinal purposes in India (Groombridge, 1992). In India, the market for herbal medicine is growing and its market is expanding at the rate of 20% annually in India (Subrat, 2002). Even weeds are employed for food, medicine, and other miscellaneous purposes (Patil, *et al.*, 2007).

India, being the treasure-house of biodiversity with its diverse ecological conditions, rich ethnic diversity, and a strong traditional knowledge base, accounts for 17,000 plant species of higher plants, out of which more than 7,500 species are used in herbal drug formulations (Ved *et al.*, 2001). According to an estimate, only 15-17% of known plant species are being systematically studied for their medicinal properties (Simes *et al.*, 2001). The plant kingdom represents a reservoir of pharmacologically important molecules to be discovered (Potterate *et al.*, 1995). Indian systems of medicines utilize 80% of material derived out of plants. The use of traditional medicine is extensive in India (Jeyachandran and Mahesh, 2007).

Medicinal plants are natural resources, yielding valuable herbal products which are often used in the treatment of various ailments (Grabley and Thiericke, 1999) and moreover, useful in minimizing adverse affects of various chemical agents due to their longevity and attaining positive general health (Kaushik and Dhiman, 2002). This is the reason for the increasing trend in sales of herbal medicines

representing a significant share drug market globally (Pal and Shukla, 2003). The effects exerted by the herbal medicine are through a diverse group of compounds called secondary metabolites which are produced by the plant cells, either in response to adverse environmental conditions or as a result of a potential defense approach against predators (Edeoga *et al.,* 2005). The most famous among these are alkaloids, phenolics, flavonoids, glycosides, saponins, tannins etc., which have the capability of bringing about a physiological effect in our body (Donald, 2000). These physiological effects produced by the secondary metabolites include, regulation of hormone action, enzymatic reactions, immune response, gene functioning and control on cellular transport and cell division (Nenad and Milan, 2015). Pharmacists are interested in these compounds because of their therapeutic performance and low toxicity (Inayatullah *et al.,* 2012). Therefore, it is the need of the hour, to explore the various phytoconstituents of the medicinal plants for their genotoxic, antimicrobial, antifungal, antioxidant studies, etc.

Cucurbitaceae is one of the largest, economically and medicinally important plant species which includes edible fruits cucumber (*Cucumis sativus*), Squash (*Cucurbita spp*), bottle gourd (*Lagenaria siceraria*), melon (*Cucumis melo*), watermelon (*Citrullus lanatus*), bitter melon (*Momordica charantia), M. balsamina* (Balsam apple), *M. dioica* (spine gourd) and wax gourd (*Benincasa hispida*). Wild Cucurbitaceae members having medicinal value are an essential part of traditional health care systems. Their cultivation provides a source of income for many rural tribes, mainly landless poor and marginalized persons. The utility of wild members of the family Cucurbitaceae in food safety has not been given much attention, particularly in India. They also contain very interesting medicinally important chemicals, like cucurbitacins, colocynthin, momordicin, lufficulin, mogrol I –IV, saponins and trichozanthin (Navot and Zamir, 1987). From the nutritional point of view, the cucurbits seeds are valued for their high oil and protein contents (Rahaman *et al.,* 2008).

The members of the family are reported with an active constituent "Cucurbitacin", which is effective in slowing or stopping cell division, and they are therefore the subject of much research in the field of medicine, especially the treatment of cancer (Lee *et al.,* 2010). *Cucumis melo* seeds are useful in painful discharges and dysuria, dyspepsia, maintaining kidney functions, reduce blood

pressure, prevent cardiac dysfunction, Possess anti-rheumatic and anti-gout properties, menorrhagia, anti-tussive, febrifuge and vermifuge (Zinchenko *et al.*, 1955). Fruit can be used as a cooling agent, cleansing agent, or moisturizer for the skin, first aid treatment for burns and abrasions. *Momordica* spp. are traditionally known for its medicinal properties, such as antidiabetic, anticancer, anti-inflammation, antivirus, and cholesterol lowering effects. It has the potential as antioxidant and antimutagen, antispermatogenic (John *et al.*, 2010; Patil and Patil, 2011).

Morphology:

The family Cucurbitaceae, being the largest family comprises of economically and medicinally important plants that comprises 800 species in 130 genera worldwide (Jeffrey 2005), primarily native to tropical and subtropical regions, with two representatives in temperate regions also (Lu *et al.,* 1986; Schaefer and Renner, 2011b). Cucurbitaceae are typically annual climbing or trailing herbaceous vines with a single tree (*Dendrosicyos socotranus*) which is endemic to Socotra (Olson, 2003). The genus *Cucumis* includes plants that are annual herbs, usually having a trailing or climbing growth habit, although some cucumber and melon cultivars have a bush habit with a single leaf and a simple tendril at each node. Leaves are simple and petiolate. Flowers are unisexual, pistillate, or staminate, but hermaphrodite flowers also occur and the inflorescence is unisexual. Fruit is spherical, oval, oblong, elongated in shape, and variable in size. In the genus *Momordica*, plants are commonly annuals or perennials. Warts and tubercles is characteristic feature of fruits with shapes ranges from ovoid to oblong with ornamentation of soft spines.

Cytology:

Cytology deals with the study of the cells, especially their structure, function, and chemistry with a focus on the chromosome. The genetic information is carried out through the generation by the process of meiosis only by chromosomes of germline cells. However, the somatic cells help only in the regeneration and maintenance within the generation by the process of mitosis. So, therefore, meiosis is required to maintain a genetic balance over generations, although it produces variations via independent assortment and crossing over between non-sister chromatids of homologous chromosomes. Homologous recombination through crossing over in the

pachytene stage (prophase-I) is a dynamic process by which DNA sequences/strands are exchanged, hence induce structural aberrations and ultimately, cause the evolution of gene/genome. Such studies provide an insight into the genetic and environmental interactions, and their possible effect on chromosomal variability in structure and number on the morphology and reproduction of the organisms and ultimately in evolution and speciation.

Biochemical studies:

As we know that plants are used as medicines since ancient times and for the scientific evaluation of traditional medicinal plants, the area of ethnobotany and ethnopharmacology play an important role. The isolated active compounds from the plants are considered natural products, which are widely recognized in the pharmaceutical industry owing their broad structural diversity as well as their wide range of pharmacological activities. Plants possess some most important secondary metabolites/phytoconstituents such as amino acids, alkaloids, carbohydrates, flavonoids, fats, glycosides, lignins, proteins, phytosterols, saponins, terpenoids, tannins that play a great role in the herbal remedies. To know about the effects of these photochemical(s) in the plants or other organisms, some biochemical studies, like antimicrobial, antioxidant, antifungal, antidiabetic, etc. studies are performed. The activity of these active constituents may differ in cytotypes/ morpho/ ecotype(s), which needs to be investigated. Therefore, to check the effect of active constituents in medicinal plants, antimicrobial and genotoxic studies have been performed in the present study.

Genotoxicic studies:

Plants have been used in the healing and prevention of diseases from ancient times, however, despite their therapeutic benefits, some of their byproducts are reported to have a carcinogenic, mutagenic, teratogenic, and toxic effect(s) (Zink and Chaffin, 1998). Such studies are not so prominent in the plants (Basaran *et al.*, 1996), raising concern about their potential toxic effects. Hence, it is necessary to evaluate the toxicological effect of plants used by humans as food. For genotoxic studies, *Allium cepa* L. assay is used, where young meristematic roots are exposed to the plant extracts for determination of anti-mitotic, cytotoxic, or genotoxic effects (Roa *et al.*, 2012). Such cytotoxicity tests, using plant test systems *in vivo*, provide information for improving human health and detecting environmental pollution (El-Shahaby *et al.*,

2003). Data on cytotoxicity and genotoxicity is rare in the Cucurbitaceae, which need further attention.

Phytochemical studies:

The major obstacle in the coalescence of herbal medicines into modern medical implementation is the dearth of scientific and clinical data and the better comprehension of the effectiveness of herbal products. To ensure the constitution and safety of its products and practices, standardization plays a significant role (Gogtay *et al.,* 2002). Owing to the sophisticated nature and inherent discrepancy of chemical ingredients of plant-based drugs, this is not easy to maintain quality control criteria. According to prescriptive guidelines, macroscopic as well as microscopic evaluation and chemical characterization of the botanical materials are used for quality control and unification. Thin layer chromatography and High-performance Thin Layer Chromatography are commonly available tools for qualitative analysis of various constituents. Many systematic techniques including volumetric analysis, gas chromatography (GC), column chromatography (CC), spectrometric methods are also being used for quality control as well as standardization (Swatantra and Kushwha, 2010). Currently, genetic fingerprinting and the use of analytical quality-control equipment(s), like HPLC and HPTLC are frequently performed in recent days on a large scale for standardization and identification of herbal drugs. Photochemistry has evolved as a major branch of Pharmacognosy in developing markers for purpose of identification and standardization. It would also be worthwhile to draw a pharmacognostic scheme for all controversial species of plants where the information could be made available officially and to make its easy access for further reference (Kumar, 2007).

Aims and objectives:

The present study covers areas of the North Indian plains, i.e. Rajasthan, Haryana, and Punjab. The area is rich in floristic diversity, as having a tremendous range of topography, rainfall, climate, etc. It includes fertile plains of Punjab and Haryana to hot desert of Rajasthan. The biological richness of the region is characterized by enormous genetic diversity with reports of the existence of intraspecific variability in the family Cucurbitaceae. Perusal of literature reveals that large scale listing of medicinal plants has been done but data on genetic diversity

within Cucurbitaceae inhabiting different ecosystems, areas, different climates, soil types, etc., are still lacking.

To meet with the above mentioned problem, there is a scientific need to screen the cytological diversity of all species and phytochemical studies in wild species *Cucumis* L. and *Momordica* L. from the North Indian Plains covering states of Punjab, Haryana, Rajasthan, and Himachal pradesh. Hence, the present study plant has been selected for the investigation with the following objectives:

- Analysis of morphological and cytological diversity in different species of *Cucumis* L. and *Momordica* L. on population basis from the North Indian Plains.
- To evaluate the phytochemical profile of different cyto-morphovariants of the wild members of *Cucumis* and *Momordica*.
- To compare bioactivity profile in different cyto-morphovariants of the wild members of *Cucumis* and *Momordica*.
- To analyze mineral elements and proximate composition in wild fruits of *Cucumis* and *Momordica*.

REVIEW OF LITERATURE

Review of literature in this or that way, is relevant objectives of the study. It makes us enable to avoid any type of duplication in research work and moreover broadens the horizon of research problem. A comprehensive review is an essential part of any scientific investigation and helps to determine the already done work, assisting in the delimiting the problem area and provide the basis for a theoretical bodywork, and provide a clear cut vision into methods and procedure. In India, there is well-documented and time tested data on herbal medicine. Besides, India is experiencing an escalating awareness in the use of natural medicine as a substitute for synthetic additives of pharmacologically suitable agents (Turek and Stintzing, 2013). The traditional health care system has suggested the use of plant as well as their products for the treatment of a wide array of diseases (Arora *et al.,* 2010). Nowadays, the plant-based medicines are regaining their importance due to the various applications like, in folk medicine, nutraceuticals, pharmaceutical intermediates, modern medicines, chemical entities, food supplements and for synthetic drugs (Pandey and Tripathi, 2014). In the present study, the data has been arranged by studies related to morphology, cytology, phytochemical analysis, biochemical analysis, Genotoxic studies, proximate and element analysis, and ethnobotanical studies.

2.1 Morphology

The family Cucurbitaceae, based on the floral character, but the tendril is the synapomorphic character, considered homologous to shoot (Wang *et al.,* 2015). The family Cucurbitaceae, being the largest family comprises of medicinally and economically important plants that comprises 800 species in 130 genera all over the world (De Wilde and Duyfjes, 2006). The family is native to tropical as well as subtropical regions, including small number of plant species, in temperate regions (Lu *et al.,* 1986; Schaefer and Renner, 2011b). Ajuru and Okoli (2013) reported the root, stem and leaf anatomy and morphology of the genus *Citrullus* Schrad. ex Eckl. & Zeyh., *Cucumis* L., *Cucurbita* L., and *Cucumeropsis* Naud. in Nigeria and Aguoru *et al.,* (2015) reported the morphology and anatomy of *Zehneria* Endl.

Wild-type bitter gourd (*Momordica charantia*) is monoecious, fertilization and flowerings vary depending on growth conditions (Reyes *et al.,* 1994). The cucurbits bear a variety of sex expressions e.g. Sex expression in *M. charantia* is affected by

environmental conditions (Behera *et al.,* 2006). Similarly, (Ekeke *et al.,* 2015) reported the importance of tendril and fruit stalk anatomy in the Cucurbit taxonomy Aguoru and Okoli, (2012) reported the petiole and stem anatomy of West African species of *Momordica* L.

The genus *Cucumis* L. was revised by Kirkbride (1993) in his Biosystematic Monograph of *Cucumis*, comprises 32 species into two new entities (Kirkbride 1994). Following the observations of Kocyan *et al.*, (2007) that Kirkbride's (1993) circumscription of *Cucumis* in its traditional concept is highly unnatural, molecular phylogenetic studies. Species relationships and inclusion of few more genera under the broad genus *Cucumis* L. was done by Renner *et al.,* (2007), on basis of reinvestigation of species. Despite these enormous works on members of Cucurbitaceae, there is little information on the morphological and anatomical description of two genera *Cucumis* and *Momordica.* Therefore, this work focuses on the morphological and anatomical description of these species to improve its delimitation.

2.2 Cytology

Cytology is the study of the structure, function, as well as chemistry of cell particularly focus on chromosomes. Chromosomes of germ-line cells, through meiosis, help in carrying the genetic information through the generations. In flowering plants, the first ever chromosome count was made in orchids by Strasburger in 1882 . In India, the cytological study was initiated with the earliest attempt in *Piper betel* by Johnson (1910). From the world and India, the chromosome number review of various plants is compiled in various chromosome atlases by Ornduff (1969), Moore (1973, 1974, 1977), Goldblatt and Johnson (2000, 2003). Besides these atlases, the chromosomal reports are available in Index to Plant Chromosome Numbers (1970-2003), IOPB/IAPT volumes (2006-2011), journals, websites of IPCN-mobot.mobot.org/W3T/ Search/ipcn.html and Chromosome count database (http://ccdb.tau.ac.il).

Chromosome numbers in family are available for about 141 species belonging to 43 of the 130 genera (Beevy and Kuriachan, 1996), including important crop plant. The haploid chromosome number vary from n=7-24, with x = 12 as the most prevalent basic number (Beevy and Kuriachan, 1996). From India, different workers contributed towards the chromosomal study of the family at a generic level like *Luffa*

species (Dutt and Roy, 1976), *Cucumis* (Rajkumari *et al.*, 2013), *Momordica* (Trived and Roy, 1972), and *Trichosanthes* (Singh and Roy, 1973). Similarly, Varghese (1973) worked on the cytology of South Indian Cucurbitaceae members.

In case of genus *Cucumis* L. the chromosome data is available for a few species, due to the small size and poor stainability of the chromosomes (Dane, 1991). Male meiotic studies in the genus *Cucumis* is also restricted to few Indian species with few details on distribution pattern of bivalents at anaphase I and II (Qian *et al.*, 2003; Cao *et al.*, 2004). *C. sativus* (2n = 2x = 14) and *C. melo (*2n = 2x = 24), are two common vegetable crops on global basis (FAO, 2008). Within the genus, two groups namely African and Asian groups are accepted on the basis of geographic region with chromosome count of 2n = 2x = 24. *C. melo* (Kirkbride, 1993*)* has been originated in Africa. Cucumber, *C. sativus*, is belonging to Asian group with chromosome count of 2n = 14 (Jeffrey, 1980). On the other hand, a recent study proposed a different opinion about the origin of *C. melo* and *C. sativus*. It was known that there are thirty sub species in *C. melo*, but only two varieties i.e. *C. sativus* var. *sativus* and C. *sativus* var. *hardwickii*), of which, C. *sativus* var. *hardwickii* (2n = 14) is wild. *C. sativus* var. *hardwickii* has the potentiality to increase the genetic diversity for breeding commercial cucumber varities (Stauba and Bacher, 1997). *C. melo* is considered to be more diverse with respect to availability of varities in genus *Cucumis* along with variation in all parameters including, taste of fruit, size, shape and texture (Robinson and Decker-Walters, 1997).

The genus *Momordica* belonging to tribe Joliffieae (Schrad.), of family Cucurbitaceae and is native of the Palaeo-tropics (Robinson and Decker-Walters, 1997) and comprises of 40 species (Chakravarty, 1982), being distributed in warm tropics of both the hemispheres, primarily in Africa and about 10 species in South East Asia (De Wilde and Duyfjes, 2002). The cytological survey of the genus *Momordica* reveals that 12 species have been explored till date, including 5 species from the India. The chromosome number varies in the genus with 2n=16, 22, 28, 33, 42, and 56. The genus is polybasic on x=8, 11, 14. The genus shows 50% polyploidy, in which intraspecific euploidy is reported in only 1 species.

According to literature Indian *Momordica* spp. (Joseph, 2005) there are 6 well known species of which 4 are dioecious *M. dioica* Roxb. 2n = 28, *M. sahyadrica* with chromosome count of (2n=2x=28) (Joseph and Antony, 2007), *M. cochinchinensis*

(Lour.) Spreng. (2n = 28), and 2 are monoecious *M. charantia* L. and *M. balsamina* L. with chromosome count of (2n = 22) (De Wilde and Duyfjes, 2002). *Momordica* species are difficult to study for cytological analysis because the chromosomes are very small with poor power of stainabilty (Bhaduri and Bose, 1947) that counterpart the cytoplasm (Trivedi and Roy, 1972). In literature, Further, the phylogenetic relationship was drawn *Momordica,* and two groups were revealed. First, the monoecious group (*M. balsamina, M. charantia*) with chromosome count 2n=22 (Mc Kay, 1930; Shibata, 1962) whereas the dioecious group (*M. dioica, M. cochinchinensis, M. denudata* and *M. subangulata)* was been found to be 2n=28 (Beevy and Kuriachan, 1996). An autotetraploid constitution (Sen and Datta, 1975) and allopolyploid origin was also reported (Mondal *et al.,* 2006) for *M. subangulata* subsp. *renigera*.

2.3 Phytochemical analysis:

Phytochemicals are natural, biologically active, chemical constituents present in plants, which give health enhancers for humans may be differentiated into macro and micronutrients (Hasler and Blumberg, 1999). Chemical analysis by quantitative approach is a crucial tool for the assurance of specifications in crude substances and intermediates. The most important concern of medicines is quality because it deals with life. For the evaluation of the potency of products, various advanced techniques, like HPTLC, HPLC, LC/MS, GC/MS and LC/SPE/NMR are used. A wide array medicinal plants have been explored for their phytoconstituents by many workers, (Sharma and Cannoo, 2013). The active principles of these medicinal plants vary with the environmental conditions. Besides these, some other factors, like environmental stress conditions, morphological variations, cytological abnormalities, and different ploidy levels of the plants also plays an important role in the variations of the active constituents in the medicinal plants. The comparative quantitative study of secondary metabolites in diploids with their polyploid cytotypes have been carried out by various workers in different medicinal plants such as *Artemisia dracunculus* L. (Eisenman *et al.,* 2011), *Datura stramonium* (Berkov and Philipov, 2001), *Solanum commersonii* Dunal (Caruso *et al.,* 2011) etc. The intraspecific morphotypes also show remarkable dissimilarity in the number of secondary metabolites, like *Achillea millefolium* (Bimbiraite *et al.,* 2008), *Eclipta alba* (Saggoo *et al.,* 2010), etc.

Cucurbits are an immense source of glucose, fructose, water-soluble polysaccharides, water- dietary fibers, vitamins, essential amino acids, phenolic glycosides, minerals and terpenoids. Apart from the mentioned chemical entities, the family is very well recognized due to the existence of a very active constituent named "cucurbitacin". Cucurbitacin consist of skelton of tetracyclic cucurbitane nucleus and a varying oxygenation functional groups present at different locations with diversifying chemical species. The cucurbitacins, classified in 12 species from A-T, consist of non-glycosylated and glycosylated triterpenoids (Chen *et al.,* 2005). Many biochemical studies revealed that cucurbitacins are employed for anti-cancer drug evolution due to their cytotoxic property (Alghasham, 2013). The cucurbitacin nucleus possesses hydrophobic property, which is a major contributing factor for the cytotoxic effects. In particular, cucurbitacin E and its glycosides are the extensively distributed chemical constituents in the food plants of the family Cucurbitaceae. Cucurbitacin E, have inflammatory (Abdelwahab *et al.,* 2011), anti-angiogenic, cytotoxic immunomodulator (Attard *et al.,* 2015), hepatoprotective, and cytostatic (Arjaibi *et al.,* 2017) properties, both *in vivo* and *in vitro* conditions.

The phytochemical investigations have been reported in many species of the family Cucurbitaceae to characterize its phytochemical potential. *Cucurbita pepo* (pumpkin) seed yields about 50% oil, (oleic acid and linoleic) and the main active constituents include 7 sterols (spinasterol, avenasterol) and, Δ5 sterols (stigmasterol, sitosterol) (Fruhwirth and Hermetter, 2008**)** Photochemistry, Pharmacognosy and pharmacological study of *Lagenaria siceraria* were studied out by Kubde *et al.*, (2010). The phytochemical studies in *Coccinia indica* reveal the presence of alkaloids, glycosides, flavonoids, proteins, Saponin, steroids, tannin **(**Hussain *et al.*, 2011). The phytochemical study in *Coccinia grandis* revealed the presence of steroids in petroleum extract of leaves and found the compounds like tannins, proteins, glycoside, amino acid, alkaloid and saponins (Tamilselvan *et al.,* 2011). Experimental study on *Trichosanthes tricuspidata* (Vidyasagar, 2012) reveals the presence of carbohydrates, flavonoids, alkaloids, proteins and glycosides while Duvey *et al.*, (2012) observed that *T. tricuspidata* contains Cucurbitacin as an active ingredient and enumerated its medicinal uses. Gupta and Wagh (2014) have studied pharmacology, phytochemistry and folklore uses of *Diplocyclos palmatus* and noted the presence of

alkaloids, flavonoids, proteins, saponins, steroids, triterpenoids, etc. The plant possesses anti-inflammatory property.

Momordica dioica and *M. charantia* were also evaluated for phytochemical analysis (Anjamma and Lakshmi, 2015; Rawat *et al.*, 2018). *M. dioica* contains Lectins, triterpenes, proteins, and vitamins (Naik *et al.*, 1951). The fruit contains a large amount of vitamin C (Bhuiya, 1977). The fruit is rich in iodine and ascorbic acid (Rao, 2001). The fruit also contains an alkaloid, flavonoids, glycosides (Ilango *et al.*, 2012). Similarly, the bitter flavour in fruits and leaves of *M. charantia* is because of the presence of alkaloids, momordicoside L and momordicin K. (Paul and Raychaudhuri, 2010). Another alkaloid, vicine, has been found in reducing blood glucose in rats (Barron *et al.*, 1982). The fruit and leaves contain alkaloids, saponin glycoside like substance (Leelaprakash, 2011). Chemical Cucurbitane-type triterpenoids, steroidal glycoside, oleanane-type triterpene from the various parts of *M. charantia* was also isolated (Jiang *et al.*, 2016). Besides these, various types of terpenoids were isolated from *M. charantia* including charantagenins E and Charantagenins D (Wang *et al.*, 2012). *M. balsamina* L. plants also contain biologically active phytoconstituents like alkaloid, flavonoids, resins, steroids, terpenes etc. (Singh *et al.*, 2009). Various types of compounds like resins, flavonoid, alkaloid, glycosides, anthroquinine, steroids, terpenes, saponin, cardiac glycoside, and carbohydrate are secreted from the fruit of *M. balsamina* are (Bot *et al.*, 2007). Tannins form complexes with proteins and carbohydrates and thereby inactivate the microbial adhesion enzymes, transport protein, cell envelope etc. (Haslam, 1996). The active principles of *M. balsamina* include glycoside, resins, flavonoid and saponins (Abubakar *et al.*, 2005).

Phytochemical studies on *Cucumis melo* L. reveals the presence of flavanoids, alkaloids, glycosides, saponins, (Mallek Ayadi *et al.*, 2018; Ibidapo *et al.*, 2019). A Cucurbitane-type triterpenoids were isolated from *C. melo* (Chen, 2009). Similarly, the phenolic compound was also studied (Zeb, 2016). *C. melo var. agrestis wa*s also analyzed for phytochemical studies by various authors (Kavita *et al.*, 2013; Memon *et al.*, 2018; Gopalasathees kumar *et al.*, 2019). Phytochemical investigation of the seed of *Cucumis callosus* showed the presence of alkaloids, Saponin, carbohydrates, proteins, flavonoids, tannins, glycosides in both water as well as alcohol extracts (Chand *et al.*, 2012). Though a good amount of phytochemical studies have been

reported in the two genera, *Cucumis* and *Momordica*, however, the comparative studies on cyto/morpho/ecotypes are still lacking, which needs further investigations.

2.4 Biochemical analysis

The biochemical analysis includes a set of methods, assays, and procedures that helps in analyzing the chemical substances found in living organisms. These studies includes, antimicrobial activities, antifungal activities, anticancerous properties, antioxidant properties, etc.

2.4.1 Antimicrobial activity

Antimicrobial chemo treatment has been a crucial medical remedy since the first antibacterial dyes were analysed by the scientist Ehrlich durung beginning of 20th century. However, during the period of late 1940s antimicrobial drug resistant bacteria were noticed that posed a serious threat in clinical environment. This situation provoked the research area to standardize the methods to inhibit the action of bacteria and also to avoid their inactivation by developing the structures, they were not capable to avert bacterial resistance. However, the use of antimicrobials for agriculture and household products has become increasing (Muto *et al.,* 2003). Medicinal plants with their active constituents like flavonoids, phenolics etc. can be a successful an alternative path to combat with bacterial diseases (Samy *et al.,* 1998). various medicinal plant formulations are being studied for their possible antimicrobial potential and to get remedies from various bacterial diseases (Elizabeth, 2002), as roots of *Arnebia nobilis* (Indrayan *et al.,* 2004). Active constituents for antimicrobial action was identified and isolated from plant parts by various authors (Chansiripornchai *et al.,* 2008). Natural polysaccharide has been found to be good therapeutic agents for immunomodulating action (Chansiripornchai *et al.,* 2008), wound healing (Chansiripornchai *et al.,* 2005, 2008), antibacterial activity (Pongsamart *et al.,* 2005), food product and pharmaceuticals (Umprayn *et al.,* 1990b) and toxicity (Pongsamart *et al.,* 2001).

Many Indian plant species were reported with antimicrobial activities (Table 2.1), including Cucurbitaceae, few of these have been discussed here. *Cucurbita maxima* show antibacterial action against the fungus *Aspergillus niger, Candida albicans, Bacillus subtilis,* and *Pseudonomas aeruginosa*. It was inactive against *Escherichia coli, Staphylococus aureus,* and *Trichophyton mentagrophytes* (Consolacion *et al.,* 2005). Antimicrobial activity of extracts from *Coccinia grandis*

was studied Dewanjee *et al.,* (2007). Anti microbial action of *Trichosanthes tricuspidata* is due to Cucurbitacins (Khare, 2007) Antibacterial activity of *Lagenaria breviflora, Coccinia grandis, C. cordifolia, C. Indica, Cucurbita pepo* and *Trichosanthes cucumerina* was evaluated by various authors (Syed *et al.,* 2009; Arawwawala *et al.,* 2011; Chonoko and Rufai, 2011; Poovendran *et al.,* 2011; Sivraj *et al.,* 2011; Gopalakrishnan *et al.,* 2012; Khatun *et al.,* 2012) against *Shigella boydi* and *Pseudomonas aeruginosa*, *Aspergillus niger and Candida albicans, Staphylococcus aureus, Streptococcus pyrogenes, Escherichia coli,* and *Pseudomonas aeruginosa* were reported (see table. 2.1)

Table 2.1. Antimicrobial potential of some common Indian medicinal plants.

S.No.	Plant name	Activity against microbe	Reference
1.	*Adiantum capillus veneris*	*E.coli, K. pneumoniae, Salmonella typhimurium, P. vulgaris, P. aeruginosa, S. aureus, Vibrio cholera*	Ishaq *et al.,* 2014
2.	*Artemisia annua*	*S.aureus, Salmonella enterica, K. pneumoniae, Shigella dysenteriae, E.coli*	Tajehmiri *et al.,* 2014
3.	*Azadirachta indica*	*S. aureus, E. coli, P.aeruginosa*	Orhue *et al.,* 2014
4.	*Calotropis procera*	*S. aureus, B. cereus*	Meena *et al.,* 2010
5.	*Capsicum annum*	*P. vulgaris, Paeruginosa, S. typhimurium,*	Keskin and Tor, 2011
6.	*Cisampelos pareira*	*S.aureus, S. typhimurium, K. pneumoniae, E. coli, P. vulgaris* and *Streptococcus pneumonia*	Ngoci *et al.,* 2014
7.	*Ocimum gratissimum*	*S. aureus*	Akinyemi *et al.,* 2005

8.	*Phylantus discoideus*	*S. aureus*	Akinyemi *et al.,* 2005
9.	*Piper pulchrum*	*E.colic, B. cereus*	Rojas *et al.,* 2006
10.	*Psidium guajava*	*E. coli, B. cereus, S. aureus,*	Biswas *et al.,* 2013
11.	*Santalum album*	*B. subtilis*	Parekh *et al.,* 2005
12.	*Tecomella undulate*	*S. epidermidis, B. subtilis*	Parekh *et al.,* 2005
13.	*Terminalia avicennioibes*	*S. aureus* (Antimethicillin resistant)	Akinyemi *et al.,* 2005
14.	*Terminalia catappa*	*S. aureus, B. subtilis*	Babayi *et al.,* 2004
15.	*Withania somnifera*	*K. pneumonia*	Bokaeian *et al.,* 2014

Further, studies in the genus *Momordica* showed antimicrobial activity against *Shigella dysenteriae, Escherichia coli, Pseudomonas multocida, Salmonella typhi,* and *Salmonella paratyphi* (Ekta and Krishnamurthy, 2013). Similarly, *M. dioica* L. showed antimicrobial action against, *Pseudomonas aeruginosa, Escherichia coli* and *Bacillu subtilis*. (Arekar *et al.,* 2013). *M. balsamina* also shows antimicrobial activity against various pathogenic organisms (Sumitra and Bhagwati, 2018).

Similarly, in these genus *Cucumis*, *C. melo* shows the highest antimicrobial activity against the bacteria *Escherichia coli* in acetone extract. In aqueous solvent, *Escherichia coli*, and in acetone extract *Staphylococcus aureus* do not show antimicrobial action (Karim *et al.,* 2016; Vishwakarma *et al.,* 2017). Despite these antimicrobial studies in the two genera, *Cucumis* and *Momordica*, the comparative studies on cyto/morpho/ecotypes are still lacking, which needs further investigations.

2.4.2 Antioxidant activity

Recently, there is an increased demand for antioxidants from natural source (Buxiang *et al.,* 1997). Natural products from medicinal plants have shown the reverse correlation between the intake of fruits and vegetables and the morbidity and mortality from the degenerative diseases (Rimm *et al.,* 1997). In the last few decades, there is an increase in the research on the health benefits of natural polyphenols (Lee

et al., 2003; Modun *et al.,* 2003). Antioxidants may up-regulate the antioxidant defenses in our body (Montoro *et al.,* 2005).

Many plants have been recommended for their consumption, having potent antioxidant action (Lee *et al.,* 2003). Previous studies have shown that plant products, mainly the secondary metabolites possesses the potentiality in reducing oxidative stress and macromolecular inhibition in our body (Margarida *et al.,* 2011; Wilson *et al.,* 2017; Xu *et al.,* 2017). The measurement of antioxidant potentiality is a difficult job due to different types of mechanism followed by the antioxidants such as hydrogen donating, chelating metal, electron-donating and free-radical scavenging ions to initiate free radical reactions. So, it is impossible to check antioxidant capacity by a single method (Prior *et al.,* 2005). Phenolic compounds has been studied extensively for their antioxidant potential by various researchers (Colla *et al.,* 2007).

Cucurbits of tropical regions plays an important role in human consumption (Rai *et al.,* 2008). Muskmelon extracts also showed high antioxidant potential as well as anti-inflammatory action (Vouldoukis *et al.,* 2004). *In-vitro* anti-oxidant of *Coccinia grandis* was studied by Ashwini *et al.,* (2012), Umamaheswari and Chatterjee, (2008). Similarly cucurbiticin was observed in *Lagenaria siceraria* by many workers (Deore *et al*., 2009; Erasto and Mbwambo, 2009; Kubde *et al*., 2010; Hossain *et al*., 2012). Ash gourd is well known for its antioxidant potential (Ziani *et al.,* 2011). Pumpkin contains an effective amount of carotenoids and tocopherols, having high antioxidant potential (Kim *et al.,* 2012). The watermelon fruits also possess free radical scavenging activity and antioxidant potential (Oseni and Okoye, 2013; Singh *et al.,* 2016) by using various solvent. Similar studies were undertaken to evaluate *Citrullus colocynthis* and *Bryonia dioica* of Cucurbitaceae for their antioxidant activity (Barreira *et al.* 2013; Echaimaa *et al.,* 2015). Antioxidant activity of *Cucumis setosus, Lagenaria siceraria, Trichosanthes tricuspidata, Coccinia grandis* and *Diplocyclos palmatus* was done by using DPPH assay (Sandhya, 2015).

In the genus *Momordica, M. charantia* (Rezaeizadeh *et al.,* 2011) showed antioxidant activity by DPPH, Superoxide radical scavenging activity and Hydrogen peroxide scavenging activity (Kubola and Siriamornpun, 2008; Choi *et al.,* 2012; Hamissou *et al.,* 2013; Raish 2017) to eliminate ROS and also showed cardio-protective activity (Rammal *et al.,* 2012; Sagor *et al.,* 2015). Similar investigations were done by many other workers (Avijeet, 2008; Bhavana *et al.,* 2010; Shreedhara *et al.,* 2011). *M. balsamina* also shows antioxidant activity at a greater rate (Souda *et al.,*

2018; Sumitra and Bhagwati, 2018). *M. indica* also possesses antioxidant activities in various extracts (Garrido *et al.*, 2004) which is due to a variety of phenolic compounds (Janet *et al.*, 2006).

Similarly, in the genus *Cucumis*, the antioxidant activity of *Cucumis melo* was studied by various authors and it showed significant results (Ismail *et al.*, 2010; Sasi Kumar *et al.*, 2015; Ibidapo *et al.*, 2019). Similarly, the antioxidant activity of *C. melo* var. *agrestis* was carried by (Manpreet and Arora, 2011; Memon *et al.*, 2018; Gopalasathees *et al*., 2019). Similar studies were done by using hydrogen peroxide assay (Mariod and Matthaus, 2008; Alagar *et al.*, 2015). The comparative studies of two genera, *Cucumis* and *Momordica* at cyto/morpho/ecotypes level are still lacking, which needs further investigations.

2.5 Genotoxicity evaluation

Genotoxicity refers to the ability of the compounds to interact with DNA/proteins apparatus including, topoisomerase enzymes and spindle fibers that regulate the functioning of the chromosomes. Genotoxicity testing is an supreme technique to assess the risk of chemicals for regulatory purpose. Medicinal plants have been used in folk medicine for longer lime. However, some plants cause adverse effects by interacting with other medications (Zink and Chaffin, 1998). It has been hypothesized that the plants or plant products recommended for medicinal formulations have low toxicity. In general, green plant act as a primary source of antimutagens as well as natural toxic agent (Tülay and Özlem, 2007). Cytotoxicity of plant extracts on both plant and animal test material is capturing scientific interest, as it can indicate their antitumor and anticarcinogenic potentials, which have always been a gateway for the development of novel anticancer drugs.

Before the medical application of plant constituents, purification, characterisation and detailed study of toxicity of compounds is very necessary. For preliminary research of toxicity, these toxicity testing procedures are very useful mechanism (Veni and Pushpanathan, 2014). However, it has been remarked that some constituents have been shown to carcinogenic, teratogenic, and mutagenic which is correlated with tumor formation in human populations (Paes-Leme *et al.*, 2005; Gadano *et al.*, 2006). Many plants used for food and traditional medicine possess mutagenic, genotoxic and cytotoxic effects, proved by *in vitro* and *in vivo* assays (van den Berg *et al.*, 2011; Ferreira-Machado *et al.*, 2014). *Allium cepa* L. have been the most extensively used plant material for cyto-toxicity and genotoxicity assay, to

describe damage due to chromosomal aberrations (Fiskesjo, 1985; Rank, 2003; Nabeel *et al.,* 2008; Tedesco and Laughinghouse, 2012; Algarni, 2018).

Cytological effects caused by caffeine and caffein derivatives were explored by Kihlman and Kronberg (1972). Raj and Rao (1972) conducted cytological studies in *Vicia faba* treated with lathryrogens. The chromosome breakage, bridge formation, micronuclei formation, stickiness, etc. were observed upon study mitosis in *Vicia faba* and *Pulicaria crispa* (Prasad and Das, 1977; Shehab, 1979). There are a number of plants which shows cytotoxic/genotoxic effects, and are listed below in table 2.2.

Table 2.2. Genotoxicity evaluation of some common medicinal plants.

Plant species	**Reference**
Spilanthes ciliate	Sreeranjini and Thoppil, 2001
Corymbia spp.	Saj and Thoppil, 2006
Artemisia nilgirica	Leeja and Thoppil, 2004
Azadirachta indica, Cymbopogon citratus,	Akinboro and Bakare, 2007
Curcumin	Ragunathan and Panneerselvam, 2007
Baccharis trimera , Baccharis articulate	Fachinetto and Tadesco, 2009
Acorus calamus	Rajkumar *et al.*, 2009
Hibiscus rosa sinensis	Ali, 2010
Inula viscose	Celik and Aslanturk, 2010
Eupatorium triplinerve	Asha and Thoppil, 2011
Erythrina velutina	Silva *et al.*, 2011
Aloe vera	Ilbas *et al.*, 2012

Despite these enormous cyto/ genotoxic studies in many plant species, the data is lacking in the family Cucurbitaceae, hence considered in the present study.

2.6 Proximate composition and mineral analysis

Nutrients are component of food required for normal development of the human body and also participate in various metabolic processes (Okiei *et al.,* 2009). About 50 nutrients that fall into six basic groups including glycosides, vitamins, protides, lipids, water and minerals are necessary for the maintenance of good health and are important for body functions (Venkatesh *et al.,* 2010).

Minerals and trace elements are necessary for various physiological and biological and physiological processes including bones, teeth, muscles, tissues, nereve cells and blood (Soetan *et al.,* 2010). They help to maintain acid-base balance, the response of nerves and blood clotting (Hanif *et al.,* 2006). Due to this, various therapeutic benefits are considered to be associated with the consumption of healthy food (Xutian *et al.,* 2009). However, in the nineteenth century, due to development of globalization, industrialization and urbanization the philosophy of "food as medicine" becomes oblivion. However, nowadays various life threatening diseases has emerged due to less focus on healthy diet (Ashakiran and Deepthi, 2012).

Further, purity and quality of crude drugs in powder form can be evaluated by ash values. The ash values have significant value are as ash may be derived from the plant parts itself (physiological ash) or from extrenal matter, adhered to the surface (non physiological ash) (Arumugam and Natesan, 2015). The determination of total ash includes both physiological as well as nonphysiological ash. The total ash varies due to variation in composition of natural and physiological ash (Bhargava *et al.,* 2013). In such cases, to obtain natural ash, it is treated with acid, and natural ash being soluble is extracted leaving behind acid-insoluble ash from contaminated soil particles (Sarvesh *et al.,* 2015).

Cucurbits, being utmost important vegetable crops are found throughout the world in both temperate as well as tropical regions (Bisognin, 2002; Sanjur *et al.,* 2002). Cucurbits being rich in nutrients are adapted to divergent climatic conditions are cultivated for seed utilization (de Mello *et al.,* 2000, 2001; El Tahir and Taha Yousif, 2004). The cucurbits are considered as orphan crops due to lack of attention from research and development point of view (IPGRI, 2002). The high amount of oil, good appearance and color, odorlessness has made these seeds suitable for oil industry (Mariod *et al.*, 2009).

Momordica charantia L. contain carbohydrate, protein, lipid, water, fiber, minerals, vitamins and minerals. *M. charantia* contain about 228 different bioactive constituents in different parts of plant, including innumerable minerals like calcium, magnesium, manganese, iron, sodium, potassium and zinc found in reasonable amounts (Bakare *et al.,* 2010; Singh *et al.,* 2011). These minerals are of immense importance for various body functions including, such as calcium plays a role in the muscle contraction, bone and teeth formation and blood clotting (Peters and Martini,

2010). Enzymatic controlled catalytic reactions are controlled by magnesium and zinc (Ahmed and Chaudhary, 2009). Sodium and potassium help in maintaining electrolytic balance and membrane fluidity across intracellular and extracellular fluid (Ahmed and Chaudhary, 2009). Iron is the basic component of myoglobin, hemoglobin, natural buffer and other metalloenzymes (Ahmed and Chaudhary, 2009; Naik, 2016) required for oxygen and CO_2 transport in respiratory activity. Potassium, calcium and zinc play an important roles in the stimulation of beta cells of islets of Langerhans to release insulin (Kar *et al.,* 1999).

Proximate composition, protein and mineral content of *Momordica charantia* L. pericarp as well as seeds was evaluated at different stages of maturity (Alvi *et al.,* 2003; Sommro and Ansari, 2005; Horax *et al.,* 2010). A reasonable amount of calcium, copper charantin, D-galacturonic acid, sterol glucoside, iron, phosphorus, vitamis etc. was isolated from bitter gourd (Sommro and Ansari, 2005; Paul and Raychaudhuri, 2010; Bakare *et al.,* 2010; Horax *et al.,* 2011). Enhancement of flavor during cooking in *Momordica* sp. is due release of alkaloids and saponins present in fruit (Schultes, 1990). Saturated fatty acids are present in the seed oil of *M. charantia* L. (Mukherjee and Barik, 2014; Yoshime *et al.,* 2016; Saini *et al.,* 2017). The immature fruits of *M. charantia* are a good source of vitamin A, vitamin C, and β-carotene (Braca *et al.,* 2008; Zhang *et al.,* 2009; Choi *et al.,* 2012). The fruits are used in the form of cooked vegetables (Nagarani, 2014). *Momordica charantia* L. also contain flavonoids, terpenes, phenols, glucosinolates, anthraquinones and isoflavones with antioxidant potential (Drewnowski and Gomez-Carneros, 2000).

M. dioica contains fiber, mineral, vitamins, fat, carbohydrate and protein (Ram *et al.*, 2004; Aberoumand and Deokule, 2009). Vitamins includes niacin, riboflavin, carotene, ascorbic acid, and thiamin (Singh, 2006). *M. dioica* contains glycosides, Lectins, Saponin, ß-sitosterol, oleanolic acid, stearic acid, and two novel aliphatic components (Sadyojatha and Vaidya, 1996; Ali and Srivastava, 1998). The nutritional significance of *Momordica dioica* was studied only to a limited extent (Aberoumand and Deokule, 2009).

Further, leaves of *M. balsamina* contains seventeen amino acids (Hassan and Umar, 2006) other minerals like like potassium, calcium, magnesium, manganese, phosphorus, sodium, iron and zinc (Hassan and Umar, 2006; Flyman and Anthony, 2007; Thakur *et al.,* 2009; Yerima and Umar, 2019).

Cucumis melo seed kernel rich in unsaturated fatty acids and consists of 40-50% fatty acids and 20-30% protein (Petkova and Antova 2015; Mansouri *et al.,* 2017). Nutritional analysis of *C. melo* reveals protein, amino acids and β-carotene (Norrizah *et al.,* 2012; Mallek-Ayadi *et al.,* 2018). *C. melo* seeds are rich in glyceride oil (30.0–50.0%) and proteins (12.0–35.0%) which is considered as the waste product (de Mello *et al*., 2000, 2001). Melon seed oil contains linoleic acid, stearic acid, oleic acid, palmitic acid, sterols and tocopherol (Milovanovic and Picuric-Jovanovic, 2005; Mian-Hao and Yansong, 2007; Azhari *et al.,* 2014). The information related to the lipid composition of the melon seeds is concerned with various physico-chemical characteristics, moreover it varies depending upon the type of the seeds used and their origin (de Mello *et al.,* 2001; Milovanovic and Picuric-Jovanovic, 2005; Mian-Hao and Yansong, 2007; Obasi *et al.,* 2012; Azhari *et al.,* 2014). In *Cucumis melo* var. *agrestis* the fruits revealed the presence of moisture, carbohydrate, ash, protein, Cu, Fe, Zn and Co (Memon *et al.,* 2018) and also reveal that use of this wild fruits aids some important minerals in diet necessary for the body (Ahmed *et al.,* 2006). *Cucumis melo* var. *momordica* good source of nutrients, sugars, vitamin C, fibers and minerals. A 100g edible fruit of snap melon contains 18.6 mg vitamin-C, 15.6 g carbohydrates, 0.3 g protein, 95.7% moisture, energy (Goyal and Sharma, 2009; Peter and Hazra, 2012). Despite many nutraceuticals values in cucurbits, the data for minerals and proximate analysis is still lacking in the family hence considered in the present study.

Need for the Study

About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants (Arun Kumar and Muthuselvam, 2009). The screening of plant products for antimicrobial activity has shown that the higher plants represent a potential source of novel antibiotic prototypes (Selvamohan *et al.,* 2012). However, a very little information is available on such activity of medicinal plants. Out of the 4, 00, 000 plant species on earth, only a small number has been systematically investigated for their antimicrobial activities (Anjana *et al.,* 2009). The traditional systems of medicine have rejuvenated for finding antioxidants and antimicrobial compounds from a natural source to control human and plant diseases due to the increasing problems with drug resistant microorganisms, side effects of modern synthetic drugs and the increasing number of emerging diseases with no proper medication. Curative, preventive and nutritive properties of the

medicinal plants provide the necessary power and energy to fight the disease and assist the action of the restorative agents in the herbal drug Bhunshan (Patwardhan *et al.,* 2004). Numerous methods have been utilized to acquire compounds for drug discovery including isolation from plants and other natural sources, synthetic chemistry, combinatorial chemistry, and molecular modeling (Geysen *et al.,* 2003). Despite the recent inventions, natural products, and particularly medicinal plants remain an important source of new drugs, and new chemical entities (Newman *et al.,* 2000), approximately one-quarter of the bestselling drugs worldwide were natural products or derived from natural products (Butler, 2004). Medicinal plants have a promising future because there are about half a million plants around the world, and most of them their medical activities have not been investigated yet, and their medical activities could be decisive in the treatment of present or future studies. Further, the phytoconstituents may vary in morpho/ cyto/ ecotypes due to genetic or environmental factor hence, need to be investigated, in search of the best chemotype.

MATERIALS AND METHODS

Natural populations of species of *Cucumis* L. and *Momordica* L. growing in different locations in northern India were collected for investigation. The information regarding the altitude, latitude and longitude of various localities from where plant populations were collected is given in table. 3.1. The voucher specimens (2411-17) are submitted to the Herbarium, Mata Gujri College, Fatehgarh Sahib, after proper identification are given in table 3.2. The parameters selected for present study were morphological analysis, cytological analysis, preliminary phytochemical analysis, genotoxic, antioxidant and antimicrobial potentials, HPLC, mineral and fruit composition of fruit extracts.

3.1 MORPHOLOGICAL ANALYSIS

The following morphological characteristics will be taken for the present study.

a. From mature samples, morphological characters like, leaf shape, leaf area, leaf length and width (cm), and from mature fruits characters like, fruit length, fruit diameter, fruit weight and fruit surface characters will be studied using standard measurement methods.
b. Plant height will be measured by using standard measurement methods.

3.2 CYTOLOGICAL ANALYSIS

For meiotic analysis, young and unopened floral buds were collected from the healthy plants and then were fixed in Carnoy' fixative i.e. Absolute alcohol: Chloroform: Glacial acetic acid::6:3:1, for 24 hours. Then the fixed buds were transferred to 90% alcohol and were kept at 4˚C in the refrigerator for further use.

The fixed anthers were squashed in 1-2 drops of 2% acetocarmine, prepared by dissolving 2gm of standard stain carmine (BDH) in 100ml of 45% acetic acid. For chromosome count, a number of freshly prepared slides were examined carefully. The incidence of various types of meiotic abnormalities, such as late disjunction, laggards, bridges at Anaphase-1 or Telophase-1, etc., were scored from various slides at random.

Detailed observations were made on PMCs at tetrad stage to study the microsporogenesis. Pollen fertility in both the species was calculated by squashing the mature anthers from flowers in 1:1 glycero- acetocarmine dye at 60˚C for 5 minutes and examined after 24 hours. Pollen grains with well stained nuclei and well filled

cytoplasm were counted as fertile, while shriveled and unstained pollen grain were considered as sterile. Photomicrographs of chromosome counts, sporads and Pollen grains were taken using Nikon 80i eclipse microscope. The percentage of pollen fertility was calculated as:

% age pollen fertility= Number of fertile pollen/ Total pollen observed X100

Table 3.1. Sampled populations of *Cucumis* L. and *Momordica* L. with geographical location

Sr. No.	Population	Latitude	Longitude	Altitude (m)
1.	Punjab , Amritsar	$31^0$20'N	76˚24'E	252
2.	Punjab , Barnala	$30^0$23'N	75˚31' E	226
3.	Punjab , Bathinda	$30^0$11'N	75˚ 00'E	210
4.	Punjab , Hoshiarpur	$31^0$32 ̍N	75˚57̍ E	295
5.	Punjab , Jalandhar	$31^0$19'N	75˚57'E	221
6.	Punjab , Kapurthla	$31^0$36̍ N	$75^0$29̍E	225
7.	Punjab , Ludhiana	30˚55'N	75˚54'E	262
8.	Punjab , Mansa	30˚46'N	76˚41'E	220
9.	Punjab , Moga	$31^0$18̍N	$75^0$16̍E	224
10.	Punjab , Mukatsar	30˚30'N	74˚43'E	184
11.	Punjab , Patiala	30˚20'N	76˚24'E	252
12.	Punjab , Sangrur	30˚12'N	75˚53'E	231
13.	Rajasthan, Sri Ganga Nagar,	$29^0$91̍N	73^0 83'E	178
14.	Haryana, Panchkula, Punchkula	$30^0$74'N	$76^0$80'E	365
16.	Haryana, Ambala, Narayangarh	$30^0$47̍N	$77^0$12'E	264
17.	Haryana, Narwana	$29^0$61̍N	$76^0$11̍E	225
18.	Himachal Pradesh, Sirmaur, Sirmaur	30.50º N	77.24º E	364
19.	Rajasthan ,Jaipur	$26^0$91̍N	$75^0$78̍ E	431

Table 3.2. Plant species with Accession number

S.No.	Plant species	Location	Accession number
1	*Cucumis melo* L.	Punjab (Barnala, Bathinda, Mansa, Moga, Mukatsar, Patiala, Sangrur)	2411
2.	*Cucumis melo* var. *agrestis* (Morphotype I)	Punjab (Amritsar, Barnala, Bathinda, Hoshiarpur, Jalandhar, Kapurthla, Ludhiana, Mansa, Moga, Mukatsar, Patiala, Sangrur) Haryana (Ambala)	2412
3.	*Cucumis melo* var. *agrestis* (Morphotype II)	Punjab (Mansa, Bathinda, Sangrur)	2413
4.	*Cucumis melo* var. *momordica* L.	Punjab (Barnala, Bathinda, Mansa, Moga, Mukatsar, Patiala, Sangrur) Haryana (Narwana)	2414
5.	*Momordica balsamina* L.	Punjab (Barnala, Bathinda, Mansa, Moga, Mukatsar, Patiala, Sangrur) Haryana (Narwana) Rajasthan (Sri Ganga Nagar)	2415
6.	*Momordica charantia* L.	Punjab (Amritsar, Barnala, Bathinda, Hoshiarpur, Jalandhar, Kapurthla, Ludhiana, Mansa, Moga, Mukatsar, Patiala, Sangrur, Haryana (Ambala, Narwana) Himachal Pradesh (Chail) Rajasthan (Jaipur, Sri Ganga Nagar)	2416
7.	*Momordica dioica* L.	Rajasthan (Jaipur)	2417

3.3. PRELIMINARY PHYTOCHEMICAL SCREENING

3.3.1. Preparation of plant extracts

The fresh plant fruits were collected and washed under the running tap water to remove dust particles and other debris.The fruits were air dried under at room temperature for few days.The dried fruits samples were ground well in to a fine powder with the help of mixer grinder. A 100gm air dry plant was soaked into 500ml organic solvents i.e. Methanol, Chloroform, Aqueous, Acetone and petroleum ether separately for 24 hrs at normal temperature..The extracts were filter through the Whatman filter paper No:1 . The extract was allowed to dry using rotary evaporator and percentage yield was calculated. The condensed extracts were stored in airtight container at 40^0 C till further investigation.

3.3.2. Phytochemical Analysis

Phytochemical analysis was determined to identify phytochemicals in the Chloroform, Aqueous, Methnol, Acetone and Petroleum ether extracts of plant fruits were used in this present work, the phytochemicals were detected by standard colour tests. Phytochemicals screening of the extracts was performed as described by (Harborne, 1984;Sofowora, 1996) as discussed below.

a. Test for alkaloids

Dragendroff's test: To 2-3 ml fruit extract, add few drops of Dragendroff's reagent. The formation of orange brown precipitate indicates the presence of alkaloids.

Iodine test: Mix 3 ml test solution and add few drops of dilute iodine solution. In the results, blue colour appears which disappears on boiling and reappears on cooling.

b. Test for Saponin

Foam test: Dilute the plant extract with 20 ml of distilled water and shake it in a graduated cylinder for 15 minutes. A layer of foam indicates the presence of saponins

c. Test for tannins

Ferric chloride test: To 1 ml of extract, add 2-3 drops of dilute ferric chloride solution, the development of green black color indicates the presence of tannins.

Lead acetate test: To 5 ml of extract, add few drops of 10% lead acetate solution. The formation of yellow or red precipitate indicates the presence of tannins.

d. Test for glycosides

Keller-Killani test: To 2 ml extract, add glacial acetic acid, one drop of 5% $FeCl_3$

and conc. H_2SO_4. The reddish brown color appears at junction of the two liquid layers and the upper layer appears bluish green indicates the presence of glycoside.

Concentrate H_2SO_4 test: To 5ml extract, add 2ml glacial acetic acid, one drop 5% $FeCl_3$ and conc. H_2SO_4. The appearance of brown ring indicates the presence of glycosides.

e. Test for triterpenoids:

Libermann-Burchard reaction: Dissolve a small amount (2-3 mg) of dried powdered extract in chloroform. To this, add few drops of acetic anhydride and two drops of H_2SO_4 along the side walls of the test tube. The formation of red/violet color indicates the presence of triterpenoids

f. Test for flavonoids

Shinoda test: To 2-3 ml extract, add few fragments of magnesium metal in a test tube, followed by drop wise addition of concentrate HCl. The formation of magenta colour indicates the presence of flavonoids.

NaOH test: To 2-3 ml of extract, add few drops of sodium hydroxide solution in a test tube. The formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicates the presence of flavonoids.

g. Test for reducing sugars:

To the extract solution (2 ml), add few drops of Fehling's solution [mix equal volumes of Fehling's A (copper sulphate solution) and Fehling's solution B (alkaline tartarate solution). Heat the mixuture for 5 min. In the results, the samples positive for reducing sugars developed brick red precipitate.

h. Test for steroids

Salkowski's test: To 2 ml of extract, add 2ml of chloroform and 2 ml of concentrated H_2SO_4 and shake well. The chloroform layer appears red and acid layer shows greenish yellow fluorescence, which indicates the presence of steroids

3.4 GENOTOXICITY EVALUATION

Allium cepa L. assay was used to study the cytotoxic potential of various extracts of *Cucumis* L. and *Momordica* L. by using the set procedure given by Fiskesjo (1985) and Rank and Nielsen (1993).

3.4.1 Preparation of plant extracts

For genotoxic studies plant fruits were collected from different sites (table

3.1). Plants were washed under running tap water. Mature healthy fruits were separated from the plants, cut into small pieces and were air dried for several days. fruits were then ground into coarse powder. The dried fine powder was stored in air-tight containers for further use.

The 5 gm of dried and ground plant fruit powder was then extracted in 100 ml distilled water at 60˚C for 6 hrs. Extracts were then filtered with help of Whatman filter paper (No.1) and centrifuged at 6000 rpm for 30 min. Then filtrate was concentrated by using rotary evaporator at low pressure and temperature (40-45˚C). Dried extract were then dissolved in distilled water and then stored at 4˚C. Similarly solvent extract (acetone and methanol) were prepared. The test conditions are given in table 3.3.

Table -3.3. Test conditions for *Allium cepa* genotoxicity test:

1.	Test organism	*Allium cepa*
2.	Parameter observed	Micronuclei in interphase and chromosome aberrations in anaphase-telophase root tip cells
3.	Test condition	Temperature 25±1˚C, light cycle 16h/8h (light/dark)
4.	Replicates	3 plants per extract.
5.	Exposition length	24hr pre treatment in control water, 48 hr of exposition with extracts.
6.	Chemical	Ethanol extract, acetone extract, aqueous extract, tap water (+ve extract), sodium azide (-ve control)

3.4.2 Procedure

Healthy equal sized bulblets of *Allium cepa* were placed over the water filled test tubes with lower part dipped in water for 48 hrs at 25±1˚C, in an incubator till the roots attain the size of about 2 cm length and the treated with various extracts, then were fixed in Carnoy' fixative i.e. absolute alcohol: chloroform: glacial acetic acid:: 6:3:1, for 24 hours. Then the fixed buds were transferred to 90% alcohol and were kept at 4˚C in the refrigerator for further use, then roots were hydrolysed in a mixture of 2% acetocarmine and 1N HCL in ration of 9:1 part, were squashed in drop of acetocarmine and covered with cover slips and observed under microscope and calculations were made as follows.

Calculations:-

The various calculations were made as follows:

Mitotic index= Total number of dividing RTC/ Total number of observed RTC*100

Percent mitotic phase= Number of cells at mitotic phase/Total number of observed RTC*100

Percentage aberration=Total number of abnormal RTC/Total number of observed RTC*100

Percent micronuclei=Total number of cells with micronuclei/Total number of observed RTC*100

3.5. ANTIBACTERIAL ACTIVITY ASSAY

The antimicrobial potential of prepared extracts of fruits of *Cucumis* L. and *Momordica* L. was evaluated in the present investigation by using method given by Baurer *et al.,* 1966. A set of bacterial strains obtained from Microbiology lab of Mata Gujri College, Fategarh Sahib was used. The details of protocols used are:

3.5.1. Preparation of Plant extracts

Plants collected from different locations were washed under running tap water. Mature healthy fruits were separated from the plants, cut into pieces and air drie and then ground into coarse powder to store in air-tight containers.

3.5.2. Extract preparation

The 5 gm of dried and ground plant fruit powder was then extracted in 100 ml distilled water at 60˚C for 6 hrs. the extracts were then filtered with help of Whatman filter paper (No.1) and centrifuged at 6000 rpm for 30 min. The filtrate was then concentrated by using rotary evaporator at low pressure and temperature (40-45˚C). Dried extract were then dissolved in DMSO and then stored at 4˚C. In the similar way solvent extract (acetone and methanol) was prepared.

3.5.3. Tested Microorganisms

The tested microorganisms used in this study were as follows:

Bacillus coagulans, Klebsiella pneumonia, Pseudomonas flourescens, Staphylococcus aureus.

3.5.5. Culture and maintenance of culture

Preparation of Mueller-Hinton agar

Mueller-Hinton agar was procured as a commercially available dehydrated medium according to the manufacturer's instructions. Immediately after autoclaving, it was allowed to cool in water bath at 45-50°C. Before using the medium in laminar air flow the outer surface of the conical flask were wiped with cotton using 70% of Iso propyl alcohol, to avoid cross contamination and then transfer about 25 to 30 ml of sterile prepared medium into 100 mm diameter petri dishes (flat bottom). The agar medium was allowed to cool at room temperature, unless the plate is used in the same day, the prepared should be stored in a refrigerator at 2-8°C. A representative sample of each batch of plates was examined for sterility by incubating at 30 - 35°C for 24 hrs.

3.5.6. Preparation of wells and performing well diffusion test

Wells of approximately 6 mm in diameter are made in plates and different concentration of plant extract solutions was loaded in them The plates were then incubated at 37º C overnight. Microbial growth is determined by measurement of zone of inhibition. The results were then compared with the standard antibiotics taken.

3.5.7. Preparation of Inoculate

The bacterial suspensions were adjusted with sterile saline. The inoculants were prepared and stored at 4ºC until use. Dilutions of the inocula were cultured on a solid medium to verify the absence of contamination and to check the validity of the inoculums. All experiments were performed in duplicate and repeated three times.

3.5.8. Inoculation of test plates

A sterile cotton swab (Hi media, readily prepared sterile swabs) was dipped into the turbid culture suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab. The dried surface of a Mueller-Hinton agar plate was inoculated by swabbing the swab over the entire sterile agar surface. This procedure was repeated by swabbing two more times, rotating the plate approximately 60° C each time to ensure an even distribution of inoculums. In final step, the rim of the agar was swabbed. The lid may be left aside for 3 to 5 minutes, but not more than 15

minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

3.5.9. Application of Discs to Inoculated Agar Plates

The wells were filled with different concentration of extracts and the plates were incubated at 5°C for 1 hr to permit good diffusion and then transferred to incubator at 37°C for 24 hrs (Salie *et al.,* 1996; Baris *et al.,* 2006). After completion of 24 hrs, the plates were inverted and placed in an incubator set to 37° C for 24 hr. In the present study, methanol, acetone and aqueous extracts of *Cucumis* L. and *Momordica* L. were screened for anti microbial activity against five different standard strains of microorganisms. Chloramphenicol was used as positive control and DMSO as negative control as standard references.

3.5.10. Reading plates

After 24 hr of incubation, each plate was examined. The diameter of zones of complete inhibition was measured, including the diameter of the disc. Zones were measured the nearest whole millimeter using a scale, which was held on the back of inverted petriplate.

3.5.11. Calculation

Observations were made in triplicates and mean values were taken for further use. Activity index was estimated by comparing the inhibition zone of plant extract with standard chloramphenicol.

Activity index =Inhibition zone of test sample or extracts / Inhibition zone of chloramphenicol.

3.6. ANTIOXIDANT ACTIVITY

The antioxidant activitiy of fruit extracts (methanol and queous) of plant material were assayed by employing the following methods. The extracts were tested for their radical scavenging capacity against free radicals by using various free radical scavenging mechanisms using spectrophotometer.

3.6.1. DPPH Scavenging Assay

Stock solution was prepared and the different dilutions were made with varying concentrations ranging from 100µg/ml to 500µg/ml by diluting the stock with methanol. The 1 ml of each concentration was taken in each test tube and 2ml of methanolic solution of DPPH was added in each test tube, then incubated at 37ºC for

30-40 minutes. The absorbance was taken at 517 nm along with the blank. Methanol and ascorbic acid was taken as blank and standard respectively. The higher the free radical scavenging activity, the lower will be the absorbance (Miliauskas *et al.,* 2004; Bozin *et al.,* 2006; Kaur *et al.,* 2012).

The DPPH radical scavenging activity of the plant extracts were calculated by the following equation:

% inhibition = $(A_0-A_s) /A_0 * 100$

Where, A_0-Absorbance of contol i.e. DPPH in methanol, A_s-Absorbance of the sample and standard

3.6.2. Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide was performed by method given by Ebrahimzadeh *et al.,* 2006. Solution of hydrogen peroxide was made in the phosphate buffer. Different concentrations of plant extracts was taken in different test tubes and then .6 mL of hydrogen peroxide solution added to these test tubes and then absorbance was taken at 230 nm. The percentage scavenging of hydrogen peroxide by the extracts and standard was calculated as follows:

% inhibition = $(A_0 –A_S)/A_0 * 100$

Where, A_0- Absorbance of H_2O_2 A_S- Absorbance of the sample and standard

3.6.3. Reducing power assay

Reducing power was measured by using method given by Oyaizu, 1986. For reducing power assay, different concentrations of extracts were taken in test tubes and to each test tube, 2.5 mL of phosphate buffer and 2.5mL of potassium ferricyanide was added. All the test tubes were then incubated at 50^0C for about 20 min. further, 2.5 ml of trichloroacetic acid (10%) was added to each test tube and then centrifuged at 3000 rpm for 15 min. 2.5 ml was taken from upper layer of solution and was mixed with 2.5 ml distilled water and and 0.5 ml $FeCl_3$ was added and absorbance was taken at 700 nm.

3.7. Estimation of Total phenolic content

Total phenolic content was measured by using Folin-ciocalteu assay (Zhou and Yu, 2006), gallic acid as standard (Benariba *et al.,* 2013; Belkacem *et al.,* 2014). Plant extracts were mixed with 1 ml of Folin-Ciocalteu phenol reagent, the incubated at room temperature for 3-4 minutes, 1ml of 20% sodium carbonate solution was

added to solution and diluted to 10 ml with double distilled water. The whole test set up was incubated in dark for 1 hour and then absorbance was measured at 765 nm. The results obtained are expressed in term of mg of Gallic acid equivalent per 100gm of the dry weight of plant extract. The total phenolic content was determined by plotting a standard curve.

3.8. Determination of total flavanoid content

The method as reported (Arvouet-Grand *et al.,* 1994) with slight modification were used to determine total flavanois content The results were expressed as quercetin equivalent as standard. The results obtained are expressed in term of mg of Quercetin equivalent per 100gm of the dry weight of plant extract. For this 0.5 ml of test solution was taken and 1.5 ml methanol was added to this, then 0.1 ml of $AlCl_3$, 0.1 ml of CH_3COONa and 2.8 ml of distilled water was added. Then whole set was kept for 30 min and absorbance was taken at 415 nm using spectrophotometer.

3.9.PROXIMATE ANALYSIS

3.9.1. Moisture content determination

For measuring the moisture content of samples (AOAC, 2000) method was used. For this, 5 g of fresh plant material and dried in hot air oven for about 3 hrs. The final weight was measured after cooling in a desiccator, and it was calculated as per the formula given below.

Moisture content (%) = FW – DW/FW*100

DW = Dry weight (g), FW = Fresh weight (g).

3.9.2. Ash value

A muffle furnace was used to analyse a 5 g sample for total ash (AOAC, 2000). The sample was heated in a silica crucible over low bunsen flame with half covered lid. When fumes were no longer produced, the crucible and lid were placed in muffle furnace and heated at 550ºC overnight. After complete heating the lid was placed on the crucible to prevent loss of fluffy ash and cooled in a dessicator. Weight was taken when the sample turned grey.

Ash value(%) = weight of ash / initial weight of sample taken*100

3.9.3. Determination of crude fat

For the determination of crude fat the (AOAC,2000) method was used. For this clean flask was preheated at 105^0 C, then cooled in desiccator and its weight was

calculated. Then, 5 g of dry sample was taken, wrapped in Whatman filter paper No.1 and was extracted in Soxhlet apparatus for 12 h with 500 mL petroleum ether. Finally, the solvent was removed using rotaevaporator and the final weight of extracted fat was measured by using the formula as follows.

Crude fat (%)=Weight of extracted fat/weight of sample *100

3.9.4. Determination of crude protein

The (AOAC, 2000) method was followed for the determination of crude protein content using the Kjeldhal apparatus. In this experiment, the sample (1 g) was taken in Kjeldhal digestion chamber and to this, conc. H_2SO_4 (20 mL) and 10 g of Kjeldhal catalyst (one part $CuSO_4$ and 9 parts K_2SO_4) was added, digested and separated by using Kjeldhal distillation apparatus. The condensed ammonia was collected and titration was performed against 0.1 N HCl. The blank test containing the entire reagents except the sample was also performed as a control. A similar process was also performed for blank solution. The nitrogen concentration was calculated as per the formula given below.

Nitrogen (%) = (A – B) × N of HCl × 14/ Weight of the sample*100

Where, A = Volume of 0.1 N HCl used in titration of sample.

B = Volume mL of 0.1 N HCl used in titration of blank.

14 = Atomic weight of nitrogen.

The protein content was calculated by multiplying the nitrogen content with 6.25 of protein conversion factor. Protein (%) = Nitrogen (%) × 6.25.

3.9.5. Determination of crude fibre

The AOAC, 2000 method was used to determine crude fibre content of the sample. For this the dry sample (1 g) was digested for 30 min with 0.25 N H_2SO_4 and it was filtered by using Whatmann filter paper. The residue was washed with hot water for three times and it was heated for 30 min with 0.3 N NaOH till it boils. After that, it was filtered again, followed by washing with hot water, 0.5 N H_2SO_4 and ethanol solution (50%). The residue obtained was completely dried using hot air oven at 130 ^oC. The dried weight obtained was incinerated in a furnace at a temperature of 600^oC for 30 min (Maynard 1970; Sadasivam and Manickam 1992). The ash obtained was weighted and the crude fibre content calculated using the formula:

Crude fibre (%) = (Dry weight of digested sample – Weight of ash)/Weight of sample*100

3.9.6. Total carbohydrate determination

The total carbohydrate amount of the plant samples was evaluated by using the difference method as prescribed by James, 1995 by using the following equation.

Total carbohydrates (%) =100 – [% of (Moisture + Ash + Crude protein + Crude fat)].

3.9.7. Determination of calorific value of plant sample

The total calorific/energy value (kcal/100 g) of the fruits was calculated (Aberoumand and Deokule, 2009). The formula for which is as follows:

Energy value (kcal/100 g) = 4 (Protein %) + 9 (Fat %) + 4 (Carbohydrate %).

3.10. Mineral contents

The mineral content such as Potassium (K), Sodium (Na), Calcium (Ca), Zinc (Zn), Magnesium (Mg), Manganese (Mn), Copper (Cu) and Iron (Fe) in the fruits samples was estimated by standard method (AOAC, 2000) and (Puwastein *et al.*, 2011). For their quantification, 1 gram of sample was taken in Teflon cup and digested with 10 mL of Nitric acid (HNO_3): percholric acid ($HClO_4$) (7:3) mixtures at temperature of 180-200°C until the transparent material was obtained. The content was then diluted with double distilled water to a volume of 100 mL and stored in Nalgene plastic bottles for further use. By running these diluted samples concentration of mineral content was estimated by using Atomic Absorption Spectrophotometer and flame photometry.

3.11. Anti nutritional factor analysis

3.11.1. Alkaloid content

For estimation of alkaloid content method given by Omoruyi *et al.*, 2012 was used. For this, 5 gram of sample was macerated in 200 mL of 10% acetic acid in ethanol and was kept undisturbed for about 4 hrs, then filtered and reduced to $1/4^{th}$ of its original volume. Then ammonium hydroxide (concentrated) was added drop wise until the precipitates are formed and allowed to settle and washed with dilute ammonium hydroxide and then was filtered. The residue obtained was dried, weighed and the alkaloid content percentage was calculated as follows:

Alkaloid content % = weight of precipitate/ weight of original sample *100

3.11.2. Oxalate content

To determine the oxalate content, titration method given by Day and Underwood (1986) was used. For this, 1 g of sample was macerated with 75 mL of 3 mol/L H_2SO_4. Then whole mixture was gently stirred magnetic stirrer for one hour and filtered. From the filtrate, 25 mL sample was collected and heated to 80–90°C then temperature was maintained to 70°C. Then hot aliquot was continuously titrated with 0.05 mol/L of $KMnO_4$ until the light pink color is obtained. The oxalate content was measured by taking 1 mL of 0.05 mol/L of $KMnO_4$ as equivalent to 2.2 mg of oxalate.

3.11.3. Saponin content

Saponin content was estimated using the method described by Otang *et al.*, 2012. 1 g of sample was mixed with 40 mL of 20% ethanol, homogenized and allowed to stand in a water bath for 4 h at 55°C. The resulting mixture was filtered using a vacuum pump. The residue was collected and re-extracted with 20 mL of 20% ethanol. The filtrates form the residue were combined and reduced to 40 mL in a water bath at 90°C; the concentrate was transferred into a 200 mL separating funnel, 20 mL of diethyl ether was added and mixed vigorously. The lower fraction was collected while the upper layer (Ether) was discarded. The lower fraction was re- introduced into the separating funnel and 20 mL of butanol was added, mixed vigorously, followed by 5 mL of 5% aqueous sodium chloride. The upper fraction (butan-1-ol) was collected and evaporated to constant weight in the oven. The saponin content in the sample was calculated using the equation:

% Saponin content = Weight of fraction/Weight of sample*100

3.12. HPLC Analysis

In every step of drug development, HPLC is an essential tool to confirm the quality of drug candidate.

3.12.1. Preparation of sample solution for HPLC analysis

The methanolic extract of samples were centrifuged for 10 minutes at 3000 rpm, then filtered by using Whatman No.1 filter paper under high pressure vacuum pump. The samples were then diluted to 1:10 with sample solvent.

HPLC method procedure given by Sharma *et al.*, 1993 was used for HPLC determination, Shimadzu LC-10 AT VP HPLC system. The fruit extracts were

compared against known chemical materials like β-sitosterol, Rutin, Quercitin and Cinnamic acid and retention time, UV spectra and area of sample were compared in each sample(s) with that of standard β-sitosterol, rutin, quericetin, and cinnamic acid.

3.12.2. Chromatographic conditions

The chromatographic analysis was performed at ambient temperature on a RP-C18 analytical column with a mobile phase composed of Acetonitrile: Methanol (50:50 v/v) and was isocratically eluted at a flow rate of 1 ml/min. A small sample volume of 20 µl was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 256 nm shown in Table 3.4.

Table 3.4. Selection of separation variables

Injection	20 µl
Flow rate	1 ml/min
Wavelength (λ)	256 nm
Temperature	Room temperature
Mobile phase	Acetonitrile:methanol (50:50)
Detector	UV
Column	Reverse phase Gemini 5u C18, 110A, Phenomenox (250 X 4.60 mm)

3.13. STATISTICAL ANALYSIS

The data in thesis was evaluated by using 2 way ANOVA and DUNCAN multiple range test.

OBSERVATION

In India, the family represents 100 species in 36 genera, most of which are of high economic importance (Chakravarthy, 1982). The genus, *Cucumis* L. comprises 30 species at word level and 20 in India, of which five are wild (Chakravarthy, 1982). Further, *Momordica* L. is a genus of 45 tropical wild and underutilized species native to Asia and Africa, of which six each occur in India and Malaysia (Schafer, 2005). North Indian germplasm of the species was investigated to know the cytological diversity within the species, also attempt was made to evaluate the cytomorphological study, preliminary phytochemical study, genotoxicity study, antioxidant assay, antimicrobial potential, mineral composition and proximate analysis of the plant.

3.9.MORPHOLOGICAL STUDY

Cucumis L. and *Momordica* L. selected for present study belongs to family Cucurbitaceae. *Cucumis melo, Cucumis melo* var. *agrestis, Cucumis melo* var. *momordica, Momordica balsamina, M. charantia, M. dioica* have been collected at random from various populations given in Table 3.1. Data regarding the morphological parameters according to species for various populations are given in table 4.1.

In case of *Cucumis melo* leaf parameter including average length and breadth varies between 13.29± 0.92 cm and 16.73± 0.63 cm respectively. Average internode length ranges from 12.32± 0.03 cm in various population on geographical basis. Fruits are smooth have average length and diameter 22.03± 0.034 cm and 41.052± 0.71 cm respectively. Weight varies with average of 1436.2±0.2 gm (Figs. 1-4).

In *Cucumis melo* var. *agrestis* two morphotypes had been observed in field. In morphotype I average petiole size is 9.20±0.73 cm. The average size of leaf length, leaf breadth varied between 5.56±0.31 cm and 15.67±0.31 cm respectively and average internodes length ranges from 4.06±0.46 cm (Fig. 5-8). The average fruit length is 5.3±0.37 and 7.8±0.65 respectively and weight with average of 278.9±0.32 gm .

In morphotype II there is noticeable large size of fruit with small sized leaves and leaf parameter including length and breadth ranges from 6.62±0.52 cm and 6.77±0.43 cm respectively. Average petiole size is 2.26±0.37 cm. Fruits are large as

compared to other morphotypes ranging from 9.1±0.43 cm in length and 8.36±0.51cm in diameter and with with average of 456.7±0.0 gm (Fig. 9-12).

In case of *Cucumis melo* var. *momordica* leaf parameter including average length and breadth varies between 9.0±0.00 cm and 12.50±0.408 cm respectively. Average internode length ranges from 8.5±0.707 cm in various population on geographical basis. Fruits are smooth have average length and diameter 27.0±1.0 cm and 32.5±0.5 cm respectively and weight with average of 1059.8±0.0 gm (Figs. 13-16).

In case of *Momordica charantia* leaf parameter including average length and breadth varies between 5.16±0.53 cm and 6.63±0.36 cm respectively. Average internode length is 5.63±0.32 cm in various populations on geographical basis. Fruits are rough and wrinked have average length and diameter 16.70±0.61 and 12.45±0.56 cm respectively and weight with average of 534.4±0.4 gm (Figs. 17-21).

In *Momordica balsamina* petiole length varies between 0.82±0.51 cm. Average length and breadth varies between 2.48±0.41cm and 3.58±0.42 cm respectively. Average internode length ranges from 4.12±0.51 cm in various populations on geographical basis. Fruits are rough and wrinked have average length and diameter 4.88±0.38 cm and 7.60±0.52 cm respectively and weight varies with average of 215±0.24 gm (Figs. 22-25).

In *Momordica dioica* petiole length is 2.6±0.38. Average length and breadth of leaf varies between 6.8±0.27 cm and 7.0±0.56 cm respectively. Average internode length is 5.2±0.45 cm in various populations on geographical basis. Fruits are rough and with spines have average length and diameter 4.56±0.801 cm and 7.8±1.21cm and weight with average variation of 239.3±.21 gm (Figs. 26-30).

Table. 4.1. Comparison of size of various plant parts (cm) in various populations of *Cucumis* L. and *Momordica* L.

		Leaf size (Average±S.D.)			Fruit size (Average±S.D.)			
Plant species	Internode (cm)	Length (cm)	Breadth (cm)	Petiole (cm)	Length(cm)	Diameter (cm)	Weight (gm)	Surface
CM	12.32± 0.03	13.29± 0.92	16.73± 0.63	11.42± 0.07	22.03± 0.034	41.052± 0.71	1436.2±0.2	Rough
CMAI	4.06±0.46	5.56±0.31	15.67±0.31	9.20±0.73	5.3±0.37	7.8±0.65	278.9±0.32	Smooth
CMAII	5.67±0.09	6.62±0.52	6.77±0.43	2.26±0.37	9.1±0.43	8.36±0.51	456.7±0.0	Smooth
CMM	8.5±0.707	9.0±0.00	12.50±0.408	7.33±0.235	27.0±1.0	32.5±0.5	1059.8±0	Smooth
MC	5.63±0.32	5.16±0.53	6.63±0.36	4.06±0.64	16.70±0.61	12.45±0.56	534.4±0.4	Rough
MB	4.12±0.51	2.48±0.41	3.58±0.42	.82±0.51	4.88±0.38	7.60±0.52	215±0.24	Rough
MD	5.2±0.45	6.8±0.27	7.0±0.56	2.6±0.38	4.56±0.801	7.8±1.21	239.3±.21	Spines

Values are mean ±SD of three measurements. Variation in the letters between samples indicates significant difference at level $p < 0.05$ by two way ANOVA Test.

Abb. CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

Table 4.2. Data matrix for numerical study based on morphology.

Character	Character state	CM	CMAI	CMAII	CMM	MC	MB	MD
Habit	0, Annual 1, perennial	0	0	0	0	0	0	1
Sex expression	0, Monoecious 1, dioecious	0	0	0	0	0	0	1
Anthesis	0, Morning 1, evening	0	0	0	0	0	0	1
Leaf lobe	0, Deeply lobed 1, angled	0	0	0	0	0	0	0
Flower size	0, Small 1, large	0	0	0	0	0	0	0
Corolla colour	0, Yellow 1, milky white	0	0	0	0	0	0	0
Stigma colour	0, Green 1, yellow	0	0	0	0	0	0	1
Anther symmetry	0, Symmetrical 1, asymmetrical	0	0	0	0	0	0	0
Fruit type	0, Fleshy 1, dry	0	0	0	0	0	0	0
Fruit shape	0, Ovoid-oblongoid 1, pyriform	0	0	0	0	0	0	0
Fruit ridges	0, Absent 1, present	0	0	0	0	0	0	0
Fruit spines	0, Absent 1, present	0	0	0	0	0	0	1
Tubercles on fruit surface	0, Absent 1, present	0	0	0	0	1	1	0
Fruit	0, Orange red	1	0	0	0	0	0	0

colour at ripening	1, green							
Placenta colour on ripening	0, Red 1, white	0	1	1	0	0	0	0
Intensity of bitterness	0, Extreme 1, light/less/nil	0	0	0	0	0	0	1
Seed sculpturing	0, Present 1, absent	0	0	0	0	0	0	0
Seed margin	0, Dented 1, smooth	1	1	1	1	0	0	0

Abb. CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

4.2. Cytological analysis

In the present study 6 species of 2 genera *Cucumis* (3 spp.) and *Momordica* (3 spp.), have been studied cytologically, of which, one species *C. melo* var. *agrestis* reveals two morphotypes.

Genus *Cucumis* L.

Cucumis melo L. plants were collected from various localities of North India (Table 3.1). Meiotic analysis from all the populations showed haploid chromosome number n=12 at diakinesis (Fig. 31). Further meiotic course was slightly abnormal with formation of cytomictic channels at the early stages of meiosis in Barnala , Bathinda, Moga and Jalandhar populations (Fig. 33-35). Laggards and stickiness were also observed at telophase-II (Fig. 32,36). Besides normal tetrads, tetrads with micronuclei (Fig. 38) and polyads (Fig.37) were also observed in Amritsar population. Heterogeneous sized pollen grains were observed (Fig. 39) and, pollen fertility was 96.32±0.21% for all the populations.

Cucumis melo var. *agrestis* L. (Morphotype I) plants were collected from various localaties of North India (Table. 3.1). Meiotic analysis showed haploid

chromosome number n=12 at diakinesis in all the populations (Fig. 40). Further meiotic course was slightly abnormal with formation of cytomictic channels at the early stages of meiosis (Fig. 41-43). Populations from Patiala, Sangrur, Ludhiana reveals normal tetrads, triad and dyad with with micronuclei (Fig. 44, 45). Heterogeneous sized pollen grains were observed (Fig. 46) and pollen fertility was maximum i.e. 98.52±0.38 in Ludhiana population.

Cucumis melo var. *agrestis* L. (Morphotype II) Plants were collected from various localaties of North India. Meiotic analysis showed haploid chromosome number n=12 bivalents at metaphase (Fig. 47). Further meiotic course was slightly abnormal with formation of triads (Fig. 48) polyad (Fig. 49), tetrad with micronuclei (Fig. 50) in Barnala and Moga, Mukatsar and Patiala populations populations. Laggards were also observed at anaphase (Fig. 51) in Narwana population. Heterogeneous sized pollen grains were observed (Fig. 52) and pollen fertility was 96.17±0.72%.

Cucumis melo var. *momordica* L. plants were collected from various localaties of North India. Meiotic analysis showed haploid chromosome number n=12:12 distribution at anaphase at diakinesis (Fig. 53). Further meiotic course was slightly abnormal with formation of chromatin bridges at the early stages of meiosis (Fig. 54, 58) in Barnala, Moga, Narwana and Patiala populations. Stickiness was also observed at metaphase (Fig. 55). Besides normal tetrads, triads, triad with micronuclei (Fig. 56, 57) were also observed. Heterogeneous sized pollen grains were observed and pollen fertility was 74.39±0.49% .

Genus *Momordica* L.

Momordica dioica L. plants of this population were collected from Jaipur. Meiotic studies revealed the haploid chromosome number n=14 bivalents at metaphase-I (Fig. 59) and 14:14 distribution of chromosomes at anaphase-I (Fig. 60). Some of the PMCs show presence of laggards (Fig. 64) at anaphase-I. Some of the PMCs observed were involved in cytomixis at tetrad and triad stages (Fig. 63). Besides tetrads, few tetrads with micronucleus (Fig. 62) were also observed.

Momordica balsamina L. plants were collected from Sangrur, barnala, Patiala, Bathinda, Jalandhar, Mukatsar, Kapurthala, Mansa, Moga, Jaipur and Narayangarh**.**

In *Momordica balsamina* L. Meiotic analysis of the species reveals the 11:11 distribution of chromosome at A-I (Fig. 65), which is in conformity with the earlier reports from India and outside India. The detailed meiotic course is found to be normal which results in high pollen fertility 99.93±1.28% (Fig. 66).

Momordica charantia L.

Plants were collected from Sangrur, Barnala, Patiala, Bathinda, Jalandhar, Mukatsar, Kapurthala, Mansa, Moga, Narwana, Chail and Narayangarh**.** Meiotic analysis showed haploid chromosome number n=11:11 distribution at anaphase (Fig. 67). Further meiotic course was slightly abnormal with formation of cytomictic channels at the early stages of meiosis (Fig. 73). Laggards were also observed in Narwana population (Fig. 71-72). Besides normal tetrads, monad (Fig. 68) diad (Fig. 69) and polyad (Fig. 70) were also observed. Heterogeneous sized pollen grains were observed with pollen fertility 92.28±0.36 %.

Table 4.3. Microsporogenesis in different populations of *Cucumis* L. *and Momordica* L.

Sr. No.	Plant	PMC observed (%)							
		Diad		Triad		Tetrad		Polyad	Abnormal PMC
		N	Mn	N	Mn	N	Mn		
1.	CM	—	—	—	—	90.10±0.56	7.69±0.51	2.19±0.36	9.89±0.932
2.	CMAI	—	—	2.17±0.05	—	92.18±0.87	4.68±0.41	—	7.81±0.47
3.	CMAII	—	—	1.40±0.23	—	94.83±0.46	2.81±0.47	0.93±1.02	5.16±0.21
4.	CMM	—	—	1.52±0.48	0.76±0.94	87.78±0.48	8.39±0.39	—	12.21±0.52
5.	MC	4.86±1.26	1.76±0.93	3.21±1.08	0.88±0.28	84.51±0.21	2.65±1.42	1.76±0.34	15.48±0.69
6.	MB	—	—	—	—	96.23±0.72	—	—	___
7.	MD	—	—	—	—	93.85±0.32	6.14±6.49	—	6.14±0.93

Abb. N = Normal , Mn = Micronuclei

Abb. CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

Values are mean ±SD of three measurements. Variation in the letters between samples indicates significant difference at level $p < 0.05$ by one way ANOVA Test.

Table 4.4. Data on chromosome number, ploidy level (n), pollen fertility and pollen size of *Cucumis* L. and *Momordica* L.

Sr. no.	Population	N	Pollen fertility (%)	Pollen size (µm)
1.	CM	12	96.32±0.21	17.58±0.72X17.10±0.41
2.	CMAI	12	98.52±0.38	17.70±0.51X17.10±0.57
3.	CMAII	12	96.17±0.72	19.25±0.28X19.21±0.36
4.	CMM	12	74.39±0.49	23.82±0.29X22.82±0.62
5.	MC	11	92.28±0.36	20.23±1.27X20.19±0.39
6.	MB	11	99.93±1.28	16.81±0.48X16.44±0.69
7.	MD	14	98.72±0.34	17.33±0.82X16.51±0.36

Abb. CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

Table 4.5. Data on cytomixis and meiotic course in the different populations of *Cucumis* L. and *Momordica* L.

Sr. No.	Population	Cytomixis			Meiotic abnormalities (%)				
		% of PMC (Mean ±SD)	PMC Associated	Meiotic stages	Lag (Mean ±SD)	Brid (Mean ±SD)	Vag (Mean ±SD)	Stickiness (Mean ±SD)	Abberant PMC (Mean ±SD)
	CM	3.82±0.32	2-3	Dia, M-I,T-II.	0.23±0.32	—	2.24±0.13	0.95±0.37	3.42±0.84
	CMAI	—	—	—	1.75±1.32	____	—	_____	1.75±0.43
	CMAII	3.30±0.48	2-3 units	Triad, tetrad	3.06±0.38	1.02±0.36	—	_____	4.25±0.29
	CMM	3.49±0.42	3-4 units	Tetrad	2.72±0.27	0.56±0.34	—	1.36±0.06	4.64±0.16
	MC	24.07±0.49	2-3unit	Dia, M-I,A-I,T-II.	—	—	—	____	—
	MB	—	—	—	—	—	—	____	—
	MD	4.65±0.38	2-3unit	T-II	1.31±0.67	—	—	____	1.31±1.32

Values are mean ±SD (Standard deviation) of three measurements. Variation in the letters between samples indicates significant difference at level $p < 0.05$.

Abb. PMC- pollen mother cell, Lag- Laggard, Brid- Bridge, Vag- Vagrant,

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

4.3. GENOTOXICITY EVALUATION

For monitoring the genotoxic potentialities of *Cucumis* L. and *Momordica* L., *Allium* assay was performed. Three types of extracts of the fruits i.e. aqueous, ethanol and acetone were tested employing the assay.

Healthy bulbils of *A. cepa* with young actively growing roots were placed on the test tubes filled with different types of fruit extracts. The treatment was given for 24 hrs. Root tips of about 1cm in length were fixed in Carnoy's fixative (ethanol:glacial acetic acid:chloroform). The cytological data on these harvested roots was recorded describing the chromosome damage and the disturbances in the mitotic cycle. Roots raised over sodium azide (0.5 mg/L) as the positive control and over distilled water served as the negative control.

The data regarding the mitotic analysis of RTCs of *Allium cepa* treated with the fruit extracts of *Cucumis* L. and *Momordica* L., and cytological aberrations induced in RTCs are given in table 4.6 and 4.7.

4.3.1. Effect on Mitotic stage frequencies and chromosome abnormalities

Genotoxicity study showed many clastogenic alterations in root tips of *A. cepa* when treated with control (negative and positive) and aqueous/ acetone and methanolic fruit extract of different species of *Cucumis* and *Momordica* (Table 4.6, 4.7). These chromosomal abnormalities includes, chromatin stickiness, chromatin bridges, laggards, polar deviation or multipolarity (Table 4.7, Figs. 1, 2), that might be reason for the mitotic depression (Table 4.6). In the negative control, *Allium cepa* roots treated with normal tap root show normal chromosomes and distribution (Table 4.6). A number of clastogenic aberrations were recorded in a number of cells (Figs. 74-98), when treated with 0.5 mgL^{-1} sodium azide solution (NaN_3), a positive control. Among the different fruit extracts, the least mitotic index (46.20±0.23%) was observed in methanolic extract of *Cucumis melo* var. *momordica*, while the maximum value (58.73±0.18) was recorded in aqueous extract of *Cucumis melo* (Table 4.6). Further, many mitotic aberrations were also recorded in fruit extracts of different plants samples (Figs. 74-98, Table 4.7). The maximum percentage of abnormal cells were recorded in methanolic extract of *Cucumis melo* var. *momordica* (Table 4.7), where abnormalities, viz. chromatin stickiness (Fig. 55), chromatin transfer and

vagrants (Fig. 77, 79, 81, 82, 83, 96) were also recorded in the maximum number of cells (Table 4.7). Further, the maximum number of cells with chromatin bridges (Figs. 74, 75, 84, 90, 94,), laggards (Fig. 86) was reported in methanolic extract of *Cucumis melo* var. *agrestis* (morphotypes I & II). Besides these, other mitotic aberrations, like un-oriented chromosomes (Figs. 77, 79, 82, 83, 91), chromatin bridges (Figs. 75, 84, 90) have also been reported in other treatments also (Table 4.7). The plant extracts induced significant ($p<0.001$) number of clastogenic alterations .

Table 4.6. Number of dividing cells and Mitotic index in in control (negative and positive) and Aqueous, Acetone and Methanolic extract of different species of *Cucumis* L. and *Momordica* L.

Treatments	No. of dividing cells	Mitotic index (%)
	Average ±SE	Average ±SE
Negative Control 1	321.00±0.578 [a]	64.20±0.12 [a]
Positive Control 1	112.67±2.03 [b]	22.53±0.41 [b]
CM-Aq.	293.67±0.88[a]	58.733±0.18[a]
CM-Ace	237.33±1.20[b]	47.47±0.24[b]
CM-MeOH	236.33±1.33[b]	47.27±0.27[b]
CM/AI-Aq.	279.67±1.45[c]	55.93±0.29[c]
CM/AI-Ace	236.33±1.33[a]	47.27±0.27[a]
CM/AI-MeOH	238.00±1.58[b]	47.60±0.31[b]
CM/AII-Aq.	279.67±1.76[a]	55.93±0.35[a]
CM/AII-Ace	237.67±1.45[b]	47.53±0.29[b]
CM/AII-MeOH	234.333±0.33[b]	46.87±0.07[b]
CM/M-Aq.	270.000±1.15[a]	54.00±0.23[a]
CM/M-Ace	237.67±1.33[b]	47.53±0.27[b]
CM/M-MeOH	231.00±1.15[c]	46.200±0.23[c]
MB-Aq.	279.33±0.83[a]	55.87±0.18[a]
MB-Ace	235.00±1.73[b]	47.00±0.35[b]
MB-MeOH	230.33±0.88[c]	46.07±0.176[c]

MC-Aq.	278.33±1.76[a]	55.67±0.35[a]
MC-Ace	285.333±0.88[b]	57.07±0.18[b]
MC-MeOH	274.00±1.73[c]	54.80±0.35[c]
MD-Aq.	270.00±0.57[a]	54.00±0.12[a]
MD-Ace	237.67±1.45[b]	47.53±0.29[b]
MD-MeOH	229.00±1.53[c]	45.80±0.31[c]

[a]NC = Negative control (distilled water); PC = Positive control (0.05 g·L−1, Na_2N_3-sodium azide), Aq = aqueous, Ace = acetone, MeOH = methanol fruit extracts of *Cucumis* spp and *Momordica* spp.

[b]Ave.±SE = Average and standard error.

[c]Values within a column followed by the same letters are not significantly different at $P < 0.001$ as determined by Duncan's multiple range tests.

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

Table 4.7. Cytological effects of aqueous, acetone and methanolic fruit extract of different species of *Cucumis* L. and L. on *Allium cepa* root tip cells.

Treatments/ Samples cells	Chromosome Stickiness	Bridges	Laggards	Polar deviation	Vagrants	Total aberrant	% of aberrations
NC	1.33±0.47	0.67±0.47	0	0.33±0.47	1.33±1.25	3.67±1.25	0.73±0.25
PC	29.33±1.70	27.00±0.81	39.33±3.09	30.3333±1.24	32±2.16	158±8.042	31.6±1.61
CM-Aq.	5.33±1.25	4±0.82	2±0.82	2.33±0.47	2±0.82	15.67±4.12	3.132±0.82
CM-Ace	5.33±0.94	4.67±1.25	3.33±0.47	3.67±0.47	4.33±0.47	21.33±2.49	4.27±0.50
CM-MeOH	7.67±0.94	5.67±0.47	4.67±0.47	5.33±1.25	5.333±1.25	28.67±2.35	5.73±0.47
CM/AI-Aq.	3.67±0.47	2±0.82	1.67±0.47	2±0.82	1±0.82	10.33±2.49	2.17±0.50
CM/AI-Ace	7.67±1.25	5.67±0.47	8.33±0.47	5.33±0.47	8±1.41	35.1±0.82	7.5±0.16
CM/AI-MeOH	9.33±0.47	6.33±0.47	10±0.82	8.67±0.47	8.67±0.47	43±2.16	8.6±0.43
CM/AII-Aq.	6.33±1.70	2.67±0.47	2.33±0.47	2.33±0.47	2.33±0.47	16±3.27	3.2±0.65
CM/AII-Ace	6.33±1.70	4.67±0.94	4.67±0.47	5.33±1.25	5.67±0.94	26.67±4.03	5.33±0.81
CM/AII-MeOH	9.33±0.47	12.69±1.25	15.83±1.26	9.73±1.25	9.67±0.45	6.33±1.25	11.27±0.25
CM/M-Aq.	5.00±0.82	1.67±0.47	0.67±0.47	1.33±1.25	1.33±1.25	10±2.83	2±0.57
CM/M-Ace	5.67±0.47	3.67±0.943	2.33±0.47	3±0.82	4±0.82	18.67±1.70	3.73±0.34
CM/M-MeOH	12±0.82	12.67±1.25	15.33±1.25	09.33±1.25	9.79±0.47	59±2.16	11.8±0.43
MB-Aq.	5.67±1.24	1.33±0.47	2±0.82	2±0.82	2±0.82	13±3.56	2.6±0.71
MB-Ace	6.33±0.94	3.33±0.47	4.67±0.47	.67±0.4	4.667±0.47	23.67±2.67	4.73±0.52
MB-MeOH	8±0.82	4.67±0.47	5.67±0.47	5.67±0.47	5.67±0.47	29.67±1.25	5.93±0.25
MC-Aq.	4.67±0.47	1.67±0.94	1.33±0.47	1.67±0.47	1.67±0.47	11±2.45	2.2±0.49
MC-Ace	6±0.82	3±0.82	3.33±0.47	4±0.82	4.33±0.94	20.67±1.70	7.47±5.04
MC-MeOH	8.67±0.47	5.33±0.47	5±0.82	6.67±1.25	6.67±1.25	32.33±3.86	6.47±0.77
MD-Aq.	5.67±1.70	1.67±0.47	1.67±0.94	2.33±0.94	2.33±0.94	13.67±4.12	2.73±0.82
MD-Ace	5.33±0.47	3.67±0.94	2.67±0.47	3±0.82	3±0.82	17.67±1.25	3.53±0.25
MD-MeOH	6.67±1.25	5±0.82	4.33±0.47	5.33±1.25	5.67±0.94	27±2.45	5.4±0.49

[a]NC = Negative control (distilled water); PC = Positive control (0.05 g·L−1, Na_2N_3, sodium azide), Aq = aqueous, Ace = acetone, MeOH = methanol fruit extracts of *Cucumis* spp and *Momordica* spp. [b]Ave.±SE = Average and standard error. [c]Values within a column followed by the same letters are not significantly different at $P < 0.001$ as determined by Duncan's multiple range tests.

4.4.ANTIMICROBIAL SCREENING

4.4.1 Primary/preliminary screening by agar diffusion assay

The genera *Cucumis* L. and *Momordica* L. under investigation were extracted in all the three solvents (water, methanol and acetone) and the extracts were subjected to primary/preliminary antimicrobial assays against 04 standard/ATCC microbial strains well diffusion method. Antimicrobial potential of different species of *Cucumis* L. and *Momordica* L.against *Klebsiella pneumonia, Staphylococcus aureus, Pseudomonas flourescens* and *Bacillus coagulans* at different concentrations has been presented in table 4.8 and activity index in table 4.9. They are expressed as diameters of inhibition zones in mm (mean ± SD; n=3).

Plant species have shown antimicrobial activity against one or more species of microorganisms. The maximum inhibitory zone of 32.3 ± 0.57 mm was reported against *Staphylococcus aureus* in *Cucumis melo* L. and 23.3 ± 0.57 mm was found against *Bacillus coagulans* in *Cucumis melo* var. *agrestis* (morphotype II) extract at 3000 ppm. A direct contact between the concentration and zone of inhibition was recorded upon increasing the concentration of the plant extracts. Most of these plants were recorded to be more effective against Gram positive bacteria as compared to Gram negative. Plants such as *Cucumis melo* var. *agrestis* (morphotype II) and *Momordica dioica* L. were found ineffective against *Klebsiella pneumoniae* even at higher concentrations, while these plants have shown a significant inhibitory affect against other test bacteria. Although all the screened plants species have shown inhibitory effect against one or more bacterial strains but plants such as *Cucumis melo* L., *Momordica charantia* L. and *Cucumis melo* var. *agrestis* (morphotype I), has been as most active plant species having medicinal properties as these exerted inhibitory action against all off the tested strains.

Table 4.8. zone of Inhibition (mm) by antibacterial activity in *Cucumis* L. and *Momordica* L.

Strains	Conc. (ppm)	CM	CMAI	CMAII	CMM	MC	MB	MD
KP	1000	14.6 ± 0.44	11.6 ± 0.24	0.0	11.3 ± 0.23	11 ± 1.0	0.0	0.0
	2000	15.6 ± 0.56	13.3 ± 0.32	0.0	12 ± 0	12.6 ± 0.59	0.0	0.0
	3000	16.6 ± 0.52	14.3 ± 0.51	0.0	14.3 ± 0.22	14.6 ± 0.27	17.00 ± 0	0.0
SA	1000	26.3 ± 0.52	21.7 ± 0.87	14.6 ± 0.29	12.6 ± 0.01	13.00±0.09	13.00±0.06	18.6 ± 0.34
	2000	28.6 ± 0.67	29.3 ± 0.19	18.33±0.55	16.3 ± 0.92	14.00±0.98	13.6 ± 0.06	21.00±0.94
	3000	32.3 ± 0.74	30.6 ± 0.18	21.3 ± 0.92	18.6 ± 0.11	16.3 ± 0.31	15.00 ± 08	24.3 ± 0.51
PF	1000	11.6 ± 0.47	12.00±0.61	12.00 ± 0.01	11.00 ± 0.0	12.6 ± 0.71	10.3 ± 0.52	12.00 ± 0
	2000	13.3 ± 0.79	13.6 ± 0.23	17.3 ± 0.57	14.00 ± 0.0	14.6 ± 0.36	11.3 ± 0.34	15.6 ± 0.74
	3000	14.3 ± 0.28	16.3 ± 0.17	21.3 ± 0.67	19.3 ± 0.19	17.00 ± 0.0	14.3 ± 0.49	19.3 ± 0.87
BC	1000	12.0 ± 0.0	11.00 ± 0.0	13.6 ± 0.42	0.0	12.00 ± 0.0	11.6 ± 0.45	10.00 ± 0
	2000	13.00 ± 0.0	12.6 ± 0.53	16.3 ± 0.59	0.0	14.00 ± 0	13.00 ± 0.0	11.3 ± 0.91
	3000	14.3 ± 0.95	14.3 ± 0.54	23.3 ± 0.47	0.0	15.3 ± 0.71	15.3 ± 0.06	13.00 ± 0

Values are mean ±SD of three measurements9n=3). Variation in the letters between samples indicates significant difference at level $p < 0.05$ by ANOVA Test.

Abb. CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

KP- *Klebsiella pneumonia*, SA- *Staphylococcus aureus,* PF- *Pseudomonas flourescens* , BC- *Bacillus coagulans*

Table 4.9. Activity index by antibacterial activity in *Cucumis* L. and *Momordica* L.

Strains	Conc. (ppm)	CM (mean±SD)	CMAI (mean±SD)	CMAII (mean±SD)	CMM (mean±SD)	MC (mean±SD)	MB (mean±SD)	MD (mean±SD)
KP	1000	0.405±0.02	0.322±0.1	0.0	0.313±0.3	0.305±0.03	0.0	0.0
	2000	0.433±0.01	0.369±0.06	0.0	0.333±0.01	0.350±0.0	0.0	0.0
	3000	0.461±0.0	0.397±0.0	0.0	0.397±0.9	0.405±0.7	0.472±0.06	0.0
SA	1000	0.821±0.52	0.675±0.04	0.456±0.09	0.393±0.0	0.406±0.4	0.406±0.5	0.581±0.0
	2000	0.893±0.0	0.813±0.6	0.572±0.0	0.509±0.4	0.437±0.0	0.425±0.4	0.656±0.3
	3000	1.009±0.5	0.850±0.31	0.665±0.73	0.581±0.6	0.509±0.1	0.468±0.2	0.759±0.0
PF	1000	0.362±0.9	0.375±0.0	0.375±0.4	0.343±0.0	0.393±0.7	0.321±0.1	0.375±0.2
	2000	0.415±0.0	0.425±0.6	0.540±0.2	0.437±0.2	0.456±0.0	0.353±0.3	0.487±0.0
	3000	0.446±0.02	0.509±0.0	0.665±0.4	0.603±0.06	0.531±0.0	0.446±0.0	0.603±0.02
BC	1000	0.4±0.05	0.366±0.0	0.453±0.4	0.0	0.40±0.07	0.386±0.9	0.333±0.7
	2000	0.433±0.01	0.420±0.5	0.543±0.07	0.0	0.466±0.0	0.433±0.24	0.376±0.09
	3000	0.476±0.0	0.476±0.87	0.776±0.0	0.0	0.510±0.42	0.510±0.06	33±0. 0.433±0.91

Values are mean ±SD of three measurements. Variation in the letters between samples indicates significant difference at level $p < 0.05$ by Anova Test.

Abb. CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

KP- *Klebsiella pneumonia*, SA- *Staphylococcus aureus*, PF- *Pseudomonas flourescens*, BC- *Bacillus coagulans*

4.5.Antioxidant activity

The present research was undertaken to measure the antioxidant potential of selected genera and the influence of methanol and aqueous solvents on polyphenolic and flavanoid compound extraction and hence also their antioxidant action.

4.5.1 Yield of extraction

Two extracting solvents i.e. methanol and aqueous was evaluated for their effectiveness in extracting antioxidants from dried plant samples. The variation in yield (5.21 to 24.34 g/100g) as well as in colour characteristics was observed in all the species. The yield of extract in methanol and aqueous was the maximum in case of *Momordica Charantia* L. and minimum yield for both extracts was found in case of *Cucumis melo* var. *momordica* L. The various attributes of selected fruit extracts are presented in Table 4.10. Extract colour is mainly brown in colour in all the species for both solvents except methanol extract of *Cucumis melo* var. *momordica* L. and *Momordica charantia* L., where it is found to be of deep green colour.

Table. 4.10. Characteristics of *Cucumis* L. and *Momordica* L. fruit extracts

Plant	**Extract**	**Physical aspect**	**Colour**	**Yield**
Cucumis melo L.	Methanol	Paste	Brown	10.87
	Aqueous	Paste	Brown	8.26
Cucumis melo var. agrestis L (I)	Methanol	Paste	Brown	17.6
	Aqueous	Paste	Brown	13.02
Cucumis melo var. agrestis L (II)	Methanol	Paste	Brown	14.28
	Aqueous	Paste	Brown	12.37
Cucumis melo var. momordica L.	Methanol	Paste	Deep green	7.93
	Aqueous	Paste	Brown	5.21
Momordica charantia L.	Methanol	Paste	Deep green	24.34
	Aqueous	Paste	Brown	15.49
Momordica balsamina L.	Methanol	Paste	Brown	14.46
	Aqueous	Paste	Brown	9.07
Momordica dioica L.	Methanol	Paste	Brown	12.29
	Aqueous	Paste	Brown	9.14

4.5.2. Phytochemical screening

The photochemical study of the selected plants revealed the existence of appreciable amounts of alkaloids, flavanoids, saponins, tannins and terpenoids (Table. 4.11). The results showed that among all the plants *Momordica charantia, Cucumis melo agretis (*Morphotype I and II) was the richest in phytochemicals including polyphenolic components, on the other hand , tannin, saponin and alkaloids were absent in *Cucumis melo* L., *Cucumis melo* var. *momordica* L., *Momordica dioica* L. and *M. balsamina* L.

A wide range of variation was observed in flavanoid and phenolic content among the selected plants species (Table. 4.12). The total phenolic content vary significantly in almost all the vegetables with respect to extracts used ($p<0.05$) and it varied in order of 81.08±05 to 23.59±0.06. Recovery of total phenolic content was found to be highest in methanolic extract and in different plant species and ranges from 81.08±05 to 36.73±0.09 and it was lowest in aqueous extract varies from 59.21±0.05 to 23.59±0.06. The flavanoid content in methanol and aqueous extract varied from 73.03 ± 0.21 to 35.43 ± 0.25 and 50.23 ± 0.15 to 27.5 ± 0.26 respectively (Table. 4.12) and it differ among all the plant species in their respective extracts.

Table. 4.11. Phytochemical screening of *Cucumis* L. and *Momordica* L.

Plant species	Flavanoids		Tannin		Saponin		Terpenoids		Alkaloids	
	ME	AE	ME	AE	ME	AE	ME	AE	ME	AE
CM	+ve	+ve	---	+ve	----	+ve	+ve	+ve	----	+ve
CM(I)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
CM(II)	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
CMM	+ve	+ve	---	+ve	---	+ve	+ve	+ve	----	+ve
MC	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
MB	+ve	+ve	----	+ve	----	+ve	+ve	+ve	+ve	+ve
MD	+ve	+ve	---	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Abb. ME- methanol extract, AE- aqueous extract

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

Table. 4.12. Total phenol and flavanoid content in different extracts

Plant species	Total phenolic content		Total flavanoid content	
	Methanol	Aqueous	Methanol	Aqueous
CM	81.08 ± 0.05^{a}	59.21 ± 0.05^{a}	73.03 ± 0.21^{a}	50.23 ± 0.15^{b}
CMAI	43.07 ± 0.05^{e}	31.72 ± 0.04^{d}	38.27 ± 0.15^{e}	34.36 ± 0.21^{e}
CMAII	55.49 ± 0.07^{c}	36.29 ± 0.06^{c}	49.43 ± 0.15^{c}	45.23 ± 0.35^{c}
CMM	69.18 ± 0.06^{b}	42.19 ± 0.02^{b}	55.97 ± 0.40^{b}	52.4 ± 0.10^{a}
MC	39.25 ± 0.12^{f}	25.12 ± 0.06^{f}	36.8 ± 0.2^{f}	27.5 ± 0.26^{g}
MB	44.53 ± 0.02^{d}	28.84 ± 0.11^{e}	42.6 ± 0.2^{d}	39.63 ± 0.15^{d}
MD	36.73 ± 0.09^{g}	23.59 ± 0.06^{g}	35.43 ± 0.25^{g}	32.56 ± 0.15^{f}
Mean	52.761	35.28	47.361	40.272

Results are means ± standard deviation (n=3) followed by different superscripts in columns indicate that they are different significantly at 5% level of significance ($p< 0.05$) as determined by Duncan's multiple range tests.

4.5.3. Antioxidant activity of plant extracts

The results obtained from antioxidant activity of different plant extracts determined using different assays is as described in Table 4.13

4.5.4 DPPH radical scavenging activity

The free radical scavenging activity of antioxidant compounds was measured using DPPH assay. Free radical activity of DPPH radical was expressed by percent scavenging as well as IC_{50}. The percent scavenging and IC_{50} values of different plants was in order of *Cucumis melo* L. > *Cucumis melo* var. *agrestis* (morphotype II)> *Momordica dioica* L.> *Cucumis melo* var. *agrestis* (morphotype I)> *Momordica charantia* L.> *Cucumis melo* var. *momordica* L.> *Momordica balsamina* L. in methanolic extracts, which was highest, whereas in aqueous extract it was comparatively less viz. *Cucumis melo* L.> *Momordica dioica* L.> *Cucumis melo* var. *agrestis* (morphotype II)> *Cucumis melo* var. *agrestis* (morphotype I)> *Momordica charantia* L.> *Momordica balsamina* L.> *Cucumis melo* var. *momordica* L. (Table. 4.13)

4.5.5. Hydrogen Peroxide Scavenging Assay

Order of scavenging in methanol extract in terms of IC_{50} was *Cucumis melo* L.>*Cucumis melo* var. *momordica* L.> *Cucumis melo* var. *agrestis* (morphotype I)>

Cucumis melo var. *agrestis* (morphotype II)>*Momordica balsamina* L.> *Momordica charantia* L.> *Momordica dioica* L. In aqueous extract the order of scavenging was *Cucumis melo* L> *Cucumis melo var. agrestis* (morphotype I)> *Cucumis melo* var. *momordica* L.> *Cucumis melo* var. *agrestis* (morphotype II)> *Momordica balsamina* L.> *Momordica dioica* L.> *Momordica charantia* L. Phenols and tannins have the ability to scavenge H_2O_2 which could donate electrons, so neutralizing it into water molecules. (Table. 4.13).

4.5.6. Reducing power assay

IC_{50} in methanol extracts was found to be in order *Cucumis melo* var. *momordica* L.> *Cucumis melo* L.> *Cucumis melo* var. *agrestis* (morphotype II)> *Cucumis melo* var. *agrestis* (morphotype I)> *Momordica dioica* L.> *Momordica balsamina* L.> *Momordica charantia* L. on the other hand this order is slight different for aqueous extracts viz. *Cucumis melo* L> *Cucumis melo* var. *agrestis* (morphotype II)> *Cucumis melo* var. *momordica* L.> *Cucumis melo* var. *agrestis* (morphotype I)> *Momordica dioica* L.> *Momordica charantia* L.>*Momordica balsamina* L. (Table. 4.13).

Table 4.13. Antioxidant activity of methanol and aqueous extracts of *Cucumis* L. and *Momordica* L. by various assays

	DPPH assay (methanol extract)						
Conc (µg/ml)	**CM**	**CMAI**	**CMAII**	**CMM**	**MC**	**MB**	**MD**
100	43.55 ± 0.25^{b}	34.35 ± 0.08^{e}	36.6 ± 0.32^{c}	45.45 ± 0.10^{a}	26.39 ± 0.10^{f}	34.76 ± 0.15^{d}	14.51 ± 0.27^{g}
200	52.93 ± 0.07^{a}	45.35 ± 0.17^{d}	49.61 ± 0.21^{c}	51.51 ± 0.17^{b}	35.09 ± 0.10^{f}	41.68 ± 0.15^{e}	30.52 ± 0.26^{g}
300	62.69 ± 0.27^{a}	54.4 ± 0.23^{d}	59.23 ± 0.12^{c}	59.82 ± 0.16^{b}	46.55 ± 0.12^{f}	52.03 ± 0.06^{e}	43.53 ± 0.09^{g}
500	77.13 ± 0.22^{c}	69.64 ± 0.12^{d}	79.38 ± 0.14^{a}	78.46 ± 0.23^{b}	62.06 ± 0.07^{g}	65.94 ± 0.05^{e}	64.55 ± 0.15^{f}
IC_{50}	43.387±0.08	67.586±0.03	59.411±0.08	74.406±0.06	71.967±0.09	78.375±0.04	64.423±0.12
	DPPH assay (Aqueous extract)						
Conc (µg/ml)	**CM**	**CMAI**	**CMAII**	**CMM**	**MC**	**MB**	**MD**
100	41.03 ± 0.05^{a}	33.62 ± 0.14^{c}	29.44 ± 0.05^{d}	36.25 ± 0.13^{b}	12.51 ± 0.19^{f}	25.05 ± 0.05^{e}	11.47 ± 0.21^{g}
200	48.93 ± 0.08^{a}	40.94 ± 0.06^{c}	38.31 ± 0.09^{d}	44.56 ± 0.33^{b}	23.69 ± 0.17^{f}	33.82 ± 2.09^{e}	22.69 ± 0.16^{f}
300	57.39 ± 0.16^{a}	52.45 ± 0.15^{b}	52.38 ± 0.26^{b}	51.36 ± 0.23^{c}	33.38 ± 0.31^{f}	41.62 ± 0.15^{d}	36.04 ± 0.06^{e}
500	73.94 ± 0.09^{b}	66.05 ± 0.06^{c}	76.44 ± 0.19^{a}	63.04 ± 0.08^{d}	50.64 ± 0.20^{g}	56.63 ± 0.13^{e}	54.75 ± 0.25^{f}
IC_{50}	67.413±0.08	85.569±0.04	82.714±0.20	125.507±0.03	101.718±0.14	110.0±0.06	73.8±0.02

	Reducing power assay (methanol extract)						
Conc (µg/ml)	**CM**	**CMAI**	**CMAII**	**CMM**	**MC**	**MB**	**MD**
100	41.8 ± 0.12^{b}	32.06 ± 0.07^{d}	35.48 ± 0.35^{c}	44.01 ± 0.08^{a}	25.2 ± 0.18^{f}	31.05 ± 0.07^{e}	21.59 ± 0.11^{g}
200	49.93 ± 0.06^{b}	44.08 ± 0.08^{d}	46.91 ± 0.08^{c}	52.42 ± 0.14^{a}	34.7 ± 0.16^{f}	38.26 ± 0.18^{e}	33.27 ± 0.18^{g}
300	64.71 ± 0.19^{a}	55.58 ± 0.18^{d}	57.06 ± 0.05^{c}	59.62 ± 0.18^{b}	42.2 ± 0.13^{e}	46.06 ± 0.09^{f}	46.24 ± 0.21^{f}
500	74.24 ± 0.14^{b}	72.63 ± 0.21^{d}	74.63 ± 0.18^{a}	73.24 ± 0.24^{c}	55.29 ± 0.16^{g}	59.94 ± 0.05^{f}	66.81 ± 0.11^{e}
IC_{50}	182.718±.195	265.249±0.108	238.655±0.145	175.579±0.266	418.200±0.045	362.647±0.198	345.560±0.362

	Reducing power assay (aqueous extract)						
Conc (µg/ml)	**CM**	**CMAI**	**CMAII**	**CMM**	**MC**	**MB**	**MD**
100	36.51 ± 1.82^{b}	27.6 ± 0.28^{d}	34.04 ± 0.05^{c}	38.09 ± 0.15^{a}	9.34 ± 0.33^{g}	24.09 ± 0.16^{e}	15.43 ± 0.20^{f}
200	49.38 ± 0.27^{a}	41.05 ± 0.05^{d}	45.95 ± 0.05^{b}	45.06 ± 0.06^{c}	18.68 ± 0.14^{g}	30.44 ± 0.23^{e}	23.3 ± 0.28^{f}
300	62.84 ± 0.17^{a}	53.36 ± 0.31^{c}	57.39 ± 0.23^{b}	52.08 ± 0.13^{d}	27.41 ± 0.29^{g}	35.44 ± 1.73^{f}	40.05 ± 0.08^{e}
500	81.06 ± 0.05^{a}	74.32 ± 0.28^{b}	73.9 ± 0.16^{c}	64.1 ± 0.16^{d}	51.05 ± 0.07^{g}	53.51 ± 0.19^{f}	57.03 ± 0.06^{e}
IC_{50}	202.441±0.331	281.345±0.271	246.631±0.631	275.637±0.074	452.764±0.191	458.461±0.226	430.201±0.524

	Hydrogen peroxide assay (methanol extract)						
Conc (µg/ml)	**CM**	**CMAI**	**CMAII**	**CMM**	**MC**	**MB**	**MD**
100	43.55 ± 0.25^{b}	34.35 ± 0.08^{e}	36.6 ± 0.32^{c}	45.45 ± 0.10^{a}	26.39 ± 0.10^{f}	34.76 ± 0.15^{d}	14.51 ± 0.27^{f}
200	52.93 ± 0.07^{a}	45.35 ± 0.17^{d}	49.61 ± 0.21^{c}	51.51 ± 0.17^{b}	35.09 ± 0.10^{f}	41.68 ± 0.15^{e}	30.52 ± 0.26^{g}
300	62.69 ± 0.27^{a}	54.4 ± 0.23^{d}	59.23 ± 0.12^{c}	59.82 ± 0.16^{b}	46.55 ± 0.12^{f}	52.03 ± 0.06^{e}	43.53 ± 0.09^{g}
500	77.13 ± 0.22^{c}	69.64 ± 0.12^{d}	79.38 ± 0.14^{a}	78.46 ± 0.23^{b}	62.06 ± 0.07^{g}	65.94 ± 0.05^{e}	64.55 ± 0.15^{f}
IC_{50}	168.313±.0443	216.757±0.079	266.172±.096	172.289±0.023	359.56±0.41	294.618±.155	373.225±.378
	Hydrogen peroxide (aqueous extract)						
Conc (µg/ml)	**CM**	**CMAI**	**CMAII**	**CMM**	**MC**	**MB**	**MD**
100	41.03 ± 0.05^{a}	33.62 ± 0.14^{c}	29.44 ± 0.05^{d}	36.25 ± 0.13^{b}	12.51 ± 0.19^{f}	25.05 ± 0.05^{e}	11.47 ± 0.21^{g}
200	48.93 ± 0.08^{a}	40.94 ± 0.06^{c}	38.31 ± 0.09^{d}	44.56 ± 0.33^{b}	23.69 ± 0.17^{f}	33.82 ± 2.09^{e}	22.69 ± 0.16^{f}
300	57.39 ± 0.16^{a}	52.45 ± 0.15^{b}	52.38 ± 0.26^{b}	51.36 ± 0.23^{c}	33.38 ± 0.31^{f}	41.62 ± 0.15^{d}	36.04 ± 0.06^{e}
500	73.94 ± 0.09^{b}	66.05 ± 0.06^{c}	76.44 ± 0.19^{a}	63.04 ± 0.08^{d}	50.64 ± 0.20^{g}	$56.6\ 0.13^{e}$	54.75 ± 0.25^{f}
IC_{50}	212.162±062	281.654±184	296.512±280	296.181±137	490.827±.080	409.583±0.160	466.466±0.253

Results are means ± standard deviation (n=3) followed by different superscripts in columns indicate that they are different significantly at 5% level of significance ($P < 0.05$) as determined by Duncan's multiple range tests.

Abb. CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

4.6 Proximate analysis and Mineral content.

The edible part and proximate composition of all the plant species are shown in Table 4.14. The major component in all the species the water (59–74 g/100 g fresh weight, FW) and the results revealed that moisture content was maximum in *Cucumis melo* var. *agrestis* L. (Morphotype II) (74.45 ± 0.35). Ash content of the sample reveals the indirect measurement of minerals in food item (Hofman *et al.*, 2002), and it was found to be significantly high in *Momordica charantia* L. (16.07 ± 0.126) among all the species and was near about (17.93). Crude fiber was maximum in case of *Cucumis melo* L. (6.67 ± 0.003), while crude fat content was considerably low, although it was maximum in case of *Momordica charantia* L. (5.8 ± 0.121). Protein content was found to be maximum in case of *Momordica dioica* L. Moreover carbohydrates were maximum in *Cucumis melo* var. *agretsis* (Morphotype I). From all these values energy efficiency was calculated, which was found to be maximum in case of *Momordica dioica* L. The mineral content of all the species is given in table results shows a great deal of variation. Calcium content was in range of (32.18 ± 0.001 to 217.76 ± 0.001) and maximum in *Momordica dioica* L. 217.76 ± 0.001.

Other important micronutrients like iron, manganese and copper were also observed from the fruit samples of all the species. These microelements ranges with average value Fe and Mn of 32.04 ± 0.001 and 6.47 ± 0.01 respectively in *Momordica balsamina* and copper was maximum in *Cucumis melo* var. *momordica* respectively i.e. 8.86 ± 0.001 (table 4.14). Manganese contents were found in a range of 8.858 mg/100 g to .842 and 6.48–.108 mg/ 100 g, respectively. Zinc amount was found to be maximum in case of *Cucumis melo* var. *agrestis* (morphotype I) (14.12 ± 0.001) and minimum in *Momordica charantia* L. (3.07 ± 0.001). Our result showed significant concentration of Mg^{+2} (57.89 ± 0.001-148.7 ± 0.001) in all the species. Out of all anti nutritional components alkaloids was maximum in case of *Cucumis melo* var. *agrestis* (Morphotype II), being 37.276 %. Oxalate was found to be maximum in case of *Cucumis melo* var. *momordica* L. i.e. 3.08% and Saponin being maximum in case of *Momordica charantia* L. i.e. 5.32 %

Table 4.14. Proximate analysis and mineral content in *Cucumis* L. and *Momordica* L.

	CM	CMAI	CMAII	CMM	MC	MB	MD
Calcium	59.08 ± 0.01	32.18 ± 0.001^{d}	47.63 ± 0.001^{b}	73.04 ± 0.001^{b}	167.97 ± 0.001^{d}	203.63 ± 0.001	217.76 ± 0.001^{f}
Copper	0.84 ± 0.001^{b}	2.43 ± 0.001^{e}	1.67 ± 0.001^{e}	8.86 ± 0.001^{f}	0.96 ± 0.001^{f}	3.7 ± 0.001^{e}	2.02 ± 0.001^{e}
Iron	4.19 ± 0.001	13.91 ± 0.001^{b}	11.08 ± 0.001^{e}	18.7 ± 0.001^{e}	18.43 ± 0.001^{e}	32.04 ± 0.001^{f}	14 ± 0.001
Magnesium	98.75 ± 0.01^{b}	57.89 ± 0.001^{e}	74.09 ± 0.001^{e}	92.43 ± 0.001^{f}	104.77 ± 0.001^{e}	148.7 ± 0.001^{f}	113.6 ± 0.1
Manganese	0.11 ± 0.001^{b}	0.65 ± 0.001^{e}	0.05 ± 0.001^{e}	2.06 ± 0.001^{e}	1 ± 0.001^{e}	6.47 ± 0.01^{e}	0 ± 0
Zinc	3.92 ± 0.001^{b}	14.12 ± 0.001^{b}	6.08 ± 0.001^{e}	11.09 ± 0.001^{b}	3.07 ± 0.001^{f}	3.71 ± 0.001^{g}	4.49 ± 0.001^{g}
Sodium/ Potassium	0.08 ± 0	0.29 ± 0	0.29 ± 0	0.15 ± 0	0.04 ± 0	0.03 ± 0	0.08 ± 0
Sodium	37.17 ± 0.002^{b}	62.07 ± 0.001^{e}	47.01 ± 0.001	26.87 ± 0.001^{e}	11.03 ± 0.001	19.78 ± 0.01^{e}	27.63 ± 0.001^{b}
Potassium	462.02 ± 0.001^{b}	212.09 ± 0.001^{e}	163.43 ± 0.001^{e}	182.61 ± 0.001	258.19 ± 0.001^{f}	624.07 ± 0.001^{f}	328.79 ± 0.001^{f}
Moisture content	66.6 ± 0.409^{b}	64.37 ± 0.325^{b}	74.45 ± 0.35	71.87 ± 0.176^{d}	56.57 ± 0.284^{f}	66.02 ± 0.325^{b}	58.85 ± 0.22^{b}
Ash value	6.43 ± 0.153^{b}	4.6 ± 0.132^{b}	6.85 ± 0.087^{b}	8.67 ± 0.029^{e}	16.07 ± 0.126^{b}	12.28 ± 0.126^{f}	8.16 ± 0.138^{g}
Crude fat	0.92 ± 0.127^{b}	0.34 ± 0.044^{c}	0.71 ± 0.096^{b}	1.06 ± 0.051^{c}	5.8 ± 0.121^{b}	4.44 ± 0.13^{b}	3.49 ± 0.407^{f}
Crude Fiber	6.67 ± 0.003^{b}	2.54 ± 0.113	3.21 ± 0.492	1.83 ± 0.075^{e}	3.87 ± 0.103^{f}	1.86 ± 0.079^{g}	4.44 ± 0.201^{e}

	CM	CMAI	CMAII	CMM	MC	MB	MD
Protein	6.61 ± 0.237^{b}	2.19 ± 0.206^{c}	6.95 ± 0.025^{b}	11.68 ± 0.04^{b}	16.49 ± 0.393	10.22 ± 0.189	21.65 ± 0.84^{f}
Carbohydrate	19.77 ± 0.926^{b}	28.51 ± 0.602^{b}	11.04 ± 0.516^{e}	6.73 ± 0.148^{f}	5.06 ± 0.858	7.05 ± 0.438	7.85 ± 0.118
Energy	112.45 ± 1.901^{b}	125.81 ± 1.504	78.35 ± 1.44^{e}	83.15 ± 0.522	138.55 ± 1.179	108.98 ± 1.45^{f}	149.37 ± 0.807
Alkaloid	27.28 ± 0.013^{b}	19.69 ± 0.002	34.28 ± 0.002^{c}	9.44 ± 0.001	17.29 ± 0.001^{c}	26.4 ± 0.001^{f}	12.34 ± 0.001
Oxalate	1.6 ± 0.01^{b}	1.24 ± 0.02^{d}	1.09 ± 0.01^{b}	3.08 ± 0.01^{d}	1.26 ± 0.01^{d}	2.7 ± 0.1^{f}	1.09 ± 0.006^{d}
Saponin	1.32 ± 0.001^{b}	2.05 ± 0.01^{c}	2.35 ± 0.026^{f}	1.13 ± 0.001^{f}	8.37 ± 0.153^{f}	5.32 ± 0.01	25 0.012^{f}

Results are means ± standard deviation (n=3) followed by different superscripts in columns indicate that they are different significantly at 5% level of significance ($P < 0.05$) as determined by Duncan's multiple range tests.

Abb. CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

4.7 Phyto chemical analysis by HPLC

The current scenario exhibits the necessity of herbal drugs throughout the world. Secondary metabolites present in the plant are not required for their survival but plays a major role in plant's antimicrobial activity (Kennedy and Wightman, 2011). These compounds can be considered as backbone of many modern pharmaceuticals because these specific metabolites produce a definite physiological action on the human body. Some of the important bioactive compounds present in plants are alkaloids, flavonoids, tannins, glycosides, steroids, saponins, resins and phenolics (Duraipandiyan *et al.,* 2006). The knowledge of the phytoconstituents by phytochemical screening of plants is valuable for authentication of folkloric remedies and is the first step towards studying the medicinal property of the plant. Development of chemical fingerprints using HPLC is an effective tool for linking the chemical constituent's profile of the plant with botanical identity for estimation of chemical and bio chemical markers. It is efficient and economic for the analysis of broad number of compounds (Mariswamy *et al.,* 2011). A comprehensive evaluation of the isolated active component of the medicinal plants can be achieved by using universal analytical techniques such as HPLC. With this background an attempt has been made to establish a profile of various secondary metabolites, their isolation and characterization, of the methanol fruit extract of various species of *Cucumis* L. and *Momordica* L. The data related to HPLC has been received in form of chromatograms and is shown in Fig 4.1-4.7.

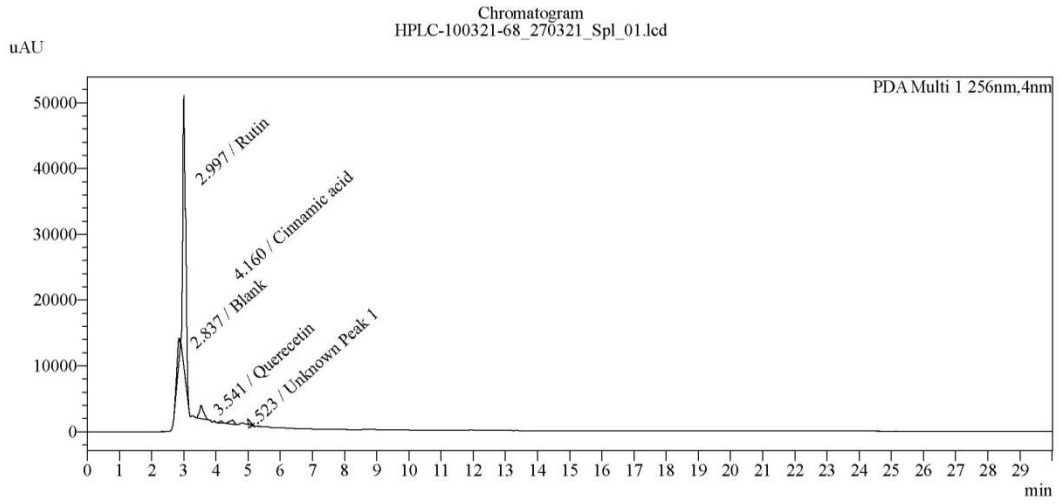


Fig . 4.1. - HPLC chromatogram of *Cucumis melo* L.

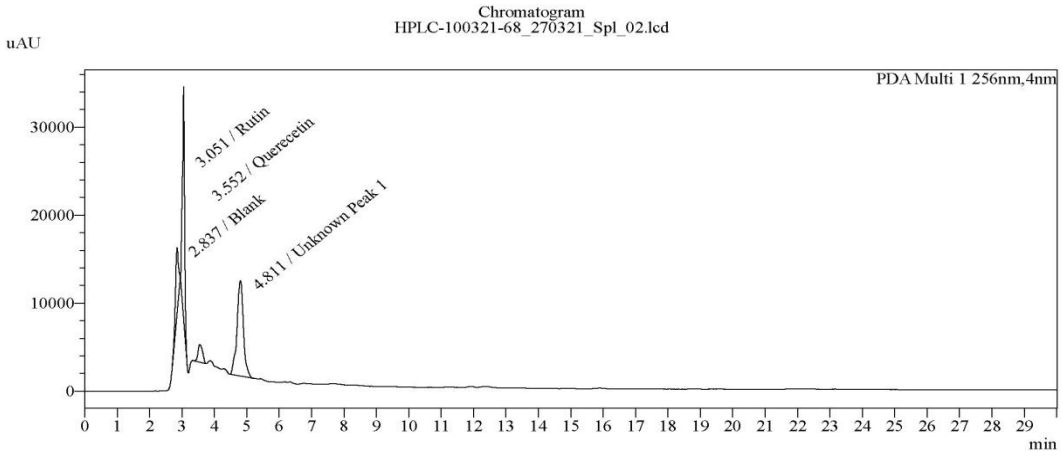


Fig. 4.2 HPLC Chromatogram of Methanol extract of *Cucumis melo* var. *agrestis* L. (Morphotype I)

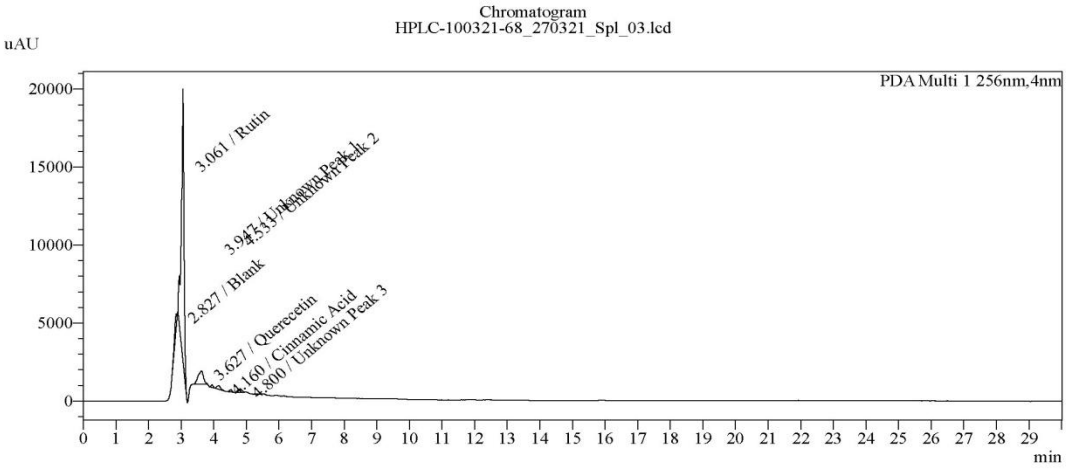


Fig. 4.3. HPLC Chromatogram of Methanol extract of *Cucumis melo* var. *agrestis* L. (Morphotype II)

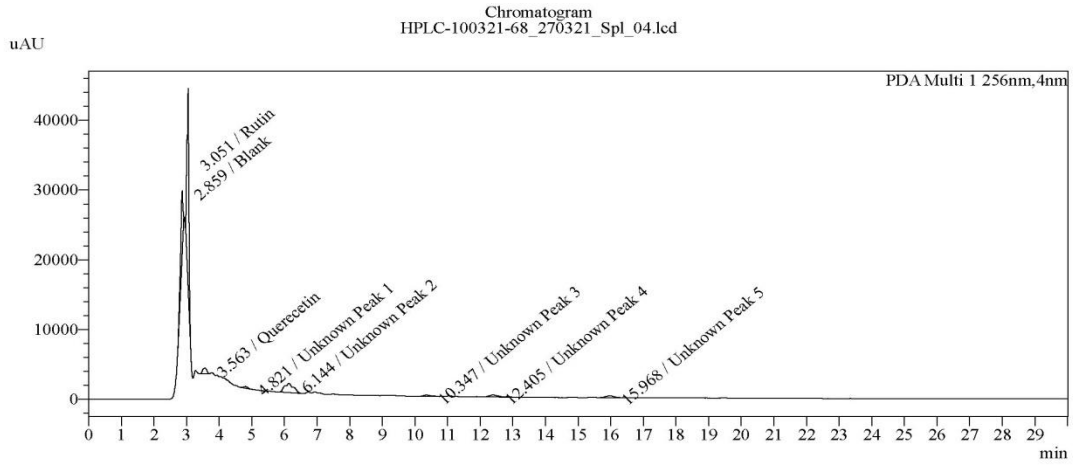


Fig. 4.4. HPLC Chromatogram of Methanol extract of *Cucumis melo* var. *momordica* L.

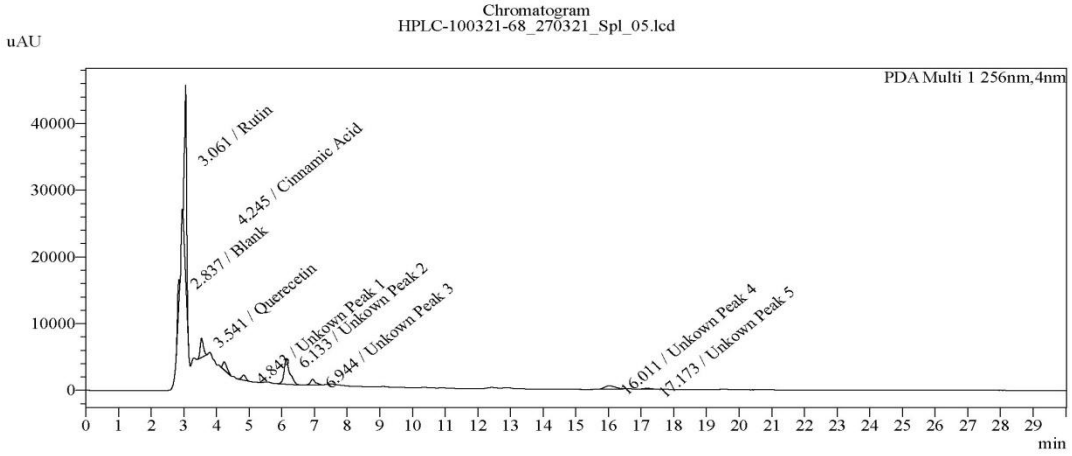


Fig. 4.5. HPLC Chromatogram of Methanol extract of *Momordica charantia* L.

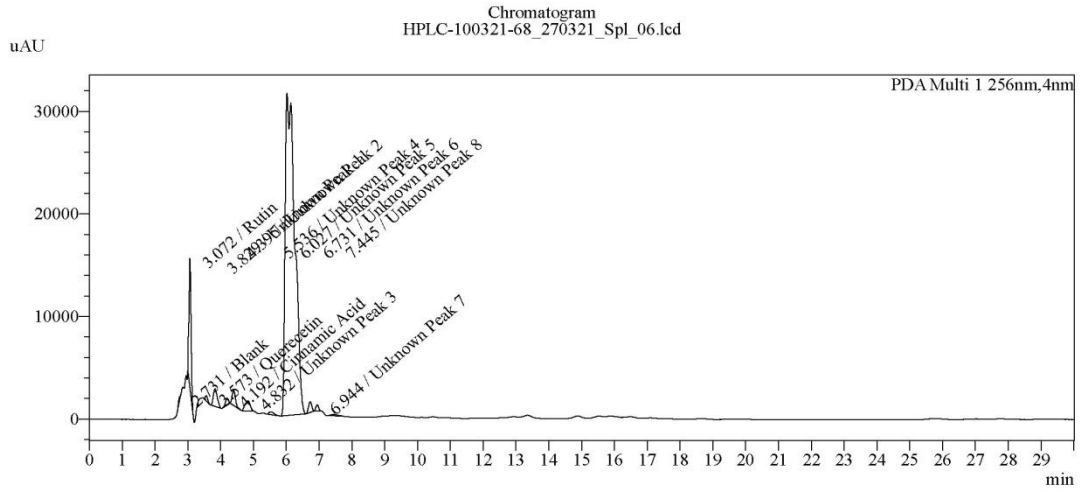


Fig. 4.6. HPLC Chromatogram of Methanol extract of *Momordica balsamina* L.

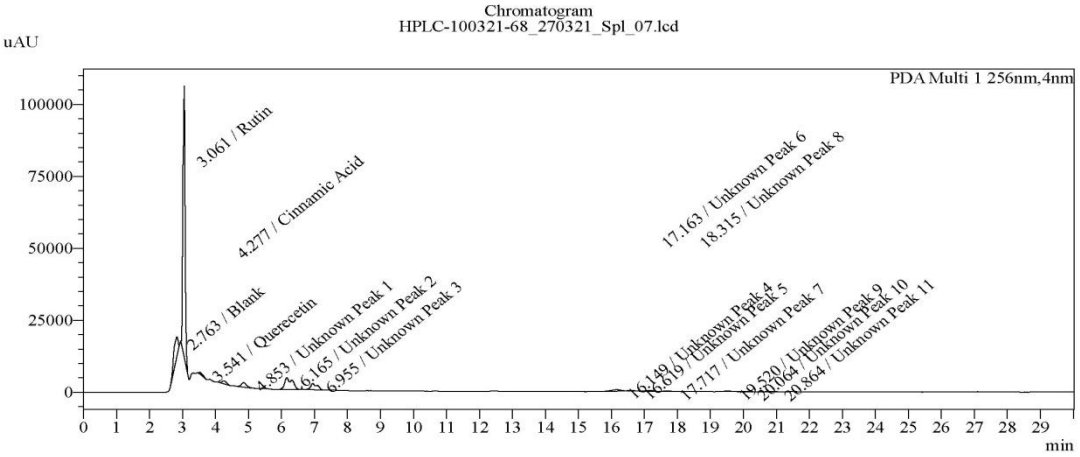


Fig. 4.7. HPLC chromatogram of *Momordica dioica* L.

4.7.1. Quantitative estimation of β-sitosterol by HPLC analysis

HPTLC of the β-sitosterol along with methanol extract of all extracts of *Cucumis* L and *Momordica* L. has been developed using acetonitrile: methanol (50:50) as mobile phase solvent system (Table 3.4) and detected in 256 nm with R_f value 3.061 as shown in 3D-chromatogram (Figure 4.1-4.7). Using different standard concentrations (0.5, 1, 2, 4, 6, 8, 10 µL) and values for slope (b) and intercept (m) were found to be 1756.42 and 846.23, respectively. In the results (Table 4.15), peak areas were obtained 37314.6, 23337.3, 25419.4, 21737.3,19526.6, 32166.5, 26599.6 for *Cucumis melo* L., *Cucumis melo* var. *agrestis* (Morphotype I) , *Cucumis melo* var. *agrestis* L. (Morphotype II) *Cucumis melo* var. *momordica* L., *Momordica charantia* L., *Momordica balsamina* L., *Momordica dioica* L. , respectively. Amount of compound in DWE were observed maximum in *Cucumis melo* L. (16.011±0.841 µg/mg) followed by *Momordica balsamina* L. (12.629±0.240 µg/mg), while it was found to be least in *Cucumis melo* var. *agrestis* (Morphotype I) (6.5±0.002 µg/mg). The limit of detection (LOD) and limit of quantification (LOQ) value is 0.866 and 2.625, respectively for the compound (Table 4.15).

Table 4.15. The amount of β-sitosterol (µg/mg DW) in genera *Cucumis* L. and *Momordica* L.

Standard	Peak area ±SE	Slope (m) Intercept (b)	R^2	Amount of compounds (µg/mg±SE)	LOD	LOQ
B- sitosterol		m = 1756.742, b= 846.23	0.998		0.866	2.625
CM	37314.6			16.011±0.841		
CMAI	23337.3			6.500±0.002		
CMAII	25419.4			9.898±0.003		
CMM	21737.3			8.414±0.003		
MC	19526.6			6.057±0.0.003		
MB	32166.5			12.629±0.240		
MD	26599.6			9.407±0.003		

Values are mean ±SD of three measurements. Variation in the letters between samples indicates significant difference at level $p < 0.05$.

CM- *Cucumis melo* L. , CMA(I)- *Cucumis melo* var. *agrestis* (morphotype I), CMA(II)- *Cucumis melo* var. *agrestis* (morphotype II) CMM- *Cucumis melo* var. *momordica* L.,

MC- *Momordica charantia* L., MB-*Momordica balsamina* L., MD-*Momordica dioica* L.

4.7.2. Quantitative estimation of rutin by HPLC analysis

HPLC of the Rutin along with methanol extract of all extracts of Cucumis L and Momordica L. has been developed using acetonitrile: methanol (50:50) as mobile phase solvent system (Table 3.4) and detected in 256 nm with R_f value 3.061 as shown in 3D-chromatogram. Using different standard concentrations (0.5, 1, 2, 4, 6, 8, 10 µL) and values for slope (b) and intercept (m) were found to be 4946.968 and 548.44, respectively. In the results (Table 4.16), peak areas were obtained 251007, 123637.633, 96540.733, 49202.233, 127710.533, 56947.466, 431731.1 for *Cucumis melo* L., *Cucumis melo* var. *agrestis* (Morphotype I), *Cucumis melo* var. *agrestis* L. (Morphotype II) *Cucumis melo* var. *momordica* L., *Momordica charantia* L., *Momordica balsamina* L., *Momordica dioica* L. , respectively. Amount of compound in DWE were observed maximum in *Momordica dioica* L. (34.19±0.004 µg/mg) followed by *Cucumis melo* L. (12.31±0.30 µg/mg), while it was found to be least in *Cucumis melo* var. *momordica* (Morphotype I) (1.73±0.017 µg/mg). The limit of detection (LOD) and limit of quantification (LOQ) value is 0.284 and 0.861, respectively for the compound (Table 4.16).

Table 4.16. The amount of Rutin (µg/mg DW) in genera *Cucumis* L. and *Momordica* L.

Standard	Peak area ±SE	Slope (m) Intercept (b)	R^2	Amount of compounds (µg/mg±SE)	LOD	LOQ
Rutin		m = 1756.742, b= 846.23	0.999		0.284	0.861
CM	251007			12.314±0.030		
CMAI	123637.633			9.494±0.028		
CMAII	96540.733			4.991±0.016		
CMM	49202.233			1.734±0.017		
MC	127710.533			10.78±0.058		
MB	56947.466			2.519±0.003		
MD	431731.1			34.19±0.004		

Values are mean ±SD of three measurements. Variation in the letters between samples indicates significant difference at level $p < 0.05$.

CM- *Cucumis melo* L. , CMA(I)- *Cucumis melo* var. *agrestis* (morphotype I), CMA(II)- *Cucumis melo* var. *agrestis* (morphotype II) CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB-*Momordica balsamina* L., MD-*Momordica dioica* L.

4.7.3. Quantitative estimation of Quercetin by HPLC analysis

HPLC of the Rutin along with methanol extract of all extracts of *Cucumis* L and *Momordica* L. has been developed using acetonitrile: methanol (50:50) as mobile phase solvent system (Table 3.4) and detected in 256 nm with R_f value 3.061 as shown in 3D-chromatogram (Figure 4.1-4.7). Using different standard concentrations (0.5, 1, 2, 4, 6, 8, 10 µL) and values for slope (b) and intercept (m) were found to be 4590.86 and 414.86, respectively (Figure Ic). In the results (Table 4.30), peak areas were obtained 18553.866, 19945.3, 9878.066, 7223.1, 8767.233, 2844.966, 4949.866 for *Cucumis melo* L., *Cucumis melo* var. *agrestis* (Morphotype I) , *Cucumis melo* var. *agrestis* L. (Morphotype II) *Cucumis melo* var. *momordica* L., *Momordica charantia* L., *Momordica balsamina* L., *Momordica dioica* L. , respectively. Amount of compound in DWE were observed maximum in Cucumis melo L. (2.794±0.014 µg/mg) followed by Cucumis melo agrestis L. (morphotype I) (2.424±0.025 µg/mg), while it was found to be least in *Momordica balsamina* L. (0.268±0.008 µg/mg). The limit of detection (LOD) and limit of quantification (LOQ) value is 0.227 and 0.801, respectively for the compound (Table 4.17).

Table 4.17. The amount of Quercitin (µg/mg DW) in genera *Cucumis* L. and *Momordica* L.

Standard	Peak area ±SE	Slope (m) Intercept (b)	R^2	Amount of compounds (µg/mg±SE)	LOD	LOQ
Quercetin		m = 4590.86 b= 414.86	0.999		0.227	0.801
CM	18553.866			12.314±0.030		
CMAI	19945.3			9.494±0.028		
CMAII	9878.066			4.991±0.016		
CMM	7223.1			1.734±0.017		
MC	8767.233			10.78±0.058		
MB	2844.966			2.519±0.003		
MD	4949.866			34.19±0.004		

Values are mean ±SD of three measurements. Variation in the letters between samples indicates significant difference at level $p < 0.05$.

CM- *Cucumis melo* L. , CMA(I)- *Cucumis melo var. agrestis* (morphotype I), CMA(II)- *Cucumis melo var. agrestis* (morphotype II) CMM- *Cucumis melo var. momordica* L., MC- *Momordica charantia* L., MB-*Momordica balsamina* L., MD-*Momordica dioica* L.

4.7.4. Quantitative estimation of Cinnamic acid by HPLC analysis

HPLC of the Cinnamic acid along with methanol extract of all extracts of *Cucumis* L and *Momordica* L. has been developed using acetonitrile: methanol (50:50) as mobile phase solvent system (Table 3.4) and detected in 256 nm with R_f value 3.061 as shown in 3D-chromatogram. Using different standard concentrations (0.5, 1, 2, 4, 6, 8, 10 µL) and values for slope (b) and intercept (m) were found to be 297560 and 1655.52, respectively (Figure Ic). In the results (Table 4.18), peak areas were obtained 2148.72, 0.0, 1779.2, 0.0, 10560, 4356.14, 10346.6 for *Cucumis melo* L., *Cucumis melo* var. *agrestis* (Morphotype I) , *Cucumis melo* var. *agrestis* L. (Morphotype II) *Cucumis melo* var. *momordica* L., *Momordica charantia* L., *Momordica balsamina* L., *Momordica dioica* L. , respectively. Amount of compound in DWE were observed maximum in *Momordica balsamina* L. (0.023±0.001 µg/mg) followed by *Momordica dioica* L. (0.020±0.0005 µg/mg), while it was found to be least in *Cucumis melo* L. (0.001±0.003 µg/mg). The limit of detection (LOD) and limit of quantification (LOQ) value is 0.551 and 1.671, respectively for the compound (Table 4.18).

Table 4.18. The amount of Cinnamic acid (µg/mg DW) in genera *Cucumis* L. and *Momordica* L.

Standard	**Peak area ±SE**	**Slope (m) Intercept (b)**	**R^2**	**Amount of compounds (µg/mg±SE)**	**LOD**	**LOQ**
Cinnamic acid		m = 297560 b= 1655.52	0.999		**0.551**	**1.671**
CM	2148.72			.001±0.0		
CMAI	0			**0**		
CMAII	1779.2			0.0003±0.0		
CMM	0			**0**		
MC	10560			0.023±0.0		
MB	4356.14			0.004±0.0		
MD	10346.6			0.020±0.0		

Values are mean ±SD of three measurements. Variation in the letters between samples indicates significant difference at level $p < 0.05$.

CM- *Cucumis melo* L. , CMA(I)- *Cucumis melo* var. *agrestis* (morphotype I), CMA(II)- *Cucumis melo* var. *agrestis* (morphotype II) CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB-*Momordica balsamina* L., MD-*Momordica dioica* L.

RESULTS AND DISCUSSION

For the development of novel industry, natural products plant extracts, provides an unlimited opportunity due to great diversity in the chemical nature. By taking this in to consideration, young research have increased attention towards the ethno-medicine, to produce new effective drugs against fungal infections, chronic disease and other infectious disease (Bhaskarwar *et al*., 2008) and this played an effective role in the health care system of many countries (Gupta *et al*., 2010).

In the present study, comparative morphological, cytological, preliminary phytochemical, genotoxicity, antimicrobial, antioxidant, mineral and proximate analysis and HPLC properties of two genera *Cucumis* L. and *Momordica* L. were undertaken Due to the destruction of environmental changes as well as extinction of medicinal plants there is great need to use conserve medicinal plant resources as an alternate pathway for medical industry. For this purpose, the present work has been undertaken.

5.1. Morphological studies

5.1.1. Quantitative/Qualitative Variations in Fruits and Seed Traits of *Cucumis* and *Momordica*

From the data (table 4.1), it is clear that there is significant differences in the two morphotypes of *Cucumis melo* var. *agrestis* morphological characteristics. The results indicate that *Cucumis melo* var. *agrestis* (Morphotype II) had the longest mean fruits of 9.1±0.43 cm in length and 8.36±0.51cm in diameter (Fig. 9-12, table 4.1) as compared to Morphotype I. Similar studies on fruits morphological traits in many tree species natural populations have also been reported by various researchers (Dadegnon *et al.,* 2015; Mkwezalamba *et al.,* 2015; Mingfei *et al.,* 2017; Okwu *et al.,* 2017).

Many research works conducted with fruits and seeds of tree species from diferent botanical families, such as cucumber tree (*Averrhoa bilimbi* L.) of Oxalidaceae family (Santos *et al.,* 2015), *Campomanesia adamantium* of Myrtaceae family (Dresch *et al.,* 2013), and majestic heaven lotus (*Gustavia augusta* L.) from *Lecythidaceae* family (Silva *et al.,* 2014), indicate that longer and wider fruits usually present higher values of fruit mass and seeds.

Fruit length and breadth are important morphological characteristics for plants characterization because phenotypical correlations allow the quantitative examination of a relevance character in relation to another (Felizardo *et al.,* 2015). However, it is not clear whether the species with long and large *Cucumis melo* var. *agrestis* (Morphotype II) fruits may be more productive than those with narrow and short fruits, because morphological dimensions and characteristics of plants are the consequence of various ecological factors on the genotype of the species.

The causes of phenotypic variation among individuals across the geographical distribution range of a species can be broadly divided into current environmental conditions within particular habitats and historical processes and phylogenesis (Merila *et al.,* 2014; Mingfei *et al.,* 2017). Plant populations of the same species growing under different environmental conditions respond to different selection pressures, producing genetic and phenotypic divergence between populations (Albarrán-Lara *et al.,* 2019). In addition, (Parmesan *et al.,* 2006; Chen *et al.,* 2011) have examined and compared intraspecific phenotypic variation across the geographic ranges of selected species. The generated signifcant variations in fruit production between provenances is an indication that superior provenances of *Cucumis melo* var. *agrestis* (Morphotype II) can be selected for domestication, breeding improvement, and commercialization purposes.

5.2. Cytology

Meiosis is a phenomenon dealing with the various important aspects of cell cycles in higher eukaryotes and act as a key step of sexual reproduction. The process involves two successive steps, first one is reductional division (meiosis-I), followed by the equational division (meiosis-II), which results in 4 haploid gametes. It involves a sequence of complex cell/ and molecular events, involving DNA and chromosome replication (S phase), chromosome pairing and genetic recombination (zygotene & pachytene stage of prophase-I), chromosome segregation (anaphase-I) and cytokinesis. These series of events are responsible for the genome integrity and stability in sexual life cycles, besides some anomalies, these also result in producing intraspecific genetic variations. These meiotic abnormalities involve, numerical (polyploidy & aneuploidy) and structural (inversions & translocations) changes in the chromosomes and have been considered as a major driving force for gene and genome evolution in nature. Because, these abnormalities lead to genetically (chromosome)

imbalanced gametes (diploid/or aneuploid gametes), which may result in genome expansion (polyploidy) or subtraction (dysploidy). The meiotic process is reported to be controlled at gene level and it is a complicated event of high evolutionary fidelity and genetically programmed cellular processes, ensures gametic viability and is regarded as one of the most sensitive stages in the life cycle of seed plants (de Muyt *et al.,* 2009). Hence, such meiotically enforced variations are key factors in studying genome evolution in plants (Cai and Xu, 2007).

In the present study the two genera *Cucumis* L. and *Momordica* L. from various locations of North India were studied for meiotic analysis. All the observations have been made by assessing detailed male meiotic course in each studied population and are discussed here in the succeeding text.The meiotic analysis in the plants of different populations of *Cucumis* L. and *Momordica* L. revealed that the majority of PMCs showed normal meiosis.

A perusal of literature revealed that in family Cucurbitaceae the existence of the haploid chromosome number varries from n=7 to 24, with x = 12 as basic number (Beevy and Kuriachan, 1996) is most prevalent. *Momordica* L. Plant collected from various regions of north India showed x=8, 11 and 14, which is is in line with previous records for these species (Dutt and Roy, 1976), *Cucumis* L. shows n=12, which is in line with the previous record given by various authors ((Jeffrey, 1980; Baratakke and Patil 2009; Bharathi *et al*., 2011; Rajkumari *et al.,* 2013).

5.2.1. Meiotic abnormalities:

A normal process of meiosis involves balanced duplication and segregation of chromosomes and results in 4 equal haploid (*n*) gametes. However, the recombination occurs in pachytene stage of meiosis-I, which is responsible for the genetic variability in the gametes. The chromosome pairing, synapsis and recombination are among the vital processes which are required for the success of meiosis (Page and Hawley, 2003), and are under the control of different genes (Li *et al.,* 2005).

In the present study, the different type of meiotic abnormalities have been observed, which include structural heterozygosity (quadrivalents in diploids), univalents, multivalent in polyploids, interbivalent connections, secondary associations, chromatin stickiness, nonsynchronous disjunction of bivalents at

metaphase-I, chromatin bridges, chromosomal laggards, un-oriented bivalents and spindle abnormalities. Microsporogenesis was abnormal to the extent that 5.16±0.21% to 15.48% of PMCs showed formation of micronuclei at tetrad as well as diad and triad stages. Polyads were also observed in some populations The frequency of species in respective meiotic irregularities is given in the table 4.5. Consequent to non-synchronous behaviour of chromosome segregation, laggards (Figure 32) were observed at anaphase and telophase in the PMCs. Formation of laggards due to non-synchronous dysjunction has been previously reported in *Zea mays* (dela Vina and Ramirez 1995) and many other plants. Intense chromatin stickiness (Fig. 55) along with broad chromatin bridges were observed towards the end of meiosis I (Figure 54, 58). The secondary chromosomal associations or bivalents/chromosomes having diffused connections existed in the species at diakinesis and pro-metaphase (Figure 32-35). Such behaviour of bivalents is attributed to polyploidy origin of the species (Stebbins, 1950; Malgwi *et al.,* 1997).

5.2.1.1. Cytomixis

Among these meiocyte studies, cytomixis, being documented a long ago, is the process of formations of cytoplasmic channels between neighboring cells, between meiotic Prophase I, through which chromatin material can migrate between the cells (Mursalimov *et al.,* 2010, 2013a; Liu *et al.,* 2012; Barton *et al.,* 2014). It has been observed in the pollen mother cells of many plant species of flowering plants at high frequency (Saggoo and Srivastava, 2009; Lone and Lone, 2013; Mursalimov *et al.,* 2013b).

Presently, the phenomenon of cytomixis has been formulated in the PMCs of as many as five species of all populations of the species where meiotic analysis was carried out. The process involved minimum of 2-3 and maximum 3-4 PMCs interconnected through cytoplasmic channels. The amount of chromatin transferred from one PMC to another varied from a small part of chromatin to sometimes complete chromatin involving the entire complement of the PMC making the donor cell completely empty (Fig. 42, 43).

The present study revealed that cytomixis was directly responsible for different sized pollen grain, abnormal meiotic behavior and pollen sterility in *Cucumis* L. and *Momordica* L. Cytomixis may resulted in the formation of unreduced,

aneuploid, polyploid sterile pollen (Pécrix *et al.,* 2011; Mursalimov and Deineko, 2015).

5.2.1.2. Chromatin stickiness:

The chromatin stickiness is a strong association/ clustering of chromosomes during any phase of the meiotic/mitotic cell cycles. The phenomenon is reported for the first time in maize by Beadle (1932) and is a result of a recessive mutant *sticky (st)* gene. But, it may be a result of partial dissociation of nucleoproteins and change in the chromosomal structure (Evans, 1962). The phenomenon is reported in different *Brachiaria* species by a number of workers suggesting their genetic or environmental control (Pagliarini *et al.,* 2008; Risso-Pascotto *et al.,* 2009). According to Ritambhara and Kumar (2010), the alterations in histones proteins that change the surface properties of the chromosomes and results in adherence among them. The chromosomal individuality is lost due to these abnormalities. In the present study, chromatin stickiness has been reported in *Cucumis melo* and *Cucumis melo* var. *momordica* L. at metaphase stage of meiosis (Fig. 36, 55). Such stickiness in the chromosomes has been found to reduce the pollen viability and has been reported by many workers (Kumar and Singhal 2011) and similar work has also been reported in the present study too (Table 4.5).

5.2.1.3 Lagging chromosomes/ laggards:

The laggards are the chromosomes that fail to reach their respective poles at anaphase or telophase stages. This phenomenon has been reported in *Cucumis melo* L. (Fig. 32), *Cucumis melo* var. *agrestis* (Morphotye II) (Fig. 51), *Momordica dioica* (Fig. 64), *Momordica charantia* (Fig. 71, 72) (Table 4.5). These laggards may be due to weak attachment of kinetochore to the spindle fibers (Nicklas and Ward 1994).

5.2.1.4. Chromatin bridges:

In some of the PMCs, the chromosome connections were observed at anaphase-I and may arise due to paracentric inversions. In some views, the chromatin stickiness may also be responsible for their origin **(**Lopez *et al.,* 2015). The occurrence of chromosomal bridges is due to failure of pairing of homologous chromosomes, resulted of a missegregation of multivalent figures (Murphy and Bass 2012). In the present study, the chromatin bridges have been observed in *Cucumis melo* var. *momordica* (0.56%).

5.2.1.5. Abnormal microsporogenesis

All the above-mentioned meiotic aberrations lead to abnormal microsporogenesis like dyads, triads, tetrads with or without micronuclei that leads to the formation of sterile pollen grains **(**Caetano-Pereira and Pagliarini 2001). Monads are formed when there is a failure of both divisions of meiosis, while dyads are formed on the failure of the second meiotic division and failure of the meiosis-II at one of the pole, results in the formation of triads. However, the polyad formation owes to spindle abnormalities. The micronuclei are formed due to the laggards and may form micro pollen or unbalanced gametes later. In *Cucumis melo*, polyad and tetrads with micronuclei were observed (Figs. 37, 38). In *Cucumis melo* var. *agrestis* (Morphotype I), dyads and triads were observed (Figs. 44, 45). Similarly, in *Cucumis melo* var. *agrestis* (Morphotype II), triads, tetrad with micronuclei and polyads were observed (Figs 48, 49, 50). In *Cucumis melo* var. *momordica* triad and triad with micronuclei were observed (Figs. 55, 56). In *Momordica dioica* tetrad with micronuclei (Fig. 62). In *Momordica charantia* Monad, dyad and polyad (Figs. 68, 69, 70) were observed. This kind of deviation from normal microsporogenesis due to abnormal meiotic behavior results in sterile pollen grains (Figs. 39, 46, 52) and hence reduced pollen viability (Table 4.4).

5.3. Antibacterial activity assay

Evolution of modern medicine from folk medicine as well as traditional system is the product of thorough screening of chemical and pharmaceuticals of plant products. Higher plants contain various substances, used to combat various diseases, act as food additives due to their versatile therapeutic actions (Mukherjee and Wahile, 2006).

Various disease causing microorganisms have led to the use of plants in the human medical field. About 3,000 constituents from 2,764 plant species has been screened for chemotherapeutic and pharmacological properties (Anon, 1988). A large spectrum of drugs is available in the market for their treatment; however, the development of drug resistance has become a huge hurdle in the treatment of these diseases and the need for new drugs has increased enormously (Giamarellou, 2010; Marasini *et al.*, 2015). Many workers have documented antimicrobial screening of plants (Dulger *et al.*, 2005; Kumar *et al.*, 2006). The plant antimicrobial drugs of the plant origin are of interest because many human and animal pathogens show

multidrug resistance and certain antibiotics have undesirable side effects (Ahmed and Beg, 2001). Higher the plants have the ability to accumulate antimicrobially active substances and other defense chemicals to themselves from microbial infection, which has been documented both *in vitro* and *in vivo* (Dixit and Trivedi, 2006). Flavonoids and phenolics reflects the antimicrobial activity of the plant extracts (Hegazi, 2000).

The present study revealed that the maximum inhibitory zone of 32.3 ± 0.74 mm was reported against *Staphylococcus aureus* in *Cucumis melo* L. (Fig. 102) and 23.3 ± 0.47 mm was found against *Bacillus coagulans* in *Cucumis melo var. agrestis* (morphotype II) (Fig. 106) extract at 3000 ppm. A direct contact between the concentration and zone of inhibition was recorded as upon increasing the concentration of the plant extract. Most of these plants showed great effectiveness against Gram positive bacteria as compared to Gram negative (Table 4.8). Plants such as *Cucumis melo* var. *agrestis* (morphotype II) and *Momordica dioica* L. were found ineffective against *Klebsiella pneumoniae* even at higher concentrations, while these plants have shown a significant inhibitory affect against other test bacteria. Although all the screened plants species have shown inhibitory effect against one or more bacterial strains but plants such as *Cucumis melo* var. *agrestis* (morphotype I), *Cucumis melo* L. and *Momordica charantia* L. has been as most active plant species having medicinal properties as these exerted inhibitory action against all the tested strains.

Biochemically the *Cucumis* L. and *Momordica* L. plants are reported to contain Lectins, triterpenes, alkaloids, saponin **(**Wang *et al.*, 2012; Jiang *et al.*, 2016; Mallek-Ayadi *et al.*, 2018; Ibidapo *et al.*, 2019**).**

5.4. Antioxidant activity

5.4.1. Yield of extraction

Biologically active compounds usually occur in low concentra-tion in plants. An extraction technique is that which is able to obtain extracts with high yield and with minimal changes tothe functional properties of the extract required (Quispe Can-dori *et al.*, 2008). Several studies have reported variations inthe biological activities of extracts prepared using different extraction techniques. Therefore, it is necessary to select the suitable extraction solvent (Hayouni *et al.*, 2007; Ishida *et al.*, 2001). On the basis of metabolites and activities a number of methods are identified for plant

extraction (Nimbalkar *et al.,* 2012; Murugan and Parimelazhagan, 2014; Pai *et al.,* 2015). The extraction yield and antioxidant potential shows a great deal of variation depending upon solvent type and method adopted (Escribano-Bailón and Santos-Buelga, 2003; Sun and Ho, 2005; Naczk and Shahidi, 2006). This might be due to the fact that low will be the viscosity of the solvent and high will be its diffusivity (Naczk and Shahidi 2006).

The two solvents used for extraction i.e. methanol and aqueous were evaluated for their potency to extract antioxidants from dried plant samples. The variation in yield (5.21 to 24.34 g/100g) as well as in colour characteristics was noticed among the selected plant samples (Table 4.10). The extraction yield was found to be highest in methanol extract indicating that the extraction efficiency favors the highly polar solvents. This result is consistent with the extraction yield of *Limophila aromatica* (Do *et al.,* 2014) and some other medicinal plants (Kuppusamy *et al.,* 2015). Extraction solvents have an effect on the extraction yield and the content of bioactive compounds, thus significantly affecting the biological activity of the extract (Turkmen *et al.,* 2006; McDonald *et al.,* 2001; Ngo *et al.,* 2017).

5.4.2. Qualitative testing of photochemical

Detection of alkaloids, flavanoids, Saponin, tannins and terpenoids in several extracts indicates that these were major secondary metabolites in these as shown in Table 4.11. Saponins are reported to have hypocholesterolemic and antidiabetic properties, while triterpenoids display analgesic and anticancer properties (Ali *et al.,* 2008). So these secondary metabolites contribute to potent use of plants in pharmacological industries. In the present study aqueous extract shows that alkaloids, flavanoids, Saponin, tannin, terpenoids are present in all the species. All these phytochemicals have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital role for good health (Bhumi and Savithramma, 2014).

5.4.3. Quantitative testing of photochemical

Phytochemical analysis on different plant extracts showed the presence of different constituents of medicinal species as well as physiological importance. Most of the secondary metabolites isolated from natural sources are used in fragrances,

pharmaceuticals, food additives, pesticides and herbicides (Pal, 2007; Ramawat and Dass, 2009).

A wide range of variation was observed among the selected plants for quantatity of tested phytochemicals. The amount of total phenolic content (table 4.12, Figs. 5.1-5.2) was found to be significantly different in both the genera and in their respective extracts ($p<0.05$) and it vary between 81.08±05 to 23.59±0.06. The results showed that in methanolic extract, the recovery rate of total phenolic content was maximum in methanol extract (36.73±0.09 to 81.08±05), while least amount was found in aqueous extract (23.59±0.06 to 59.21±0.05) previous studies revealed that total phenols content in cucurbits ranges from 51.6 to 750 mg/100 g in various cucurbits (Nagarani *et al.,* 2014). Similar studies revealed in *Rumex crispus* in which ethanolic extracts have high phenolic content when it was compared with aqueous extract (Gulcin *et al.,* 2003). Plant Phenolic compounds had antioxidant activity chiefly attributed to their redox properties to scavenge free radicals (Duenas *et al.,* 2006; Kilani-Jaziri *et al.,* 2011). The mechanism behind this is to activate antioxidant enzymes, enhance DNA repair system and to reduce oxidative DNA injury prevent (Stoner *et al.,* 2008). Pharmacists usually target the plant with high phenolic content to treat different diseases (Petti and Scully, 2009)

The flavanoid content in methanol and aqueous extract varied from 35.43 ± 0.25 to 73.03 ± 0.21 and 27.5 ± 0.26 to 50.23 ± 0.15, respectively (Table 4.12, Figs. 5.3-5.4) and it differ among all the plant species in their respective extracts. Flavanoids are main secondary metabolites in many plants (Kozlowska and Szostak-Wegierek, 2014), contributing to the antioxidant potential (Csepregi *et al.,* 2016). The protective functions of flavanoids include suppression of ROS formation, up regulating antioxidant defense, scavenging ROS, protection of lipid against oxidative damage, inhibiting enzyme involving in ROS generation (Kumar *et al.,* 2013). Many workers revealed the presence of flavanoid in genus *Momordica* L. (Wu and Ng, 2008; Zhu *et al.,* 2012; Kenny *et al.,* 2013)

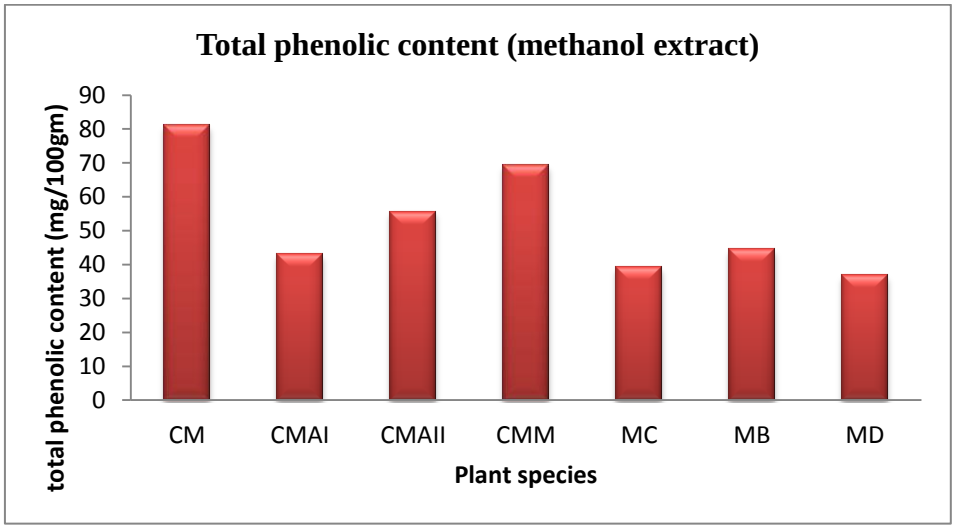


Fig. 5.1. Histogram showing total phenolic content in methanol extract

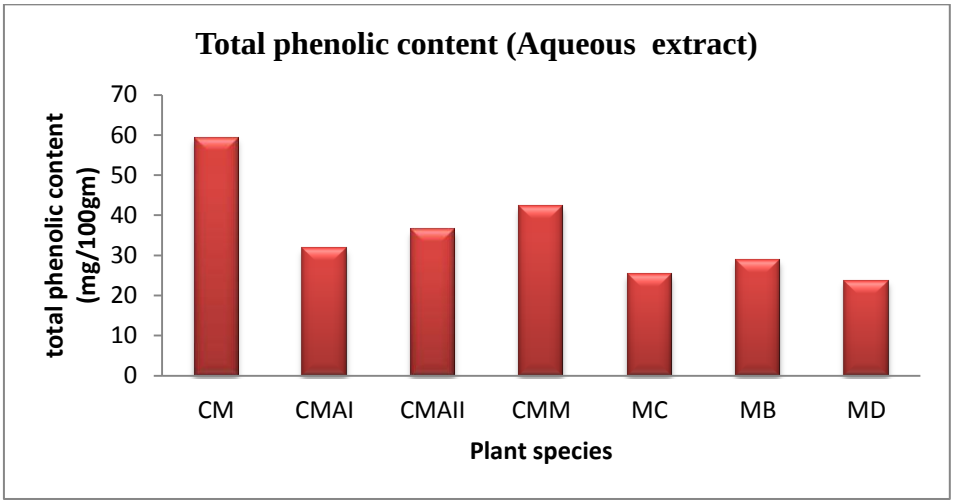


Fig 5.2. Histogram showing total phenolic content in aqueous extract

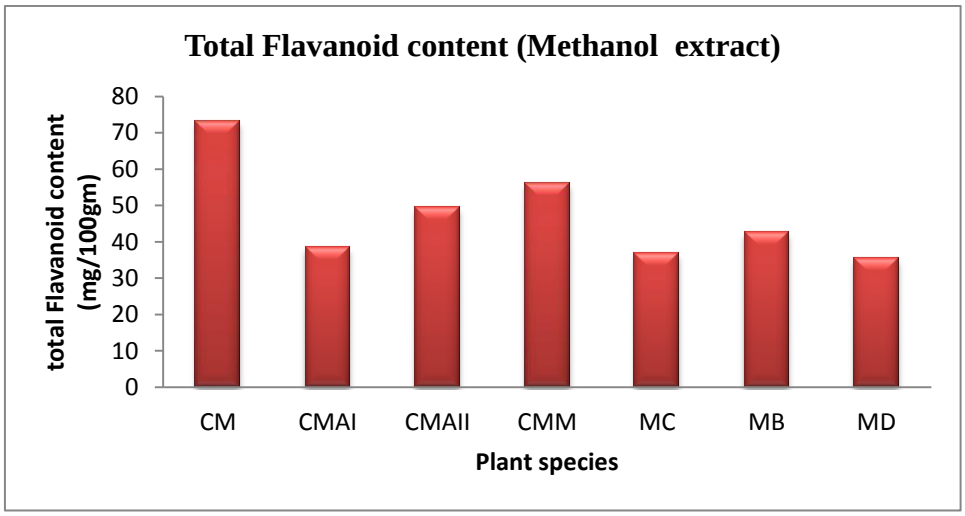


Fig 5.3. Histogram showing total flavonoid content in methanol extract

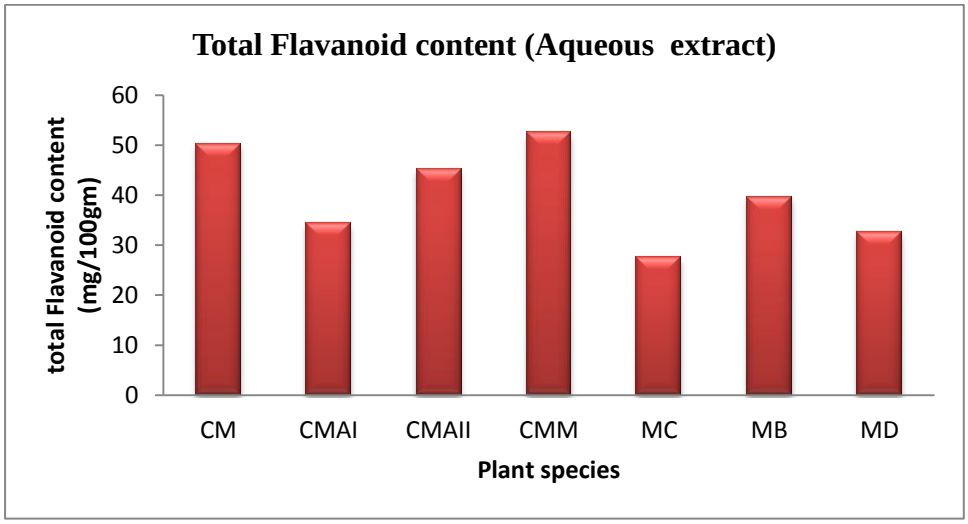


Fig 5.4. Histogram showing total flavonoid content in aqueous extract

Abb. CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

5.4.3. Antioxidant activity of plant extracts

The antioxidant capacity is influenced by several factors and it could not be fully described by using a single assay. A number of methods with variations have been prescribed to assess the antioxidant potential (Niki, 2011). Therefore, in the present study, an antioxidant potential was assessed by using different assays based on different procedures. The results of antioxidant activity of different extracts by using different methods are as presented in table 4.13.

5.4.4. DPPH radical scavenging activity

The DPPH method is employed to determine the radical scavenging activity of antioxidant constituents. Free radical scavenging activity by using DPPH assay expressed by the percent scavenging as well as IC_{50} value. The percent scavenging and IC_{50} values of different plants was in order of *Cucumis melo* L. > *Cucumis melo* var. *agrestis* (morphotype II)> *Momordica dioica* L.> *Cucumis melo* var. *agrestis* (morphotype I)> *Momordica charantia* L.> *Cucumis melo* var. *momordica* L.> *Momordica balsamina* L. in methanolic extracts, which was highest, whereas in aqueous extract it was comparatively less viz. *Cucumis melo* L.> *Momordica dioica* L.> *Cucumis melo* var. *agrestis* (morphotype II)> *Cucumis melo* var. *agrestis* (morphotype I)> *Momordica charantia* L.> *Momordica balsamina* L.> *Cucumis melo* var. *momordica* L. (Table 4.13, Figs. 5.5-5.6). The trend of percent scavenging

increase with increase in concentration was revealed by various authors (Shah *et al.,* 2010; Al-Adhroey *et al.,* 2010; Thambiraj and Paulsamy, 2012).

In the present study the results of DPPH free radical scavenging potential is shown in table 4.13. It was observed that DPPH free radical scavenging activity vary with concentration as well as with phenolic compounds (Moure *et al.,* 2001). Antioxidants by interacting with DPPH, transfer an electron/hydrogen atom to DPPH, so in this way neutralize free radical character of DPPH (Naika *et al.,* 2003).

5.4.5. Hydrogen peroxide scavenging assay

Hydrogen peroxide have the capacity to inactivate different enzymes by the oxidation of thiol groups and to oxidize cell compounds by moving accross cell membranes (Sen *et al.,* 2013). Hydrogen peroxide have the tendency to react with toxic metal ions like iron and copper to form hydroxyl radicals (Hazra *et al.,* 2008). A fast reduction in the concentration of hydrogen peroxide, caused by antioxidant can be assessed at 230 nm (Sen *et al.,* 2013). The measurement of reducing ability of extract serves as an important tool to calculate the antioxidant potential (Dorman *et al.,* 2003). The results are shown in (table. 4.13, Figs. 5.7-5.8). Hydrogen peroxide (H_2O_2) scavenging activity of natural antioxidants present in plant extracts has been determined widely by various researchers (Rajamanikandan *et al.,* 2011; Keser *et al.,* 2012; Shahriar *et al.,* 2013;Vinodhini and Lokeswari, 2014; Ahmad *et al.,* 2014).

5.4.6. Reducing power assay

Principle behind the working of the reducing power method is that increase in the absorbance of the reaction mixture is an indicator of antioxidant potential of the extracts (Nabavi *et al.,* 2009). In this assay, the presence of antioxidants in the plant samples results in the reduction of Fe^{+3} to Fe^{+2} by donating an electron which is measured by the formation of Perl's Prussian blue at 700nm (Chung *et al.,* 2002).

IC_{50} in methanol extracts was found to be in order *Cucumis melo* var. *momordica* L.> *Cucumis melo* L.> *Cucumis melo* var. *agrestis* (morphotype II)> *Cucumis melo* var. *agrestis* (morphotype I)> *Momordica dioica* L.> *Momordica balsamina* L.> *Momordica charantia* L. on the other hand this order is slight different for aqueous extracts viz. *Cucumis melo* L> *Cucumis melo* var. *agrestis* (morphotype II)> *Cucumis melo* var. *momordica* L.> *Cucumis melo* var. *agrestis* (morphotype I)> *Momordica dioica* L.> *Momordica charantia* L.>*Momordica balsamina* L. (Table

4.13, Figs. 5.9-5.10). The results showed the existence of hydrophilic polyphenolic compound in plant extracts that are responsible for reducing power (Karawita *et al.*, 2005). Different reducing power values of plants and variable behavior of same plant in different solvents suggest that nature of plant and solvent system used for the extraction are very crucial to study antioxidant activity as observed various researchers (Katalinic *et al.*, 2006; Qader *et al.*, 2011). Similar observations were recorded for the relation between the polyphenolic components and reducing power activity for several plant extracts (Amarowicz *et al.*, 2004).

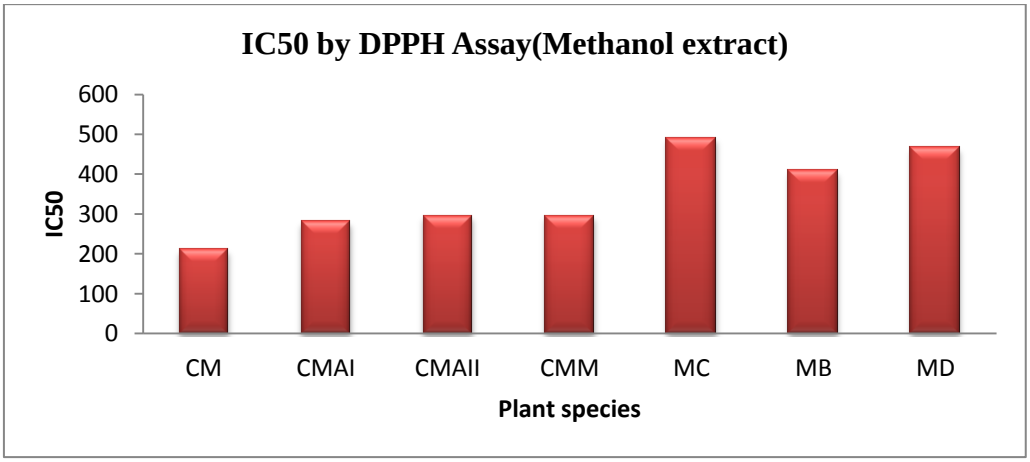


Fig 5.5. Histogram showing IC_{50} by DPPH Assay using methanol extract

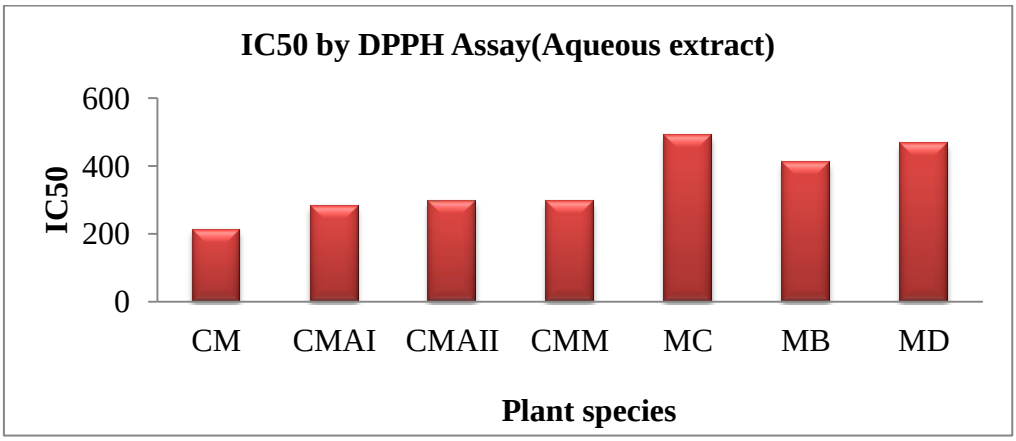


Fig 5.6. Histogram showing IC_{50} by DPPH Assay using aqueous extract

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

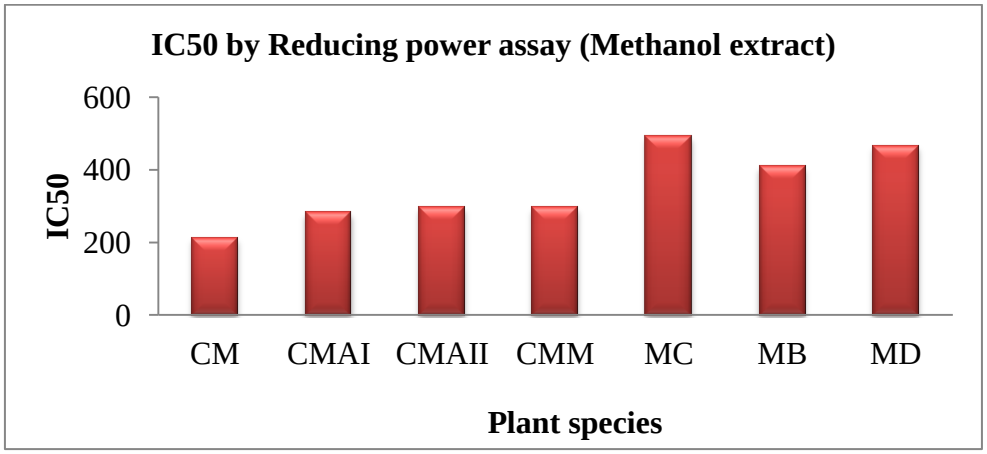


Fig 5.7. Histogram showing IC_{50} by reducing power Assay using methanol extract

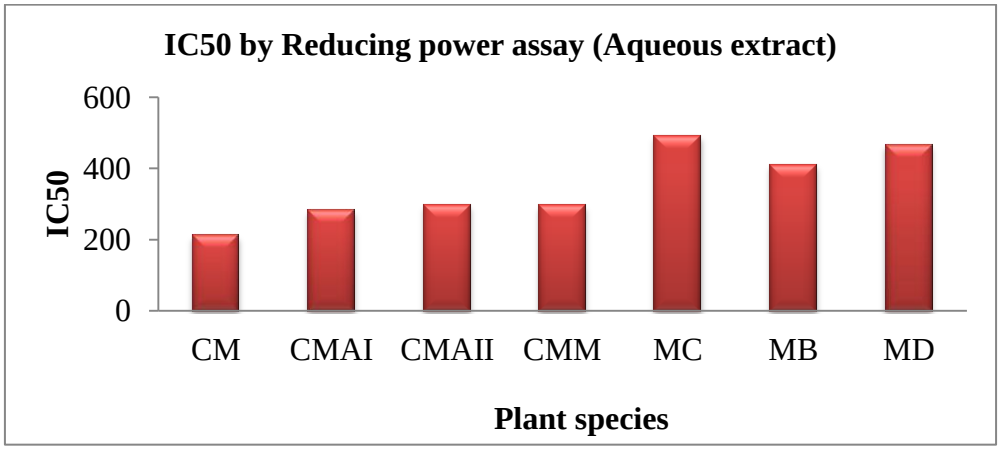


Fig 5.8. Histogram showing IC_{50} by reducing power Assay using aqueous extract

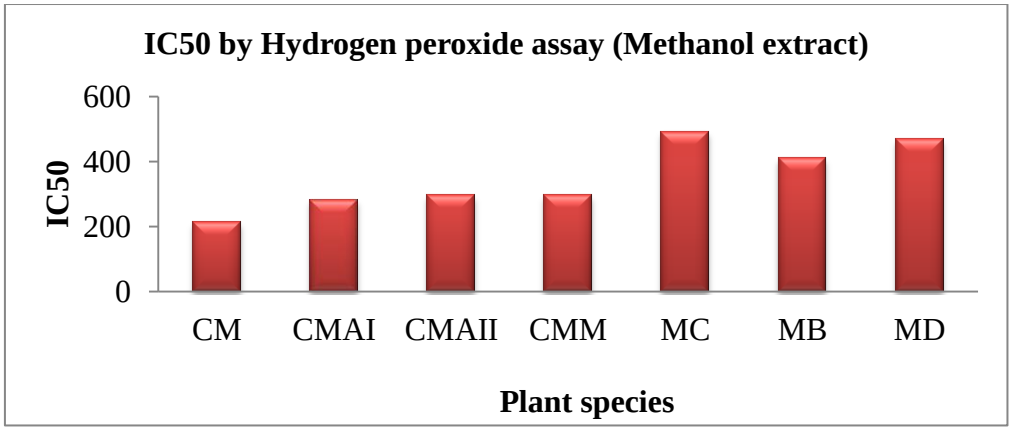


Fig 5.9. Histogram showing IC_{50} by hydrogen peroxide Assay using methanol extract

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

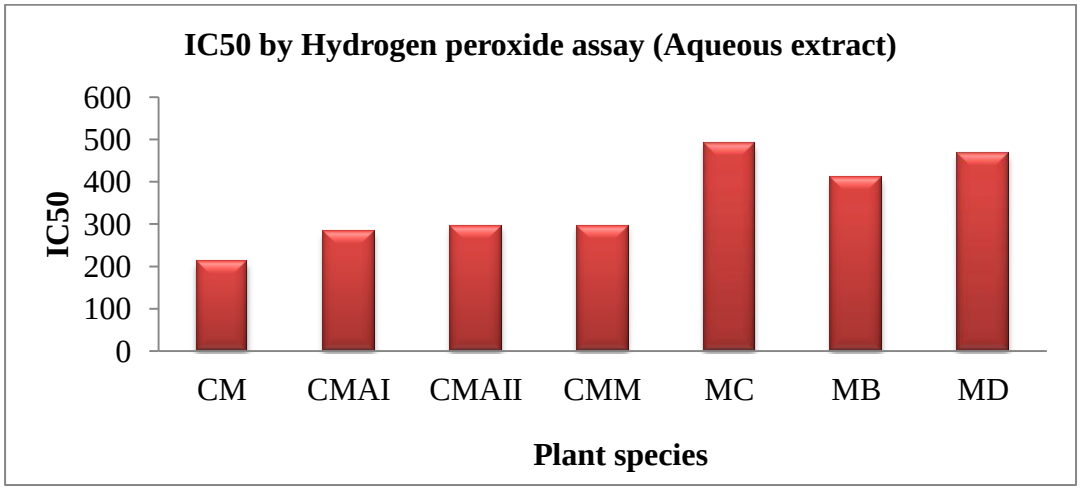


Fig 5.10. Histogram showing IC_{50} by hydrogen peroxide Assay using aqueous extract

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

According to the data given by Jun *et al.,* (2003) (Table. 5.1), antioxidant activity fall into five categories inactive, weak, moderate, active and highly active. According to Molyneux, (2004), the IC_{50} value in range of 200-1000 µg/ml was declared less active but still have the potential to act as an antioxidant. Based on this data, we can conclude that in our research, IC_{50} of methanol extract of *Cucumis melo* L. value is 43.387 µg/ml (DPPH scavenging assay) so it comes under very strong antioxidant category on the basis of average mean of IC_{50} indicated in table 4.13, and IC_{50} value in methanol and aqueous extract of all other species, for other two assays comes under the range of 50-100µg/ml i.e. active antioxidant potential. On the basis of IC_{50} value obtained, it is clear that the fractions of methanol extract have the greatest antioxidant activity in DPPH free radical scavenging assay, which is regarded as the most significant method because it has the ability to accommodate many samples in very short period of time and moreover, it is sensitive enough for detection active ingredients even at low concentrations (Sanchez-Moreno, 2002).

Table 5.1. Power levels of antioxidant by DPPH method (Jun *et al.*, 2003)

S.No.	Intensity	IC_{50}
1.	Inactive	>500 µg/ml
2.	Weak	250-500 µg/ml
3.	Moderate	101-250 µg/ml
4.	Active	50-100 µg/ml
5.	Highly active	<50 µg/ml

Antioxidant potential (in mg/ml) in many members of family Cucurbitaceae have already been observed like, *Momordica charantia* (Samir *et al.,* 2017), *M. balsamina* (Sumitra and bhagwati, 2018), *M. dioica* (Shreedhara *et al.,* 2011), *Cucumis melo* var. *agrestis* (Gopalasathees kumar *et al.,* 2019), *Cucumis melo* (Ibidapo *et al.,* 2019). *Cucumis melo* L. var. *agrestis* (Smell melon or wild melon), is considered as one of the major weed in crops of soyabean, peanut and cotton (Grichar, 2007a, 2007b; Sohrabi *et al.,* 2013) and it can be explored as natural antioxidant.

The extracts of both the genera possess significantly higher percentage of scavenging activity with increase in dosage. Similar observation has been reported for several plants in terms of dose dependent manner (Jamuna *et al.,* 2012). On the other hand there was a differential response of methanol and aqueous extracts of all the 6 species of *Cucumis* L. and *Momordica* L. and its fractions in various antioxidant tests. This can be explained on the basis variability of structure of antioxidants, and moreover, on the basis of electron transfer from antioxidants at varying redox potential in different systems (Loo *et al.,* 2008). In the present study, despite of methodological limitations to determine antioxidant potential, it is advisable to determine it by using several test systems to find the accuracy (Kaur and Kapoor, 2001). This is due to the reason that bioactive compounds participating in antioxidant potential of fruits and vegetables is influenced by various factors, such as storing condition, degree of ripeness, geographical region and climatic conditions (Wang, 2006).

As per literature studies, no earlier reports are available regarding the antioxidant potential of the plant extracts of the selected genera on the basis of cyto-morphovariation as well as comparison of wild and cultivated ones, in these genera.

Our study found that two morphotypes of *Cucumis melo* var. *agrestis* shows a great variation with respect to its total phenolic and total flavanoid content and hence, antioxidant potential in terms of μg/ml.

Various researches found that antioxidant potential of Cucurbitaceae seeds was correlated with total phenolic content (Ismail *et al.,* 2010; Achu *et al.,* 2013; Talukdar and Hossain, 2014)

5.5. Genotoxicity evaluation

Plant based assay systems are recognized as sensitive biomonitors of the genotoxic effects of the environmental chemicals and can be used both *in situ* situations as well as in the laboratory (Grant, 1999). The *Allium* anaphase /telophase chromosome ablteration assay was employed as a method for the rapid screening of chemicals as well as environmental samples. The utmost important benefit of the *Allium* test is due to its 'low budget' nature, which being fast, easy to access, gives reliable results. *Allium* test has been employed to determine the cytotoxic and genotoxic effects of various types of environmental hazards, wastes, etc. (Smaka-kincl *et al*., 1996).

The present work deals with the comparative analysis of genotoxicity in 6 species of the family Cucurbitaceae, normally consumed by local inhabitants or tribal people. There is always a possibility that some chemicals can contribute to the carcinogenic process, by interfering in inter/ intracellular signaling/ communications, patters of gene expression, and acetylation of DNA/ histones proteins (Trosko and Upham, 2005; Prajitha and Thoppil, 2016). *Allium cepa* assay is widely used for evaluation of genotoxicity potential of chemicals and different plant extracts (El-Shabbaby *et al.,* 2003; Ventura *et al.,* 2008), and has many benefits, like the capability of activating promutanges and mixed oxidase system. Further, this test is also important in studying possible damage to DNA *in vivo* (Nefic *et al.,* 2013).

In the present study, the dried fruit extract of different species (Table 3.1), have been tested in the genotoxicity assay and are reported with mitodepressive effect (lowers Mitotic Index) on cell division (Table 4.6), and the results are quite similar to the previous studies (Lamsal *et al.,* 2010; Frescura *et al.,* 2013; Khanna and Sharma, 2013). Mitotic Index is the quantitative assessment of mitotic actions in a particular part organism/ or even whole organism (Smaka-Kinel *et al.,* 1996; Sreeranjini and

Siril, 2011) and, the cyto/-genotoxicity can be determined by decreased MI rate. A decline in MI as compared to the control values may have a lethal effect on an organism (Antonsiewicz, 1990), while, sublethal effects are reported as this value reduces below 50 percent (Panda and Sahu, 1985), and known as cytotoxic limit value (Sharma, 1983).

Similarly in the present study maximum MI index was represented in aqueous extract of *Cucumis melo* and minimum value was reported in methanol extract of *Momordica dioica* (Table 4.6). The reduction in MI may be due to potential cytotoxicity, that could inhibit DNA synthesis, block cell cycle in G2 phase or in check cell to enter M-phase (Sudhakar *et al.,* 2001). Further, it may also be due to impairment of nucleoprotein synthesis or reduction in ATP level, which will affect chromosomal movement, microtubule assembly, and spindle elongation (Majewska *et al.,* 2003). The reduction in MI value in leaf extract of cytotoxic, may be used as an anticancer alternative and for suppression of cell proliferation in research.

Further, some clastogenic aberration, are also reported in this study (Table 4.7), which are either numerical or structural, where later arises due to the break/ exchange of a part of chromosomal material (Timothy *et al.,* 2014). Cytomixis, a phenomenon of chromatin transfer from one cell to another through intracellular cytomictic channels, other than plasmodesmata (Mursalimov *et al.,* 2013) have been reported (Figure). It is considered that cytomixis generally originated by physical factors (Narain, 1976), chemicals (Kravets, 2011; Sidorchuk *et al.,* 2007) or genetic factors (Baptista-Giacomelli *et al.,* 2000; Ghanima and Talat, 2003), or by genetically controlled stress factors (Haroun *et al.,* 2004). Further, cytomixis will result in the formation of micronuclei (Fig. 78), nuclear bridges that will connect adjacent cells directly, binucleate cell, and enucleated cells (Negron-Ortiz, 2007; Mursalimov and Deineko, 2011, Mursalimov *et al.,* 2013). Besides this, chromatin stickiness, which seems to be a result of mutated sticky (st) gene (Utsunomiya *et al.,* 2005; Risso Pascotto *et al.,* 2006, 2009; Pagliarini *et al.,* 2008), is also observed in few cells of(Fig. 95, Table. 4.7), that normally remains held together and results in another chromosomal aberration called as chromatin bridges (Figs.75, 84, 90, 94). However, these bridges, also form due to inoperative non-histone proteins and paracentric inversions (Gaulden, 1987) or unequal chromatid exchange during translocation (Khanna and Sharma, 2013) or even by the activity of telomeres (Jones, 2005). The

observed chromosomal anomalous behaviour may be due to the presence of phytochemicals, viz. as alkaloids, tannin, flavonols, polyphenols etc. in the these species (Prajitha and Thoppil, 2016), which have been reported in several plant plants earlier (Ene and Osuala, 1990; Yamanaka *et al.,* 1997; Hayakawa *et al.,* 1999; Perez-Carreon *et al.,* 2002), however, few species also show some anitmutagenic potential. This opposite effect, on the other hand, should be considered as therapeutic agent (Perez-Carreon *et al.,* 2002).

Cucurbits are widely consumed as food products by humans (Dhiman *et al.,* 2012), but genotoxic studies are lacking in the family. The present study indicated genotoxicity of aqueous, acetone, and methanol fruit extracts of Cucurbits, which is reported for the first time for *Cucumis* and *Momordica* spp. There remains a need for genotoxicological and risk assessment of possible effects that can be detrimental to humans. To reach a final conclusion on the cytotoxicity and genotoxicity potential of the extract, it has to be tested in different cytotoxicity and genotoxicity test systems, especially on animal and human cells (*in vitro* and *in vivo*).

5.6.Proximate analysis and mineral composition

5.6.1. Energy and Proximate Compositions

Plants play a significant role for providing energy and nutritional requirements to human beings. The carbohydrates, proteins, and fats are the nutrients found in plants play a vital role in creating a healthier organ control system in human beings (Rahmatollah and Mahbobeh, 2010). The experimental plants were characterized using various physicochemical parameters.

The edible part and proximate composition of all the species are shown in table 4.14. The major component in all the species the water (59–74 g/100 g fresh weight) and the results revealed that moisture content was maximum in *Cucumis melo* var. *agrestis* L. (Morphotype II) (74.45 ± 0.35) (Fig. 5.11, Table.4.14), but was relatively less as observed by Memon *et al.,* (2018) and was high in comparison to that as reported for *Durio zibethinus* (58–69 g 100 g^{-1} FW) (Charoenkiatkul *et al.,* 2016). The high moisture content indicates that the plant species is easily susceptible to spoilage by microbes (Kwenin *et al.,* 2011; Ooi *et al.,* 2012), if not well preserved (Omoregie and Osagie, 2011; Ozioma *et al.,* 2013) it provides greater activity to water soluble

enzymes and that are needed for metabolic activities of vegetables (Iheanacho and Ubebani, 2009; Arasaretnam *et al.*, 2018).

Nutrients and bioactive components are measured on a dry weight basis (dry matter, DM). Ash content of the sample gives the indirect measurement of minerals in food item (Hofman *et al.,* 2002), and it was found to be significantly high in *Momordica charantia* L. (16.07 ± 0.126) (Fig. 5.12, Table 4.14) among all the species and lie in accordance with previous studies where it was observed (17.93 % by DW) (Amina and Mohammed, 2018).

Crude fiber was maximum in case of *Cucumis melo* L. (6.67 ± 0.003) (Fig. 5.14, table 4.14) and was less in percentage as reported by (Oluwatoyin and Oluwaseun, 2014), which aids in digestion, absorption of water from the body. Fibre help in softening the stool to prevent constipation (Olowokudejo *et al.*, 2008), while crude fat content was considerably low, although it was maximum in case of *Momordica charantia* L. (5.8 ± 0.121) (Fig. 5.13, table 4.14), which can be easily incorporated in weight reducing diet and prevent arteriosclerosis and aging (Shaheen *et al.*, 2013). High protein content is required to for replace dead tissues and also provide energy and adequate amount of required to form amino acids (Emebu and Anyika, 2011) which was found to be maximum in *Momordica dioica* (Fig. 5.15, table 4.14). Moreover carbohydrates being essential nutrient produce energy to body, muscle, brain and blood (Emebu and Anyika, 2011; Ejelonu *et al.*, 2011) was maximum in *Cucumis melo* var. *agretsis* (Morphotype I) (Fig. 5.16, table 4.14). From all these values energy efficiency was calculated, which was found to be maximum in case of *Momordica dioica* L. (Fig. 5.17, table 4.14) but is was somewhat less as reported by previous workers (Aberoumand, 2011).

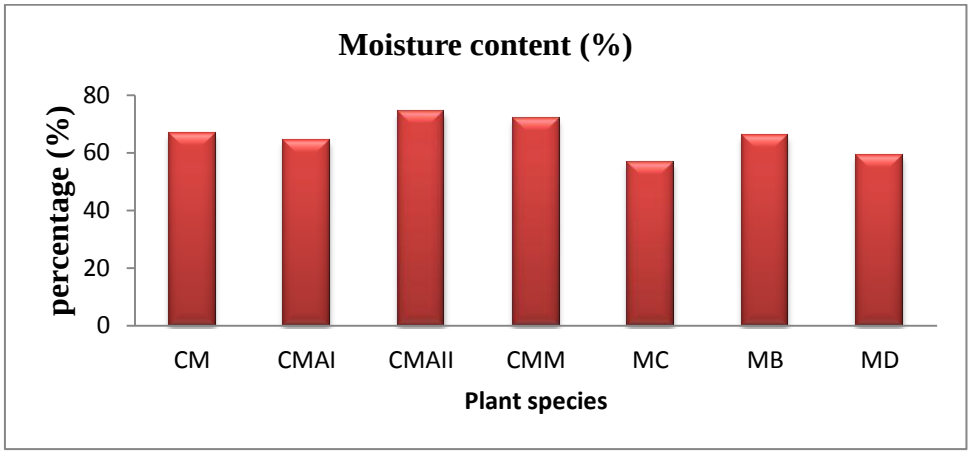


Fig 5. 11. Histogram showing Moisture content (%) in studied fruit samples.

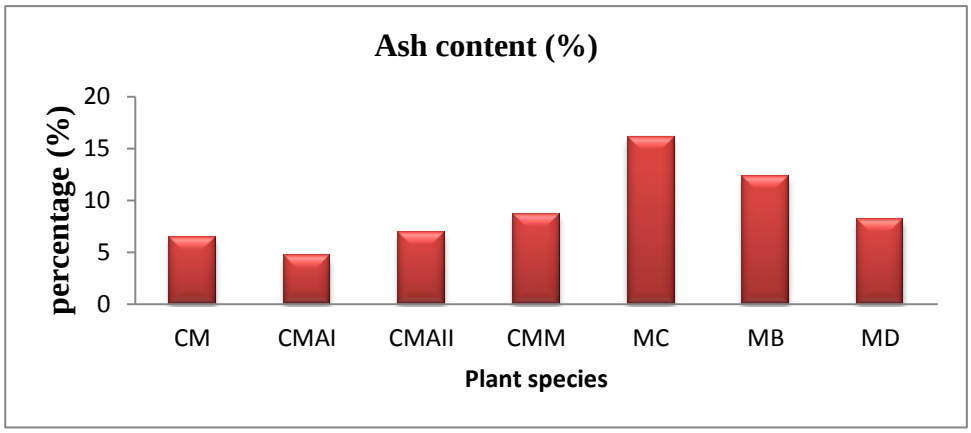


Fig 5.12. Histogram showing crude ash content (%) in studied fruit samples.

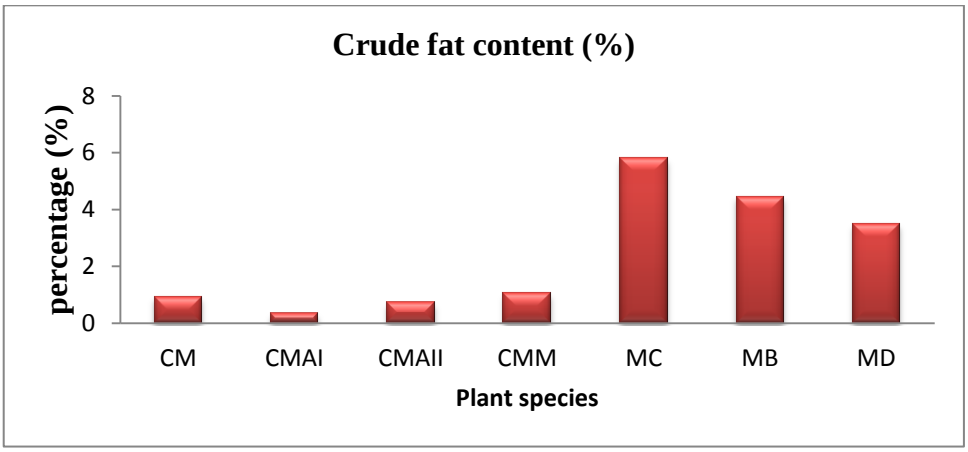


Fig 5.13. Histogram showing crude fat content (%) in studied fruit samples.

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L

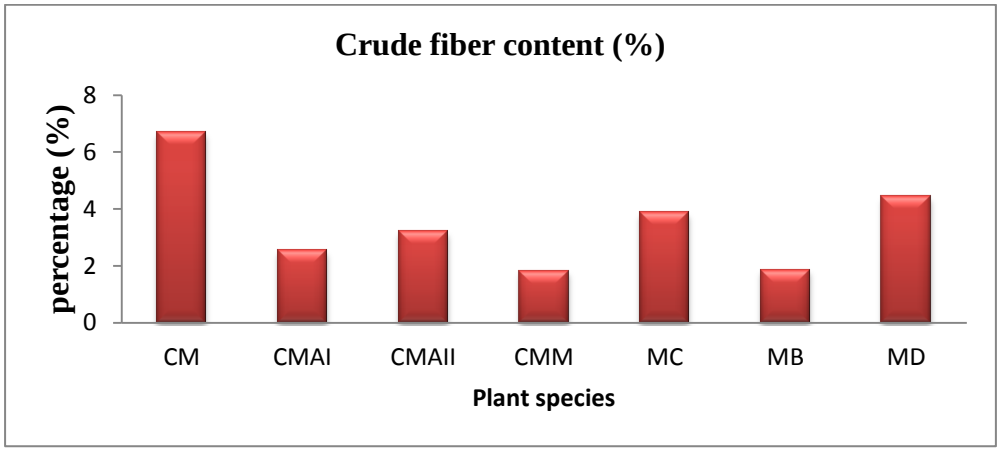


Fig 5.14. Histogram showing crude fiber content (%) in studied fruit samples.

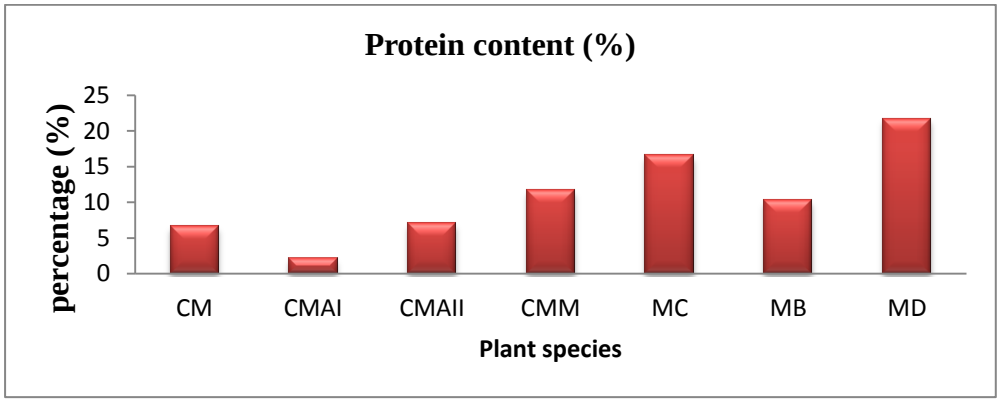


Fig 5.15. Histogram showing Protein content (%) in studied fruit samples.

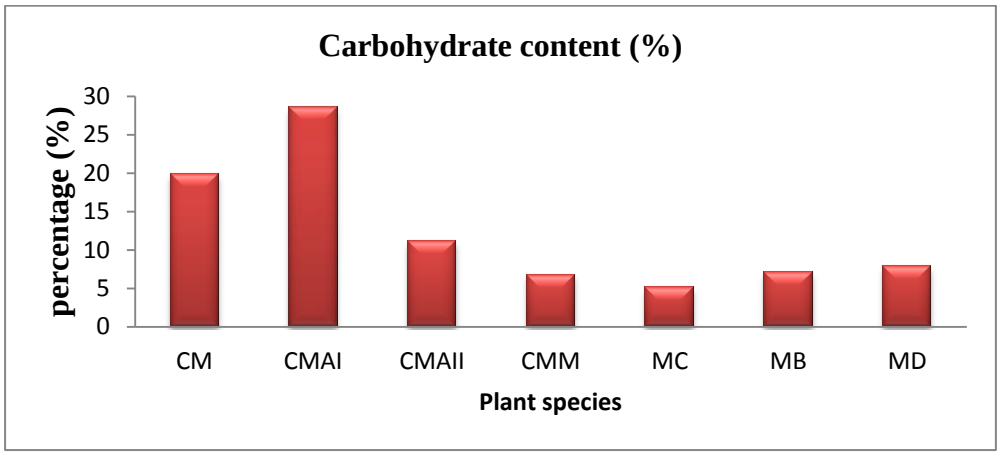


Fig 5.16. Histogram showing carbohydrate content (%) in studied fruit samples.

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L

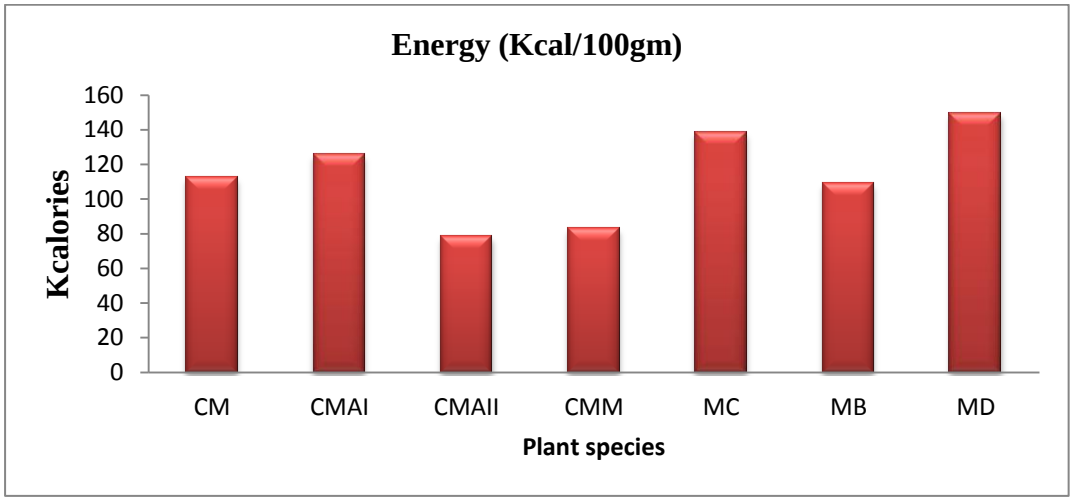


Fig 5. 17. Histogram showing energy content Kcal/100gm) in studied fruit samples.

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

5.6.2 Mineral content

The analysis for several micro- and macro-elements in the plants indicated that these were present in all plant samples which are responsible for curing different types of diseases. A variety of factors have been attributed to the increasing public interest in herbal remedies, some of which include the high cost and side effects of most modern medications (Ahmad, 2007). Biochemical processes in the human body are affected by macro and trace elements. The metabolism is influenced by active ingredients of medicinal plants, such as metabolic products of plant cells and a number of mineral elements (Dhyan *et al.,* 2005). Some minerals are still chelated with organic ligands, making them accessible to the body (Mohanta *et al.,* 2003).

5.6.2.1 Macro elements

The elements when present in large quantity (100 mg/g) are known as macronutrients/major elements. The content of macronutrients (Ca, Fe, K, Mg, Na) were determined. The mineral content of all the species is given in results shows a great deal of variation. Calcium content was in range of (32.18 ± 0.001 to 217.76 ± 0.001) (Fig. 5.18, table 4.14) and maximum in *Momordica dioica* L. 217.76 ± 0.001 and was found to be less as reported by earlier workers but was found to be greater than orange and apple, that contain 200 mg/100 g and 90 mg/100 g respectively (Mahapatra *et al.,* 2012). Calcium is required for vital functions of body including blood

coagulation, bone, teeth, cell integrity, muscular contractions, apoptosis, etc. (Pu *et al.*, 2016).

Iron, an important constituent is beneficial for pregnant and nursing women, convalescing and anaemic patients, oxygen carrier and DNA synthesis (Haimi and Lerner, 2014; Thomas and Krishnakumari, 2015; Gharibzahedi and Jafari, 2017) is found to be maximum in species *Momordica balsamina* (Fig. 5.20, table 4.14). Magnesium plays an important role in reactions involving phosphate transfer, structural stability of nucleic acids and absorption of electrolyes (Thomas and Krishnakumari, 2015). Our research depicted significant concentration of Magnesium with average value of 148.7 ± 0.001 (Fig. 5.21, table 4.14) and is much higher when compared with fruits like *Prsopsis cineria* and *Ficus glomeratai* but was similar to *I. gabonensis* and less than *Spondias mombin* (Olayiwola *et al.*, 2013; Ayessou *et al.*, 2014). Sodium is responsible to establish homeostasis in the body by maintaining electrolytic balance in the body in the body by cellular (Farquhar *et al.*, 2015). In the entire plant species low Na+ /K ratio was recorded to be low i.e. less than one (Figs. 5.24, 5.25, Table. 4.14), thus helps to reduces the risk of hypertension and other coronary heart diseases (Yang *et al.*, 2011; Weaver, 2013).

5.6.2.1 Micro elements

The elements when present in small amounts (>1 mg/g) referred as micronutrients or minor elements. The content of macronutrients (Cu, Mn, Zn) were determined. Manganese is required for normal functioning of the brain and nervous system by being a co-factor to many enzymes (Au *et al.*, 2008) and is found to be maximum in case of *Momordica balsamina* (Fig 5.22, table 4.14). Copper is essential for strengthening the immune system and as a co-factor for many enzymes to regulate their functioning and it deficiency leads to cardiac abnormalities and poor lipid metabolism (DiNicolantonio *et al.*, 2018) is recorded maximum in case of *Cucumis melo* var. *momordica* (fig. 5.19, table 4.14). Zinc is required for sexual maturation, foetal development, protein and nucleic acid synthesis (Roohani *et al.*, 2013). Zinc content was recorded maximum in case of *Cucumis melo* var. *agrestis* (morphotype I) (14.12 ± 0.001) and minimum in *Momordica charantia* L (3.07 ± 0.001), which was much higher when compared with *Carica papaya*, *Musa paradisiacal* and *Psidium guajava*. (Francisco Torrens Zaragoza, 2015).

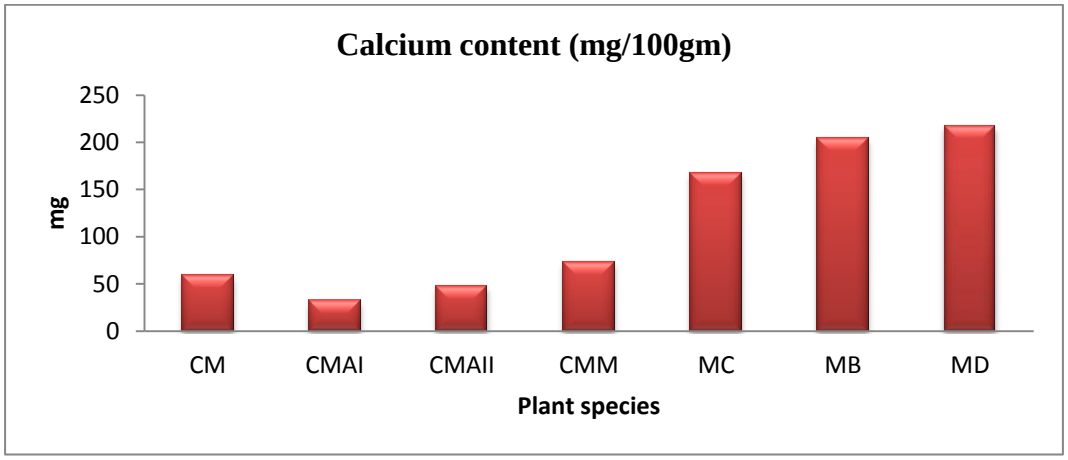


Fig 5.18 Histogram showing calcium content (mg/100gm) in studied fruit samples.

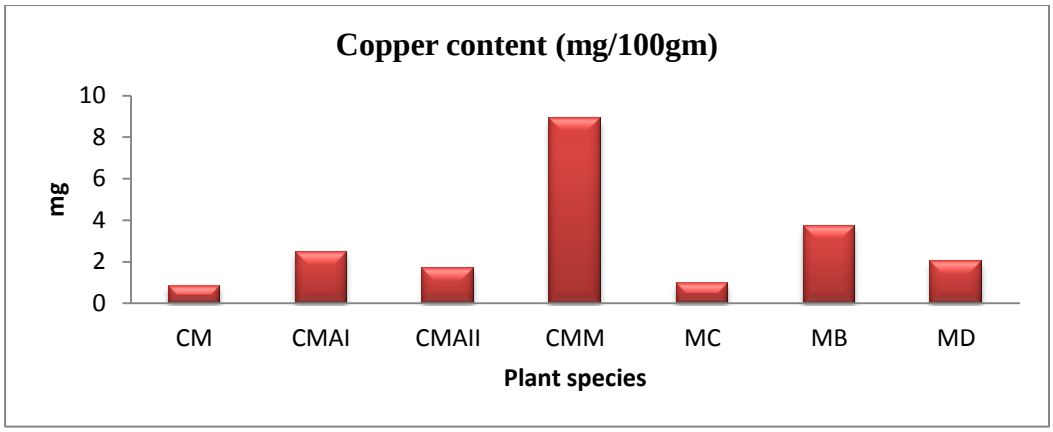


Fig 5.19. Histogram showing copper content (mg/100gm) in studied fruit samples.

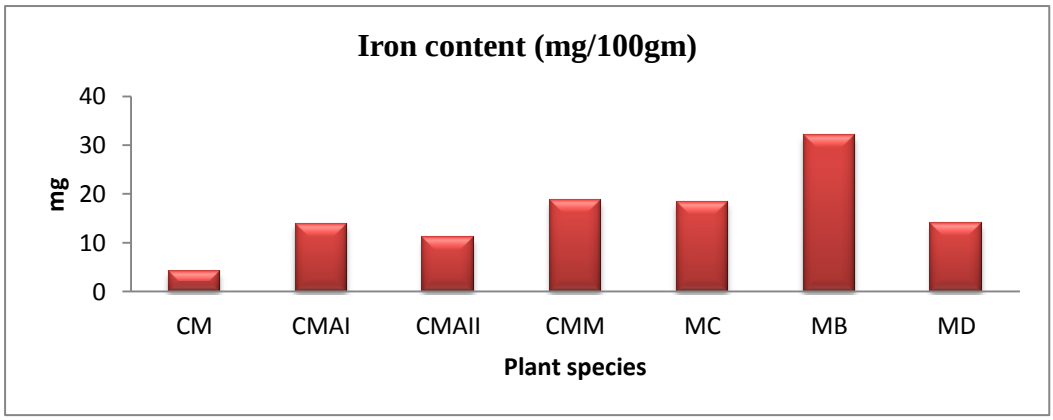


Fig 5.20. Histogram showing Iron content (mg/100gm) in studied fruit samples.

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

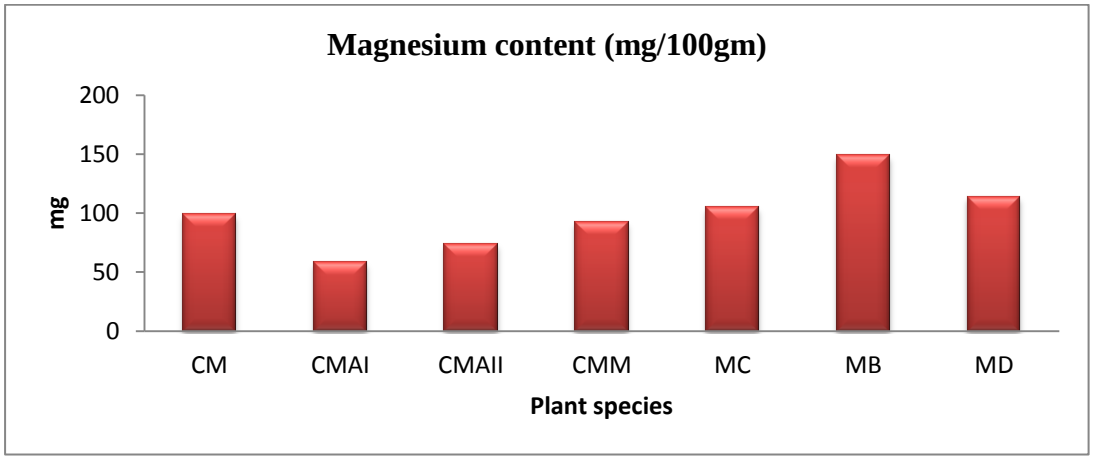


Fig 5.21. Histogram showing Magnesium content (mg/100gm) in studied fruit samples.

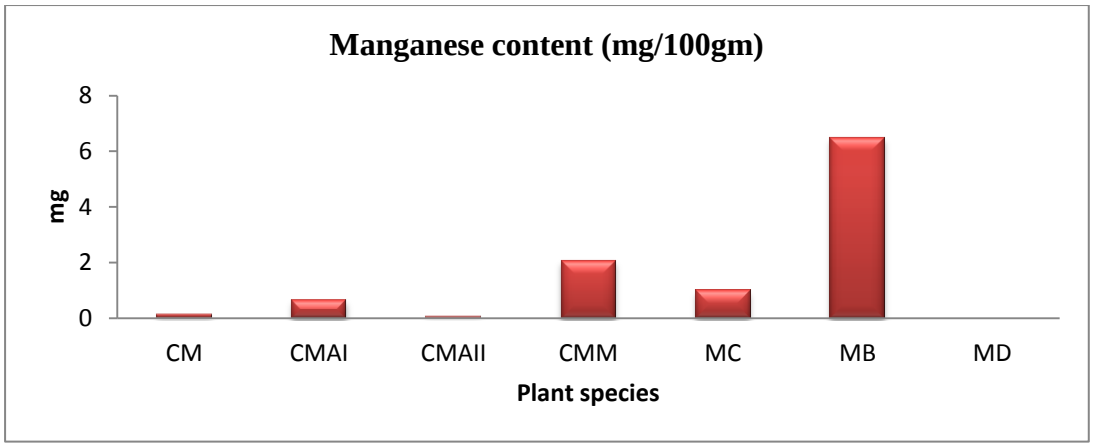


Fig 5.22. Histogram showing Mangenese content (mg/100gm) in studied fruit samples.

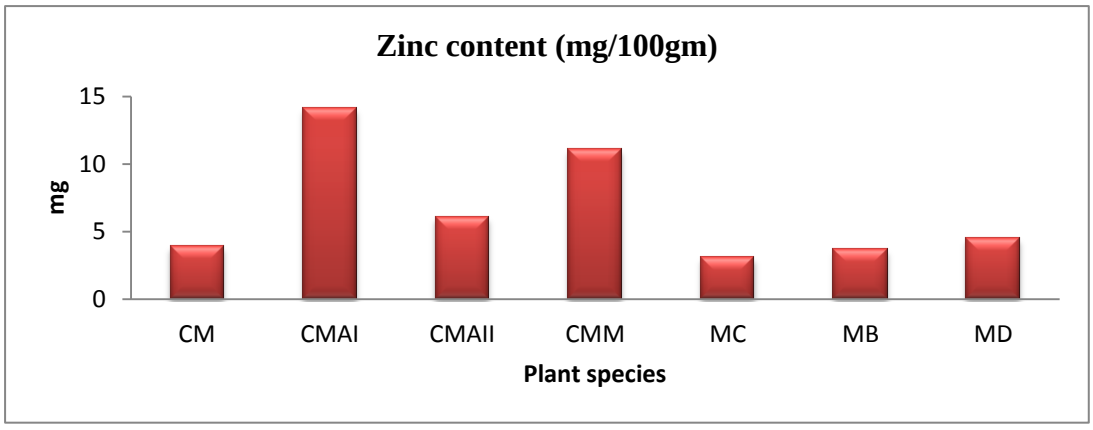


Fig 5.23. Histogram showing Zinc content (mg/100gm) in studied fruit samples.

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

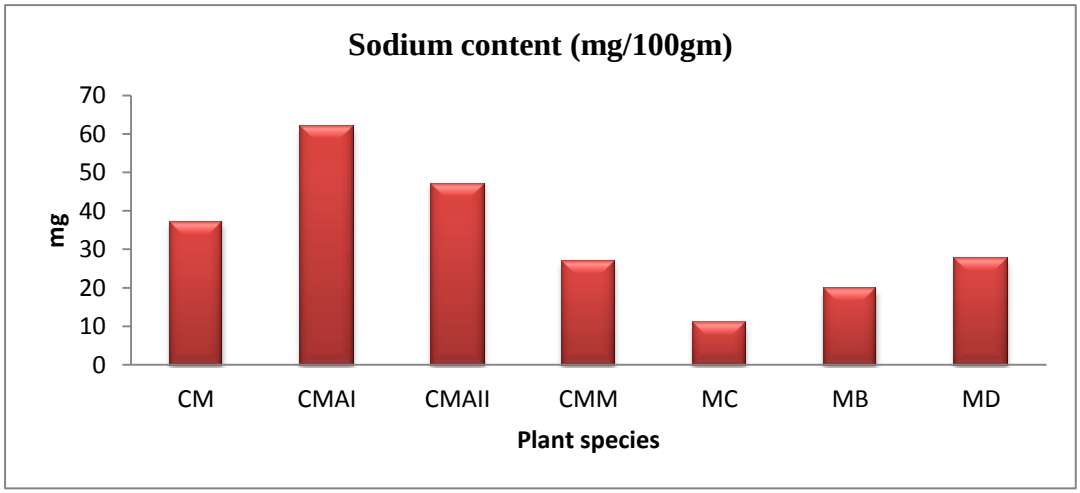


Fig 5.24. Histogram showing Sodium content (mg/100gm) in studied fruit samples.

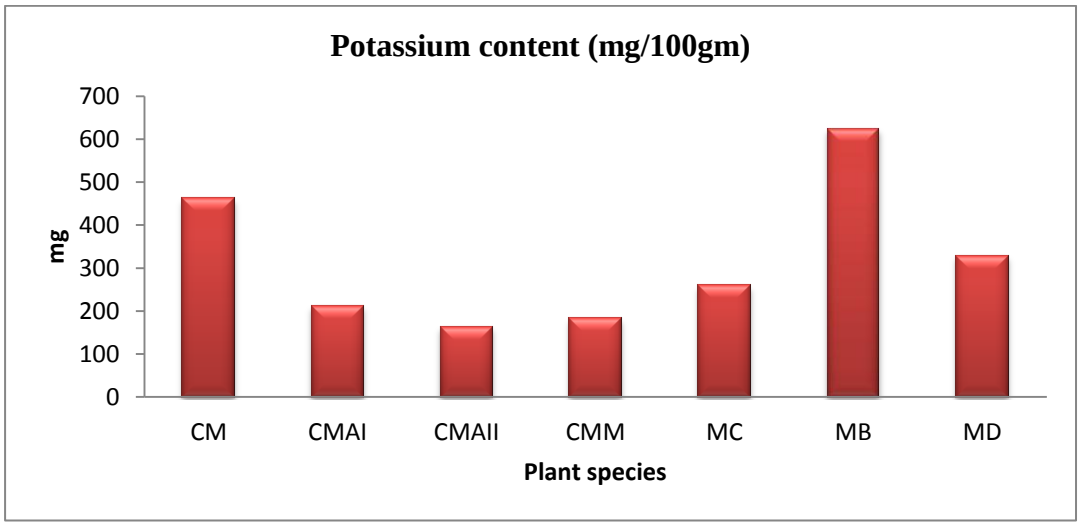


Fig 5.25. Histogram showing potassium content content (mg/100gm) in studied fruit samples.

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

5.6.3. Antinutritional factors

Antinutrients in foods interfere with the absorption of some minerals and other micronutrients in the digestive system which may have a negative impact on the functioning of certain organs (Gemede and Ratta, 2014).

Out of all anti nutritional components alkaloids was maximum in case of *Cucumis melo var. agrestis* (Morphotype II), being 37.276 % (Fig 5.26, table 4.14).

These are naturally occurring chemical compounds having basic nitrogen atoms and are active components of medicinal plants. However, high percentage of alkaloid is toxic to human and animals (Matsuura and Fett-Neto, 2017) and it is is within the range of alkaloids content that is perceived to have more pharmacological effect rather than toxicity (Makkar *et al.,* 2007).

On the other hand oxalate was maximum in case of *Cucumis melo var. momordica* L. i.e. 3.08% (Fig. 5.27, table 4.14) which affects the human body by forming a strong chelate with dietary calcium and other minerals thereby rendering such nutrients unavailable for absorption and assimilation (Jiru and Urga, 1995). This insoluble calcium oxalate in the crystal form is stored in the kidney causing serious health-related problems called kidney stone (Natesh *et al.,* 2017). Therefore, diets high in oxalic acid need supplementation of minerals to avoid deficiency (Uusiku *et al.,* 2010). This insoluble calcium oxalate in the crystal form is stored in the kidney causing serious health-related problems called kidney stone (Natesh *et al.,* 2017).

Saponins at high concentrations can affect nutrient absorption by inhibition of metabolic and digestive enzymes as well as binding with nutrients such as zinc Saponins have properties of precipitating and coagulating red blood cells and they also have cholesterol binding properties, formation of foams in aqueous solutions and hemolytic activity (Sodipo *et al.,* 2000). Various studies have shown that saponins although non toxic can generate adverse physiological responses in animals that consume them. They exhibit cytoxic effects and growth inhibitions against a variety of cells, making them have anti-inflammatory and anticancer properties. They also show tumour inhibiting activities on animals (Akindahunsi and Salawu, 2005). When saponin in a diet is less than 10%, it is believed to be harmless to the body (Hortwitz, 2003) and are recorded maximum in case of *Momordica charantia* L. i.e. 5.32 % (Fig. 5.28, table 4.14).

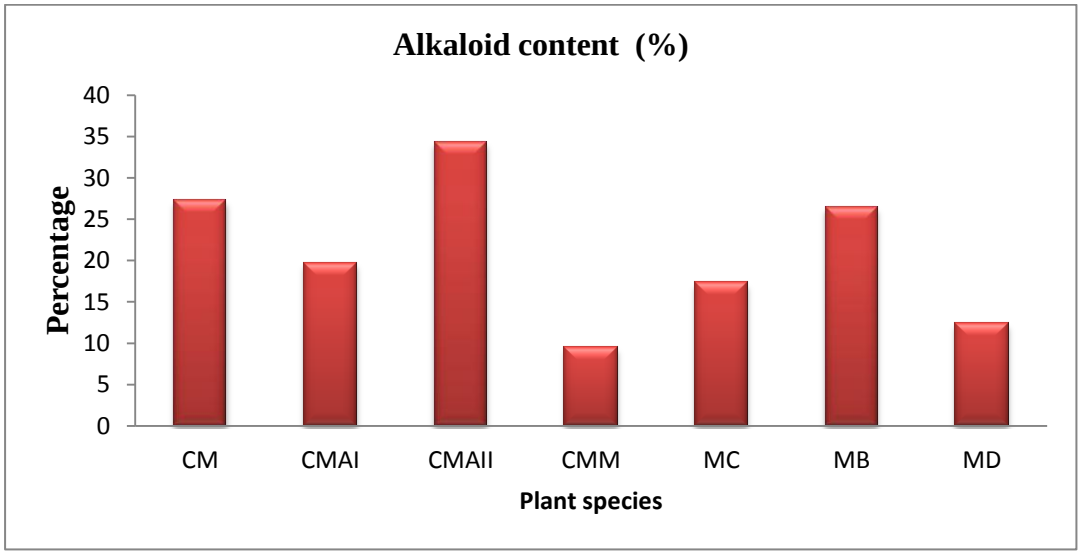


Fig 5.26. Histogram showing percentage of alkaloid content in studied fruit samples.

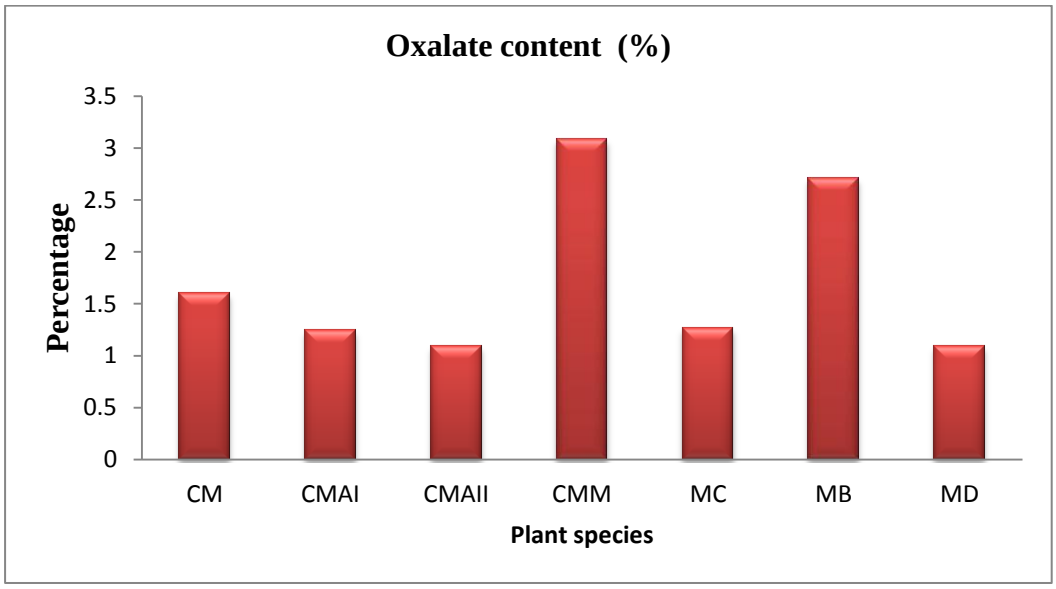


Fig 5.27. Histogram showing percentage of oxalate content in studied fruit samples.

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

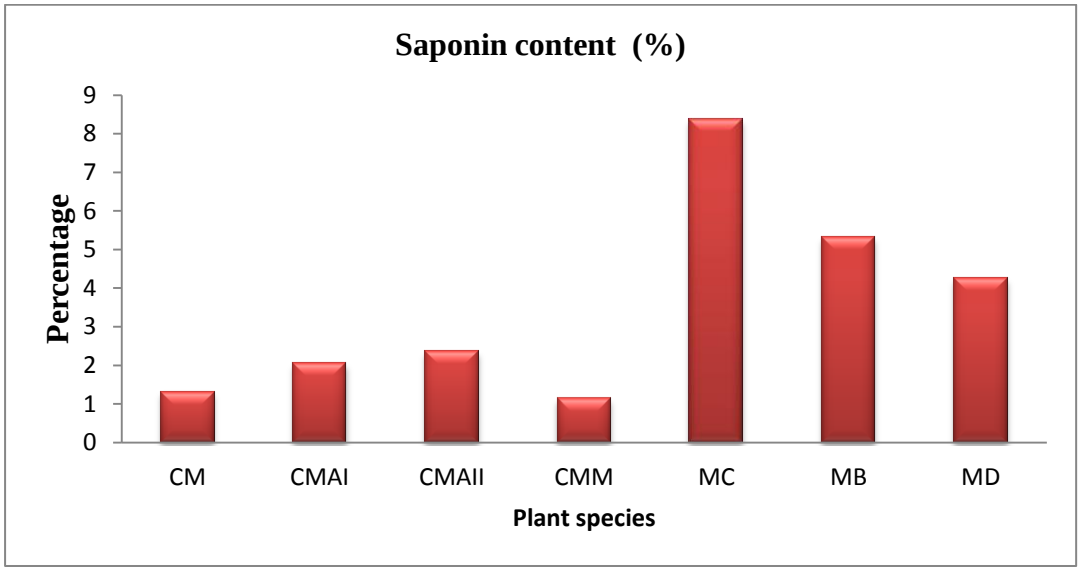


Fig 5.28 Histogram showing percentage of Saponin content in studied fruit samples.

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

The study revealed that the analyzed samples are the significant sources of K, Ca, Mg, Cu, Fe, Na, Mn and Zn. The content of potentially toxic elements are present below the provisional tolerable intake values. Hence, the analyzed plants could also be potential sources of mineral elements. This research further concludes that the analyzed samples are effective nutritional source.

5.7.HPLC Analysis

Phytochemical analysis:

The plants produce a wide range of secondary metabolites which are used frequently by humans for medicinal purposes, especially in drugs. Such drugs are very common these days because of low cost and reduced adverse effects. A large number of plant species have been explored in search of these secondary metabolites with the help of modern biotechnological and phytochemical techniques. But, nowadays, these secondary metabolites are explored even in intraspecific levels, on cytomorphovariation and population basis. In the present study, afterwards the detailed cytomrphological study, all the species are selected for the phytochemical screening against 5 different standard compounds. These standards fall under 3 main

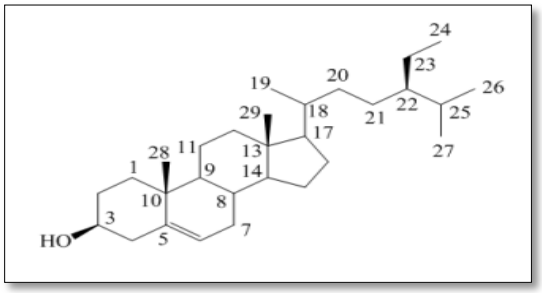
categories i.e. phytosteroles, flavanoids and phenolcarboxylic acid which have been discussed below.

5.7.1. Phytosterols

These are sterols of plants frequently used in human diet and known to reduce cholesterol absorption in guts. These phytosterols are also reported with anti-cancerous properties (Awad *et al.,* 2000). In the present study, only a single representative of this category of secondary metabolite has been screened in all species of genera, *Cucumis* L. and *Momordica* L. i.e. β-sitosterol.

5.7.1.1.β-sitosterol:

β-sitosterol, most ubiquitous phytosterols in the plant kingdom, is known to possess a wide array of biological activities and is reported in numerous plants including rice, wheat, corn, nut, peanut, etc.



***Structure of β-sitosterol (Schneider *et al.*, 2009)**

It is recorded with some health beneficiary effects, such as antioxidant and antipyretic, anti-inflammatory (Gupta *et al.*, 1980), benign prostatic hypertrophy, breast cancer (Awad *et al.*, 2001), colon cancer (Awad *et al.,* 1998), inflammatory disorders, immune-modulatory, hepato-protective and for rheumatoid arthritis (Bouic *et al.,* 1996). The compound is earlier reported in many plants, such as *Vanda roxburghii* (Usman *et al.,* 2012), *Withania coagulans* (Rashid *et al.,* 2012), etc. Hence there high medicinal values make them a practical subject of research interest.

The amount of compound in DWE was observed to be maximum in *Cucumis melo* (16.011±0.841 µg/mg), followed by *Momordica balsamina* (12.629±0.240 µg/mg), while it was found to be least in *Cucumis melo* var. *agrestis* (Morphotype I) (6.500±0.002 µg/mg) (Fig. 5.29, table 5.1).

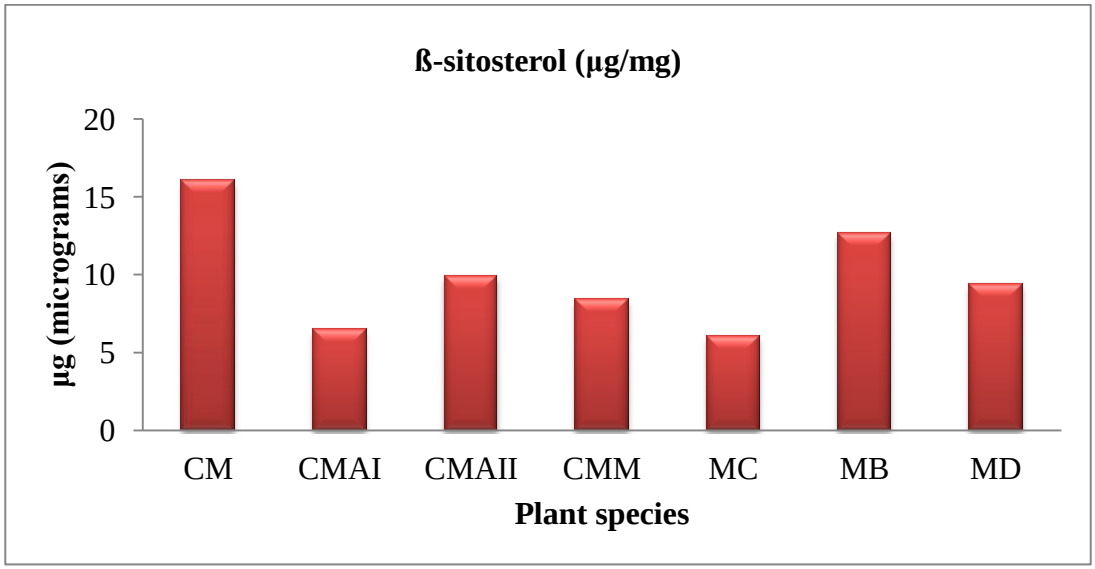


Fig. 5.29 Histogram showing the amount of β-sitosterol in fruit extracts of different plant species

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

5.7.2 Flavonoids

Flavonoids are the most diverse and widespread group of natural phenolic compounds. Hydroxyl position in the flavonoid molecule determines antioxidant properties and it depends on the ability to donate hydrogen or electron to a free radical (Liaudanskas *et al.*, 2014). In the present phytochemical study, two flavanoid, namely Rutin and Quercitin have been screened from all the selected plant species.

5.7.2.1. Rutin

Rutin is a flavonol, abundantly found in plants, such as passion flower, buckwheat, tea, and apple. It is a vital nutritional component of food stuff (Harborne, 1986). Rutin, also called as rutoside, quercetin-3-rutinoside, and sophorin is a citrus flavonoid glycoside found in buckwheat (Kreft *et al.*, 1997). The name 'rutin' comes from the plant *Ruta graveolens*, which also contains rutin. Chemically it is a glycoside comprising of flavonolic aglycone quercetin along with disaccharide rutinose. It has demonstrated a number of pharmacological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective activities (Javed *et al.*, 2012; Richetti *et al.*, 2011; Nassiri-Asl *et al.*, 2010; Mellou *et al.*, 2006).

The amount of compound in DWE was observed to be maximum in *Momordica dioica* (34.19±0.004 µg/mg), followed by *Cucumis melo* (12.314±0.030µg/mg), while it was found to be least in *Cucumis melo* var. *momordica* (1.734±0.017 µg/mg) (Fig. 5.30, table 5.1).

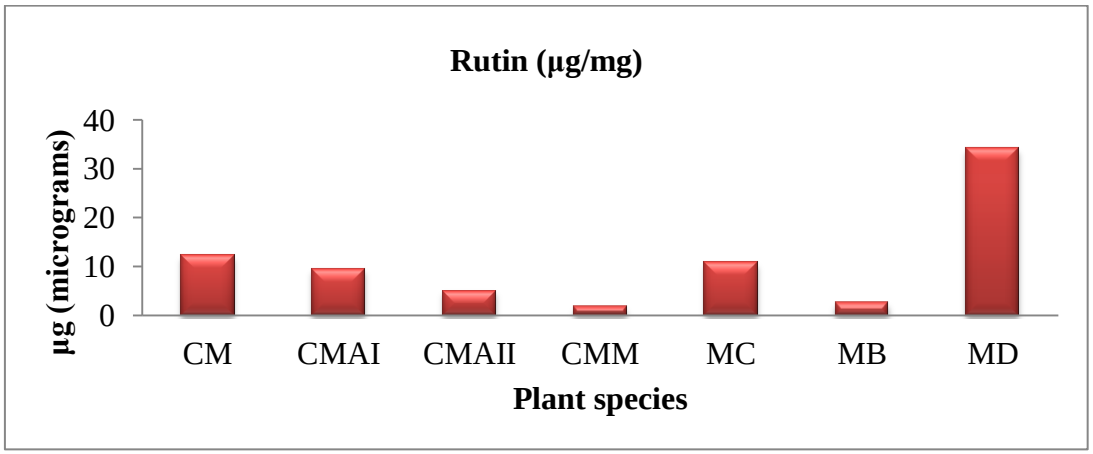


Fig. 5.30 Histogram showing the amount of Rutin in fruit extracts of different plant species

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

5.7.2.2.Quercetin

Quercetin is one of the important bioflavonoids present in plants and which is known for its anti-inflammatory, antihypertensive, vasodilator effects, antiobesity, antihypercholesterolemic and antiatherosclerotic activities (Lakhanpal and Rai,2007; Sultana and Anwar,2008; Salvamani *et al.*, 2014). It has potential protection against coronary heart disease (Pace-asciak *et al.*, 1996) and hypertension (Larson *et al.*, 2010). These health claims are merely supported by in vitro evidence related to the cytoprotective ability of quercetin to modulate the activity of numerous enzymes involved in energy metabolism (An *et al.*, 2010), signal transduction, cell growth (Nakamura *et al.*, 2011) and antioxidant activity (Zhang *et al.*, 2011b). Quercetin is an emerging prospective anticancer drug candidate and its prodrug QC12 has entered phase-I clinical studies (Hirpara *et al.*, 2009). Quercetin showed neuronal cell protective effect against glutamate-induced neurotoxicity (Yang *et al.*, 2012), and recovered the mitotic index and chromosomal instability after treatment with hydrogen peroxide (Boligon *et al.*, 2012).

The amount of compound in DWE was observed to be maximum in *Cucumis melo* (2.794±0.014 µg/mg), followed by *Cucumis melo* var. agrestis (Morphotype I) (2.424±0.025 µg/mg), while it was found to be least in *Momordica balsamina* (0.268±0.008 µg/mg) (Fig. 5.31, table 5.1).

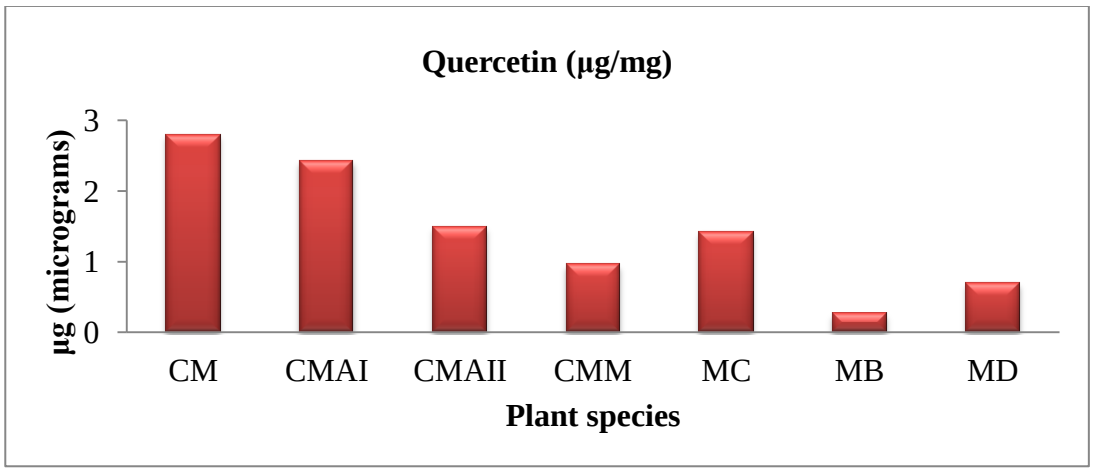


Fig. 5.31 Histogram showing the amount of quercetin in fruit extracts of different plant species.

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

Phenol carboxylic acids

These are found in the variety of plant-based foods *viz*. seeds, skins of fruits and leaves of vegetables contain them in highest concentrations. Typically, they are present in bound from such as amides, esters, or glycosides and rarely in free form (Pereira *et al.,* 2009). Phenolic acids possess much higher *in vitro* antioxidant activity than well known antioxidant vitamins (Tsao and Deng, 2004). There are numerous epidemiological and experimental evidences present describing the protective role of phenolic acids in degenerative diseases such as cardiovascular, cancer, diabetes, inflammation and many more (Scalbert *et al.,* 2005; Kumar *et al.,* 2015; Kumar *et al.,* 2016; Kumar *et al.,* 2019). In the present phytochemical study, one phenol carboxylic acid, namely cinnamic acid have been screened from all the selected plant species.

5.7.3.1 Cinnamic acid

Cinnamic acid, a natural aromatic carboxylic acid, is a key chemical found in plants such as Cinnamomum cassia and Panax ginseng, fruits, whole grains, vegetables and honey (Chandra *et al.,* 2019). The previous studies have reported that cinnamic acid exhibit antioxidant, antimicrobial, anticancer, neuroprotective, anti-

inflammatory and antidiabetic properties (Gibel *et al.*, 2019; Wang *et al.*, 2019; Guo *et al.*, 2019).

The amount of compound in DWE was observed to be maximum in *Momordica charantia* (0.023±0.0 µg/mg), followed by *Momordica dioica* (0.020±0.0 µg/mg), while it was found to be least in *Cucumis melo* var. *agrestis* (Morphotype II) (0.0003±0.0 µg/mg). It is absent from two species i.e. *Cucumis melo* var. *agrestis* (Morphotype I) and *Cucumis melo* var. *momordica* (Fig. 5.32, table 5.1)

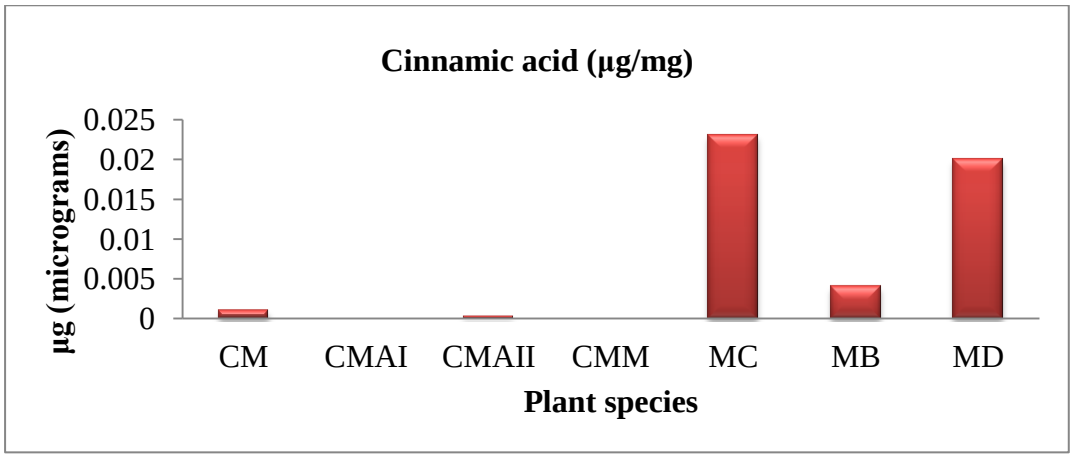


Fig. 5.32 Histogram showing the amount of Cinnamic acid in fruit extracts of different plant species.

CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

Table. 5.2 Comparison of compounds in all the selected species of genus *Cucumis* L. and *Momordica* L.

Standard compound	**CM**	**CMAI**	**CMAII**	**CMM**	**MC**	**MB**	**MD**
β-sitosterol.	16.011±0.841	6.500±0.002	9.898±0.003	8.414±0.003	6.057±0.0.003	12.629±0.240	9.407±0.003
Rutin	12.314±0.030	9.494±0.028	4.991±0.016	1.734±0.017	10.78±0.058	2.519±0.003	34.19±0.004
Quercitin	2.794±0.014	2.424±0.025	1.492±0.022	0.952±0.006	1.422±0.027	0.268±0.008	0.699±0.011
Cinnamic acid	.001±0.0	0.0	0.0003±0.0	0.0	0.023±0.0	0.004±0.0	0.020±0.0

Abb. CM- *Cucumis melo* L. , CMAI- *Cucumis melo* var. *agrestis* (Morphotype I), CMAII- *Cucumis melo* var. *agrestis* (Morphotype II), CMM- *Cucumis melo* var. *momordica* L., MC- *Momordica charantia* L., MB- *Momordica balsamina* L. , MD- *Momordica dioica* L.

Among all the four compounds analyzed, β-sitosterol and rutin was detected maximum in all the species (table 5.1). (Figs. 5.29 to 5.32) shows representative data obtained in the HPLC analysis of β-sitosterol, rutin, quercetin and cinnamic acid in the fruit extracts of the genera *Cucumis* L. and *Momordica* L. Among all the species, the fruit extract of *Momordica dioica* had the highest concentration of rutin, *Cucumis melo* L. have high concentration of β-sitosterol. Other two compounds namely quercetin and cinnamic acid were found in less amount in all the species.

SUMMARY

Cucumis L. and *Momordica* L. selected for present study belongs to family Cucurbitaceae. The plants are found primarily in temperate zone throughout the world and can be seen growing in subtropical regions during summer.

North Indian germplasm of the species was investigated to know the cytological diversity within the species and also efforts were made to evaluate the genotoxic, antimicrobial potential, antioxidant potential, proximate and mineral analysis and phytochemical analysis of the plants. Populations of *Cucumis* L. and *Momordica* L., growing in different locations in various districts of Punjab, Haryana, Himachal and Rajasthan were collected for investigation.

CYTOLOGICAL ANALYSIS

Meiotic analysis of different populations of *Cucumis* L. and *Momordica* L. growing at different areas was carried out and following points of interest imaged.

1. Plants belonging to populations of *M.charantia*, *M.dioica* and *Momordica balsamina* L. from various populations shows 2n=22, 2n=28, 2n=22 respectively. The present count of chromosome number of 2n=22 is in line with the earlier records for the species. A perusal of literature revealed the existence of 2n=22, 2n=28, 2n=22 in the species. In *Cucumis* L the 2n=24. Meiosis, by and large, was normal with the equal distribution of chromosomes at both the poles in anaphase-I. All the populations show the presence of bridges, Laggards and vagrants chromosomes in PMCs. Among the analyzed populations 3.79% -22.07% PMCs showed the phenomenon of cytomixis.
2. The abnormalities in chromosome segregation and cytomixis leads to the development of microspores. Micronuclei at tetrad stage were also observed. Besides tetrads the presence of diads, triads and polyads was also reported.
3. Heterogeneity in pollen size was also recorded with variation in pollen size range from 23.82±0.29X22.82±0.62 to16.81±0.48X16.44±0.69, with majority of pollen grains of medium size.
4. Pollen fertility ranges from 74.39±0.49 to 98.93±1.28. The small sized pollens grains were sterile and contribute to pollen infertility.

MORPHOLOGICAL STUDIES

Various morphological parameters like plant height, leaf parameters were evaluated during the study. The following points were noted during the study:

1. In case of *Cucumis melo* leaf parameter including average length and breadth varies between 13.29± 0.92 cm and 16.73± 0.63 cm respectively. Average internode length ranges from 12.32± 0.03 cm in various populations on geographical basis. Fruits are smooth have average length and diameter 22.03± 0.034 cm and 41.052± 0.71 cm respectively.
2. In *Cucumis melo* var. *agrestis* two morphotypes had been observed in field. In morphotype I average petiole size is 9.20±0.73 cm. The average size of leaf length, leaf breadth varied between 5.56±0.31 cm and 15.67±0.31 cm respectively and average internodes length ranges from 4.06±0.46 cm. The average fruit length is 5.3±0.37 and 7.8±0.65 respectively.
3. In morphotype II there is noticeable large size of fruit with small sized leaves and leaf parameter including length and breadth ranges from 6.62±0.52 cm and 6.77±0.43 cm respectively. Average petiole size is 2.26±0.37 cm. Fruits are large as compared to other morphotypes ranging from 9.1±0.43 cm in length and 8.36±0.51cm in diameter.
4. In case of *Cucumis melo* var. *momordica* leaf parameter including average length and breadth varies between 9.0±0.00 cm and 12.50±0.408 cm respectively. Average internode length ranges from 8.5±0.707 cm in various population on geographical basis. Fruits are smooth have average length and diameter 27.0±1.0 cm and 32.5±0.5 cm respectively .
5. In case of *Momordica charantia* leaf parameter including average length and breadth varies between 5.16±0.53 cm and 6.63±0.36 cm respectively. Average internode length is 5.63±0.32 cm in various populations on geographical basis. Fruits are rough and wrinked have average length and diameter 16.70±0.61 and 12.45±0.56 cm respectively.
6. In *Momordica balsamina* petiole length varies between .82 cm -.52 cm. Average length and breadth varies between 2.48±0.41cm and 3.58±0.42 cm respectively. Average internode length ranges from 4.12±0.51 cm in various populations on

geographical basis. Fruits are rough and wrinked have average length and diameter 4.88±0.38 cm and 7.60±0.52 cm respectively.

7. In *Momordica balsamina* petiole length is 2.6±0.38. Average length and breadth of leaf varies between 6.8±0.27 cm and 7.0±0.56 cm respectively. Average internode length is 5.2±0.45 cm in various populations on geographical basis. Fruits are rough and with spines have average length and diameter 4.56±0.801 cm and 7.8±1.21cm (Figs. 26-30).

GENOTOXICITY EVALUATION

For monitoring the genotoxic potential potentialities of the *Cucumis* L. *and Momordica* L., *Allium* assay was performed. Three types of extracts of leaves i.e. aqueous, ethanol and acetone were used for genotoxicity monitoring. Healthy bulbils of *Allium sativum* with young actively growing roots were placed for 24 h on test tubes containing different extracts. The cytological data of these roots was recorded. The roots raised over Sodium azide as positive control and tap water served as negative control.

Salient features of our observation are:

1. Among the different fruit extracts, the least mitotic index (46.20±0.23%) was observed in methanolic extract of *Cucumis melo* var. *momordica*, while the maximum value (58.73±0.18) was recorded in aqueous extract of *Cucumis melo.*
2. The maximum percentage of abnormal cells were recorded in methanol extract of *Cucumis melo* var. *momordica.*
3. The maximum number of cells with chromatin bridges, laggards was reported in methanol extract of *Cucumis melo* var. *agrestis* (morphotypes I & II).
4. Cytological aberrations were of very low amount, which is insignificant.

ANTIMICROBIAL ACTIVITY

The antimicrobial action of fruit extracts of crude methanolic, acetone and aqueous extracts of 6 species of genra *Cucumis* L. and *Momordica* L. against *Staphylococcus aureus*, *Pseudomonas flourescens*, *Bacillus coagulans* and *Klebsiella pneumoniae*. Fruits collected from plants of all the different populations of

Cucumis L. and *Momordica* L. were used for the preparation of extracts and the assay. The results were

1. Two types of extracts of the fruits i.e. acetone extract, aqueous extract of all plants populations showed no antimicrobial activity against any of the test organism. No inhibition zone was formed.
2. The maximum inhibitory zone of 32.3 ± 0.57 mm was reported against *Staphylococcus aureus* in *Cucumis melo* L. and 23.3 ± 0.57 mm was found against *Bacillus coagulans* in *Cucumis melo* var. *agrestis* (morphotype II) extract at 3000 ppm in methanol extract.

ANTIOXIDANT ACTIVITY

1. The variation in yield and colour range (5.21 to 24.34 g/100g) was observed among the selected plant samples. The yield of aqueous and methanol extract was found to maximum in case of *Momordica charantia* L.
2. The total phenolic content vary significantly ($p<0.05$) in both of the genera and it ranges from 81.08±05 to 23.59±0.06. The flavanoid content in methanol and aqueous extract varied from 73.03 ± 0.21 to 35.43 ± 0.25 and 50.23 ± 0.15 to 27.5 ± 0.26 respectively and it differ among all the plant species in their respective extracts.
3. IC_{50} of methanol extract of *Cucumis melo* L. value is 43.387 µg/ml (DPPH scavenging assay) so it comes under category of very strong antioxidant.

PROXIMATE AND MINERAL ANALYSIS

1. Both the genera were compared for their nutrients and mineral composition. The results revealed the variation of carbohydrates, fibre content, ash content, macronutrient and micronutrients and it fulfills the human's nutritional requirements and provides ethnomedicinal benefits to human health.

HPLC ANALYSIS

By observing the chromatograms of *Cucumis* L. and *Momordica* L. it was observed that amount of β-sitosterol compound in DWE were observed maximum in *Cucumis melo* L. (16.011±0.841 µg/mg). Amount of rutin compound in DWE were observed maximum in *Momordica dioica* L. (34.19±0.004 µg/mg) Amount

of Quercetin compound in DWE were observed maximum in *Cucumis melo* L. (2.794±0.014 µg/mg) Amount of cinnamic acid compound in DWE were observed maximum in *Momordica balsamina* L. (0.023±0.001 µg/mg).

CONCLUSION

1. The comparative analysis in wild and cultivated cucurbits reveals significant difference in the morphology and cytology. Such studies are beneficial for solve taxonomic enigma(s). The generated signifcant variations in fruit production between provenances is an indication that superior provenances of *Cucumis melo* var. *agrestis* (Morphotype II) can be selected for domestication, breeding improvement, and commercialization purposes.
2. Genotoxic studies reveal significant results, which needs to be studies in animal models.
3. Further, these cucurbits also differ in bacterial inhibition, scavenging activity, secondary metabolites and nutrient composition. The plants are wild in occurrence and are easily available. These plants may be studied pharmacologically to generate low cost generic medicine for human healthcare.

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Fig: 1. Plant of *Cucumis melo* L.
Fig: 2. Fruit of *Cucumis melo* L.
Fig: 3. Leaf of *Cucumis melo* L.
Fig: 4. Flower of *Cucumis melo* L.

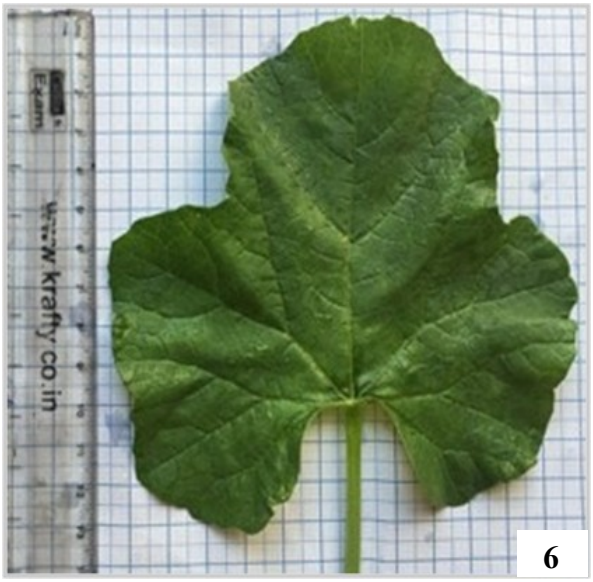


Fig: 5. Plant of *Cucumis melo* var. *agrestis* L. (Morphotype I)
Fig: 6. Leaf of *Cucumis melo* var. *agrestis* L. (Morphotype I)
Fig: 7. Leaf of *Cucumis melo* var. *agrestis* L. (Morphotype I)
Fig: 8. Fruits of *Cucumis melo* var. *agrestis* L. (Morphotype I)

Fig: 9. Plant of *Cucumis melo* var. *agrestis* L. (Morphotype II).
Fig: 10. Leaf of *Cucumis melo* var. *agrestis* L. (Morphotype II).
Fig: 11. Fruits of *Cucumis melo* var. *agrestis* L. (Morphotype II).
Fig: 12. Flower of *Cucumis melo* var. *agrestis* L. (Morphotype II).

Fig: 13. Plant of *Cucumis melo* var. *momordica* L.
Fig: 14. Fruit of *Cucumis melo* var. *momordica* L.
Fig: 15. Leaf of *Cucumis melo* var. *momordica* L.
Fig: 16. Flower of *Cucumis melo* var. *momordica* L.

Fig: 17. Plant of *Momordica charantia* L.
Fig: 18. Fruit of *Momordica charantia* L.
Fig: 19. Split fruit of *Momordica charantia* L.
Fig: 20. Leaf of *Momordica charantia* L.
Fig: 21. Flower of *Momordica charantia* L

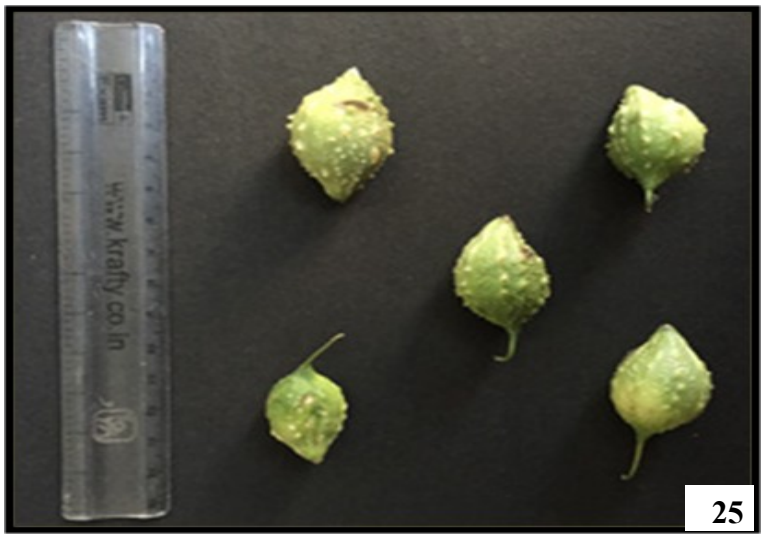


Fig: 22. Plant of *Momordica balsamina* L.
Fig: 23. Leaf of *Momordica balsamina* L.
Fig: 24. Flower of *Momordica balsamina* L.
Fig: 25. Fruits of *Momordica balsamina* L.

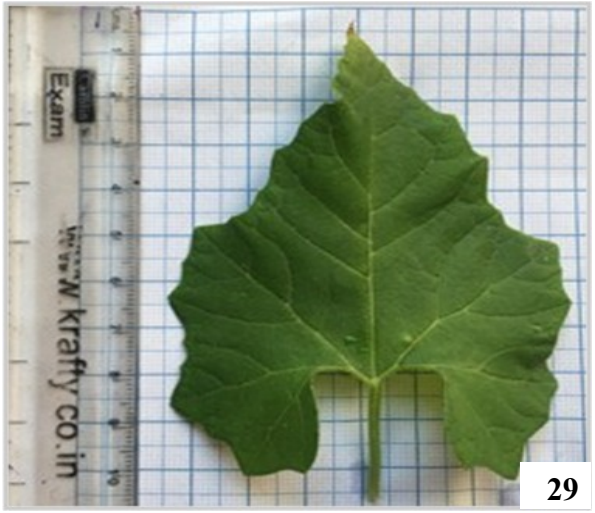
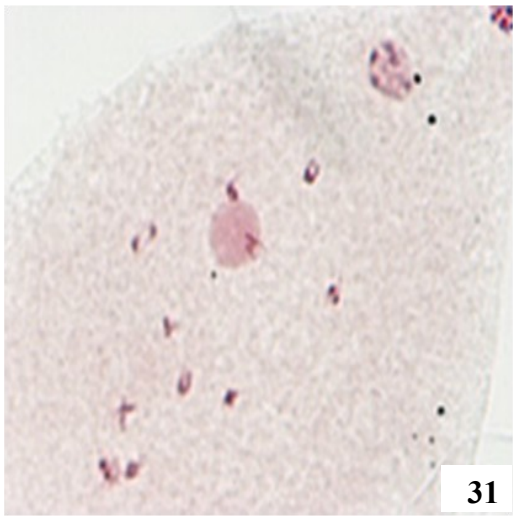
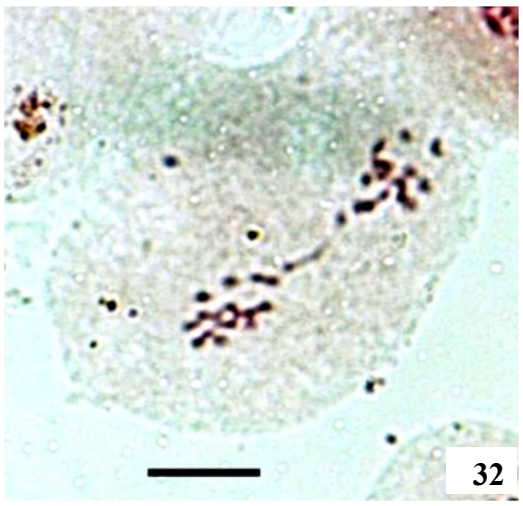
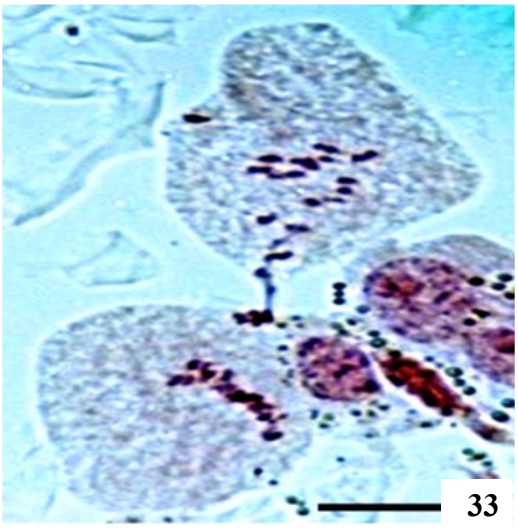
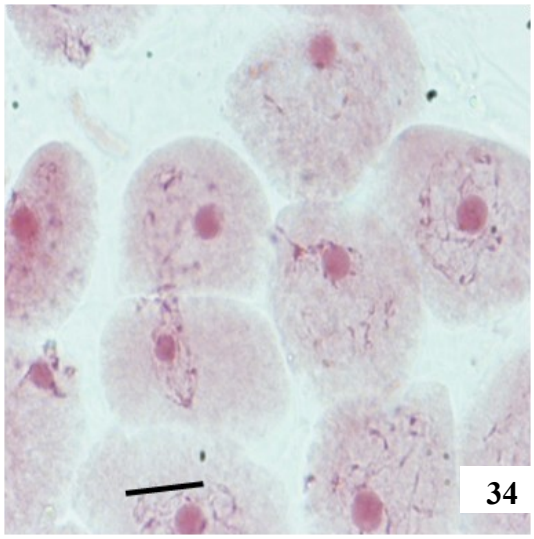


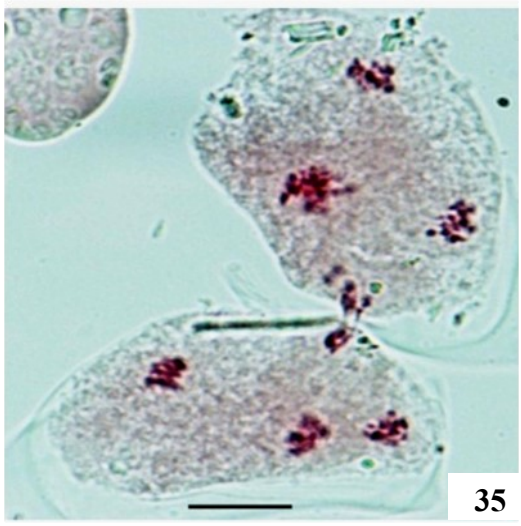
Fig: 26. Plant of *Momordica dioica* L.
Fig: 27-28. Fruit of *Momordica dioica* L.
Fig: 29. Leaf of *Momordica dioica* L.
Fig: 30. Flower of *Momordica dioica* L.











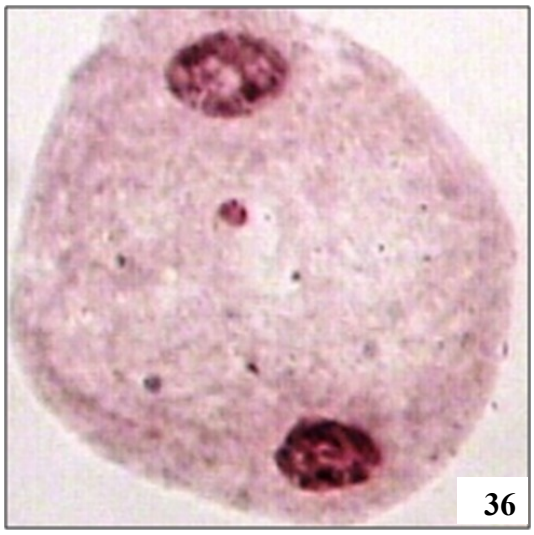
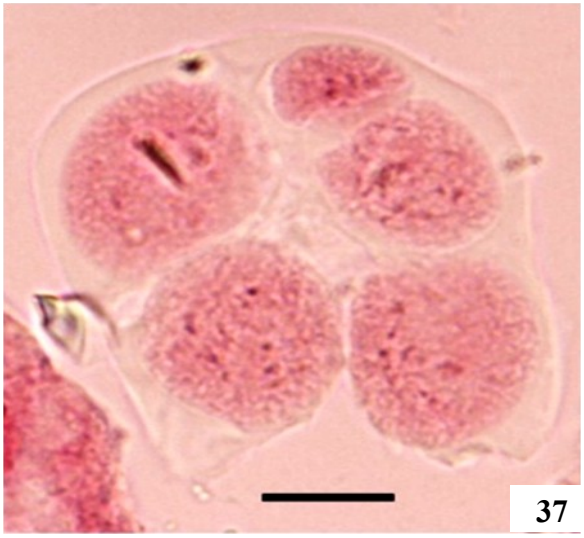
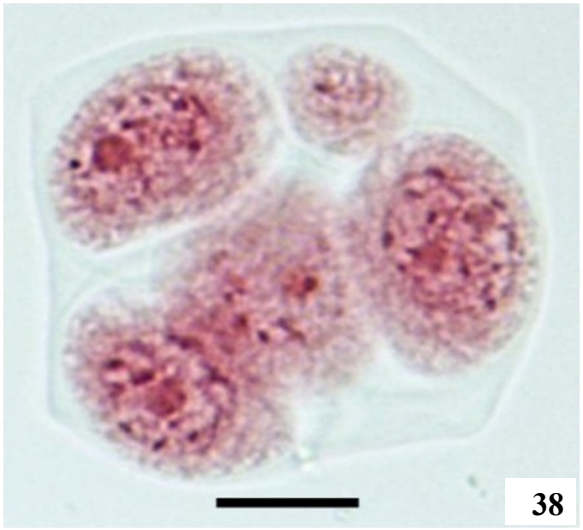
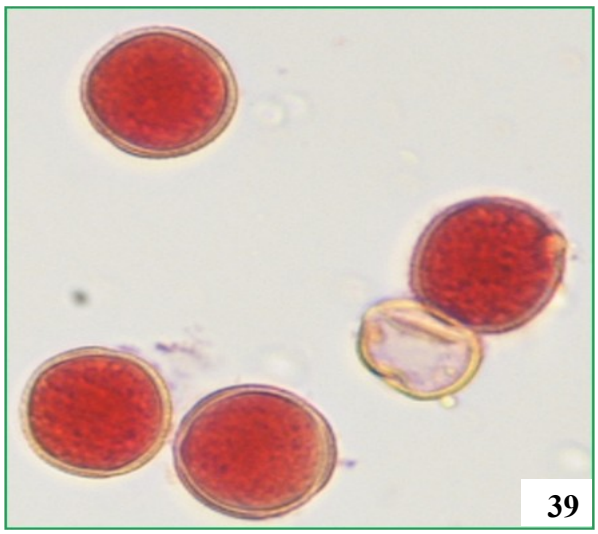
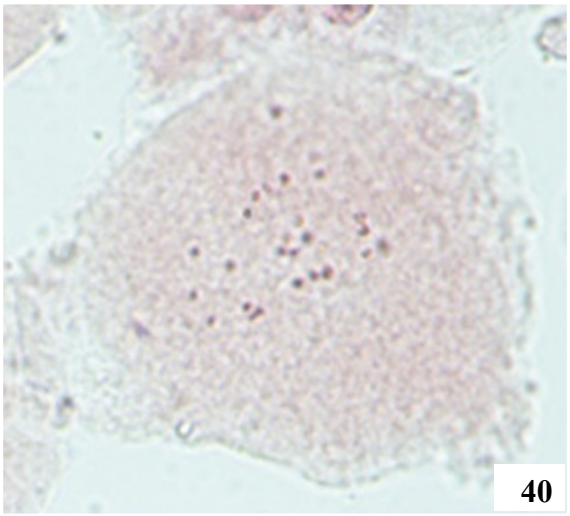


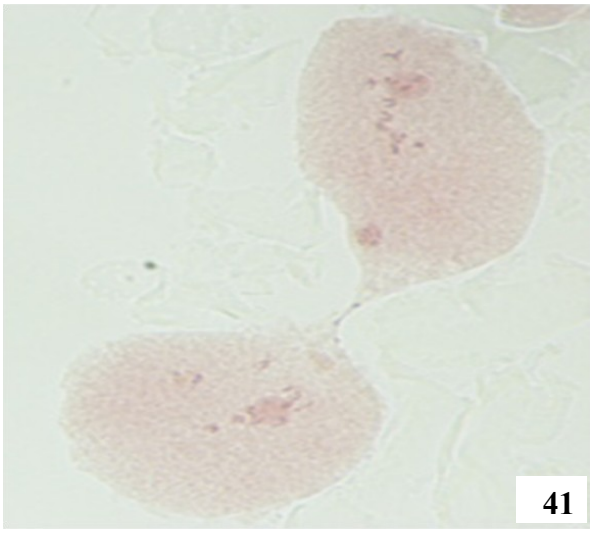
Fig: 31. PMC showing 12 bivalents at Diakinesis in *Cucumis melo* L.
Fig: 32. PMC showing laggards at Anaphsae I. In *Cucumis melo* L.
Fig: 33-35. PMC showing cytomixis in *Cucumis melo* L.
Fig: 36. PMC showing stickiness in *Cucumis melo* L.











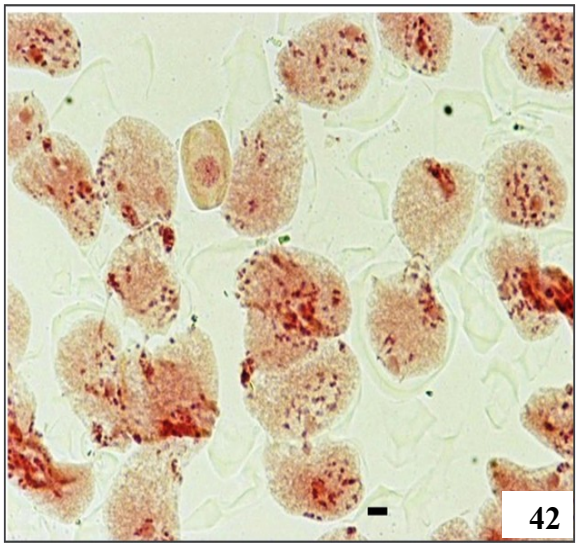
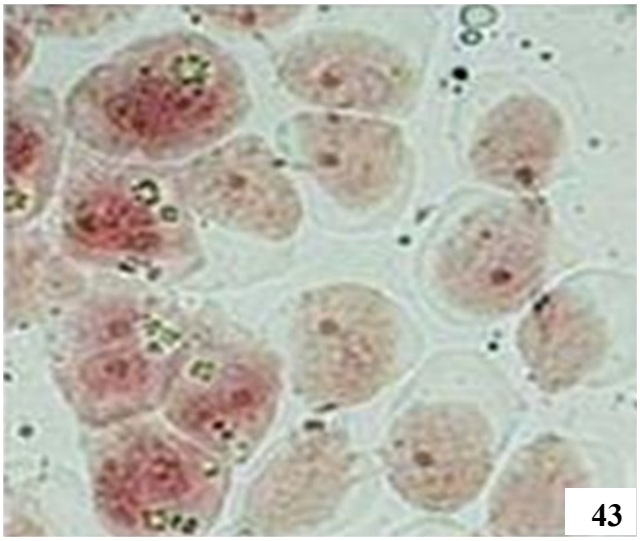
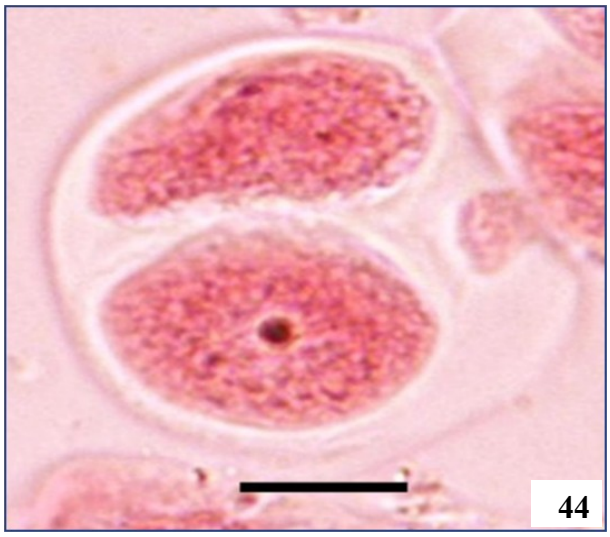
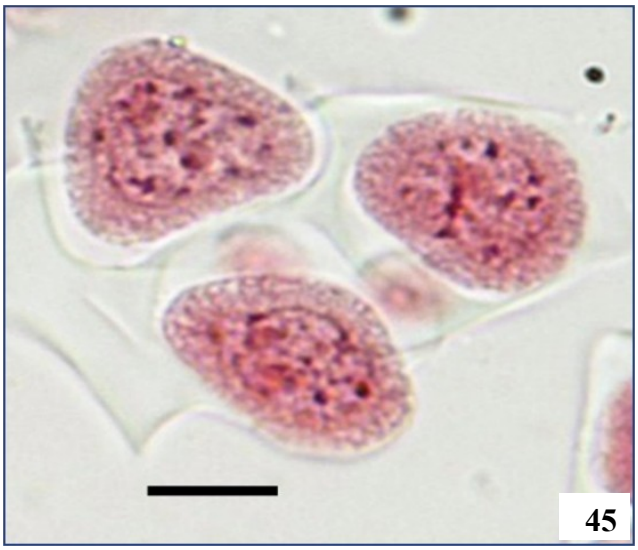
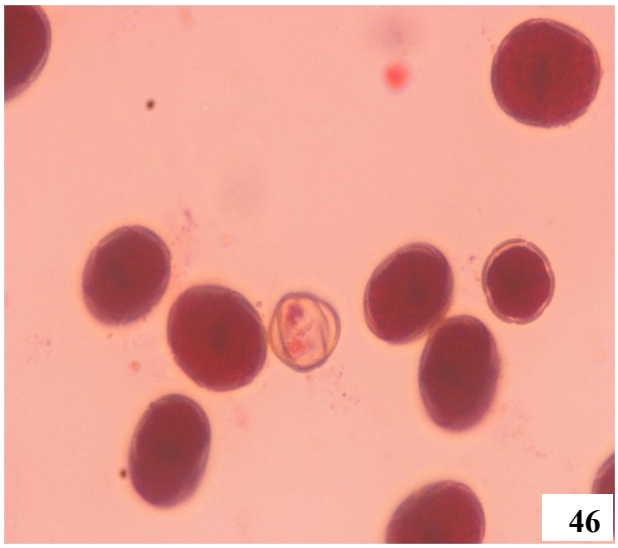


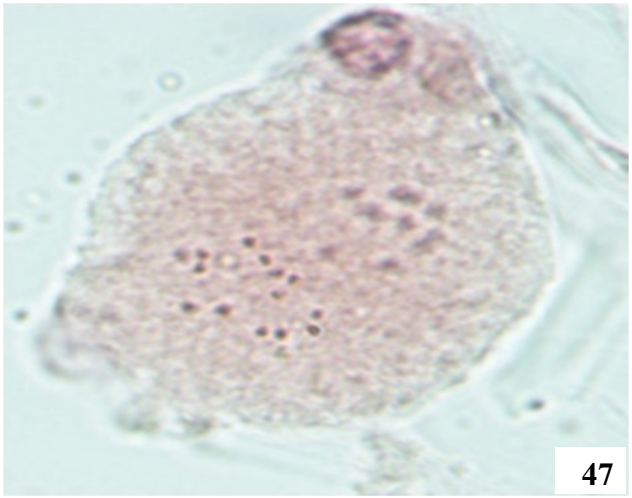
Fig: 37. Polyad in *Cucumis melo* L.
Fig: 38. Tetrad with micronuclei *Cucumis melo* L.
Fig: 39. Unequal sized apparently fertile (dark) and sterile (light) pollen grains *Cucumis melo* L.
Fig: 40. PMC showing Anaphase with 12:12 distribution of chromosomes in *Cucumis melo* var. *agrestis* (Morphotype I).
Fig: 41. 41-42. PMC showing cytomixis in *Cucumis melo* var. *agrestis* (Morphotype I).











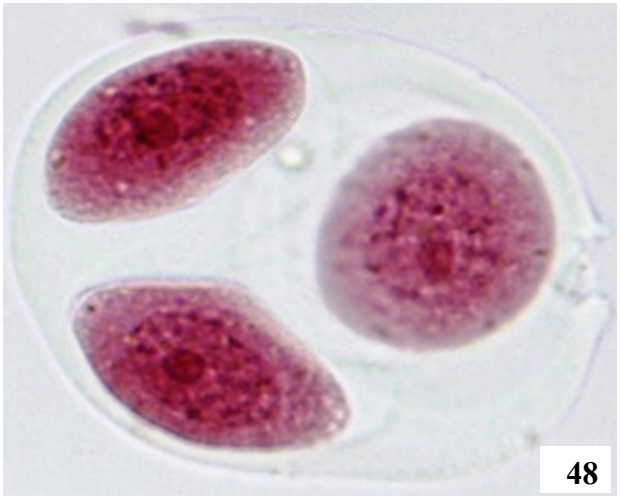
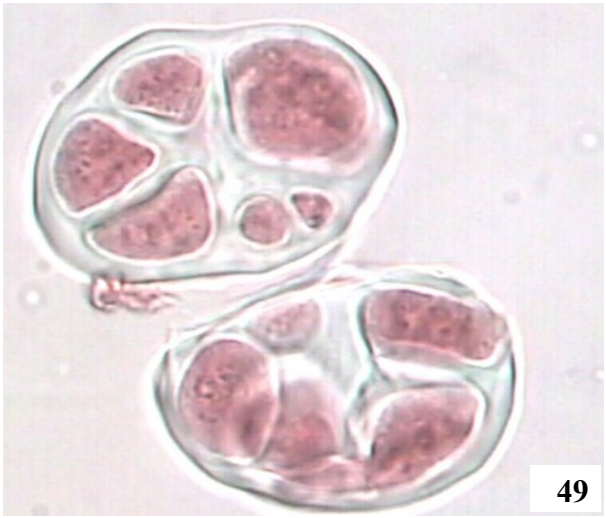
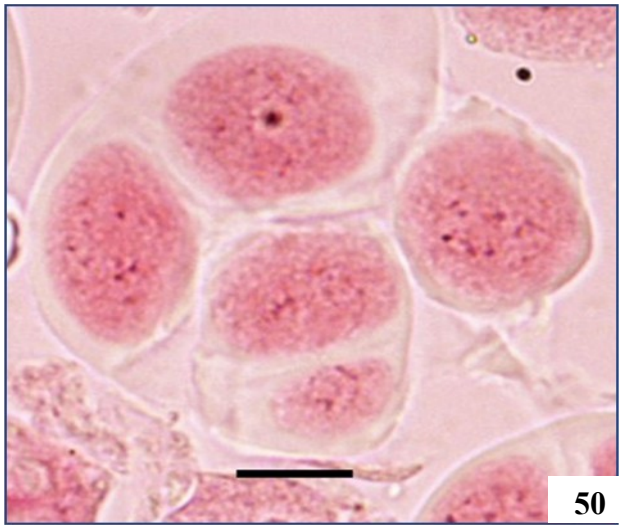
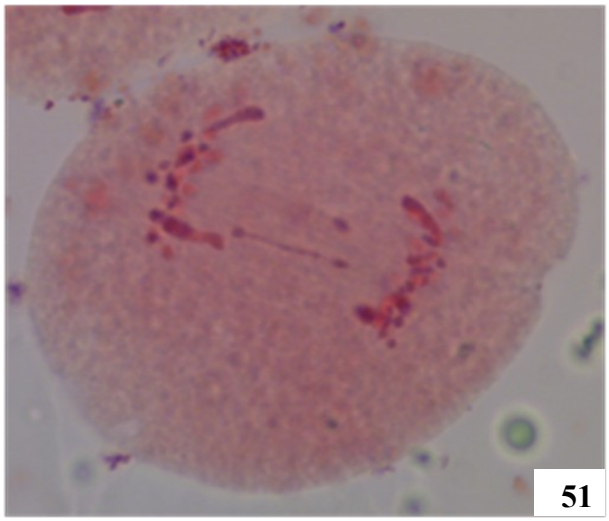
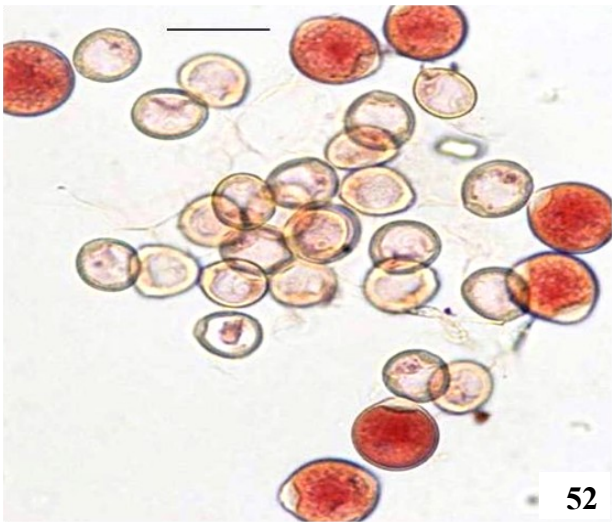


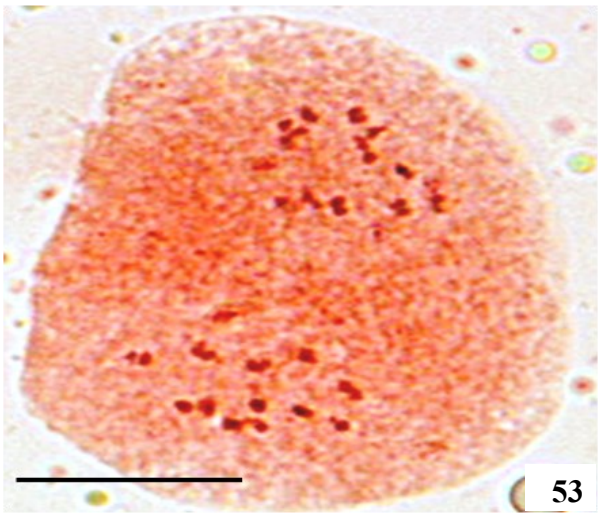
Fig: 43. PMC showing cytomxis in *Cucumis melo* var. *agrestis* (Morphotype I).
Fig: 44. Diad with micronuclei in *Cucumis melo* var. *agrestis* (Morphotype I).
Fig: 45. Triad with micronuclei *Cucumis melo* var. *agrestis* (Morphotype I).
Fig : 46. Unequal sized apparently fertile (dark) and sterile (light) pollen grains in *Cucumis melo* var. *agrestis* (Morphotype I).
Fig: 47. PMC showing 12 bivalents at Metaphase in *Cucumis melo* var. *agrestis* (Morphotype II).
Fig: 48. Triad in *Cucumis melo* var. *agrestis* (Morphotype II).











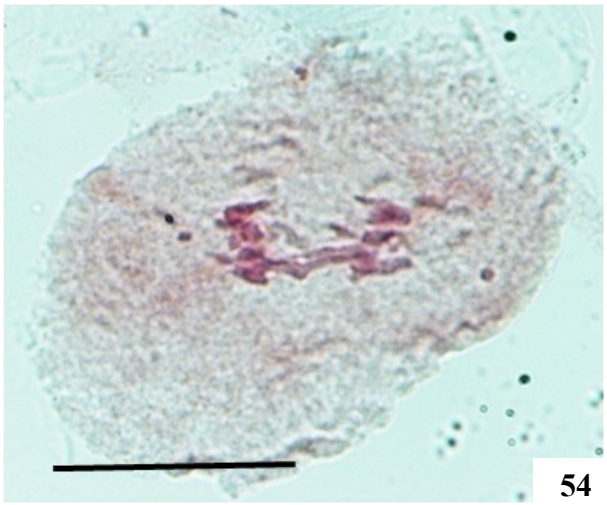
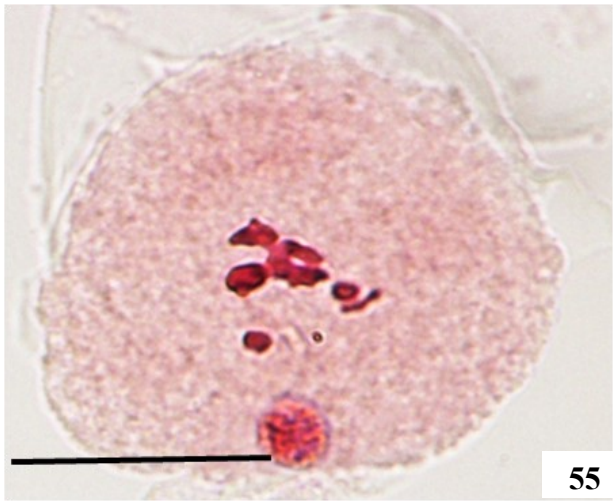
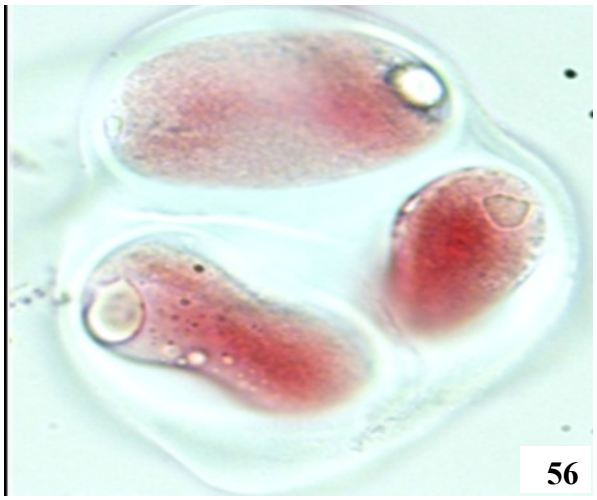
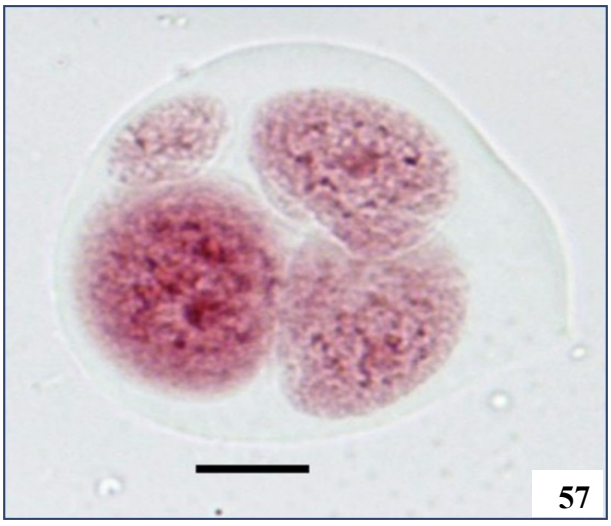
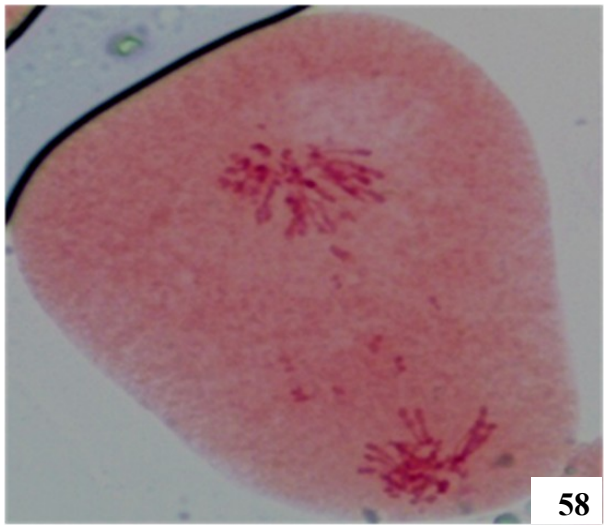


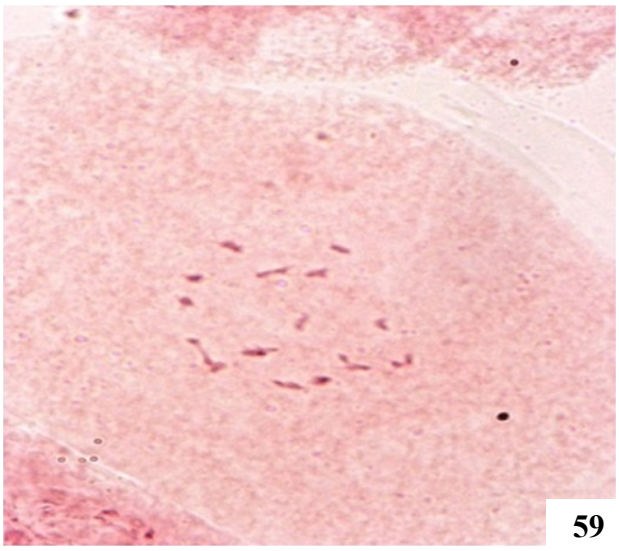
Fig: 50. Tetrad with micronuclei in *Cucumis melo* var. *agrestis* (Morphotype II).
Fig: 51 PMC showing laggard at Anaphase *Cucumis melo* var. *agrestis* (Morphotype II).
Fig: 52. Unequal sized apparently fertile (dark) and sterile (light) pollen grains *Cucumis melo* var. *agrestis* (Morphotype II).
Fig: 53. PMC showing equal distribution of 12:12 chromosomes at Anaphase in *Cucumis melo* var. *momordica* L.
Fig: 54. PMC showing chromatin bridges in *Cucumis melo* var. *momordica* L











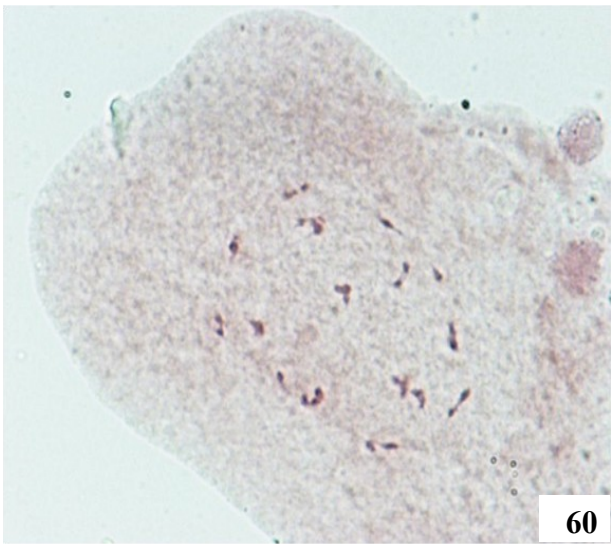
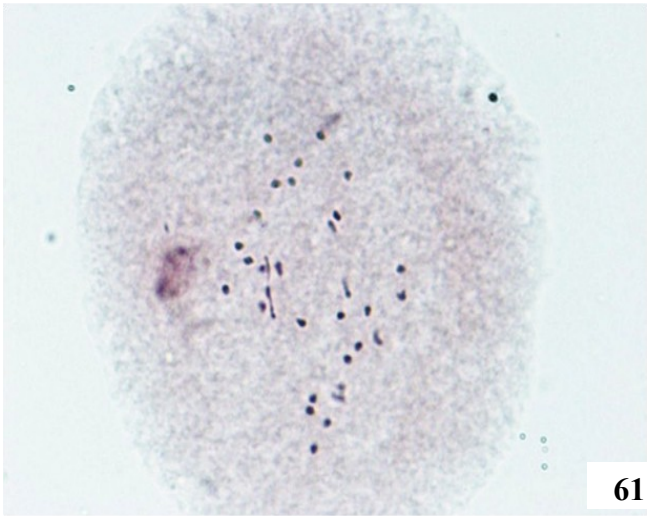
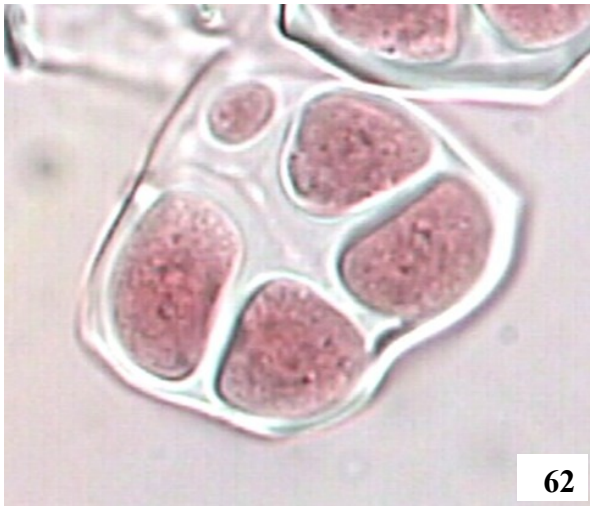
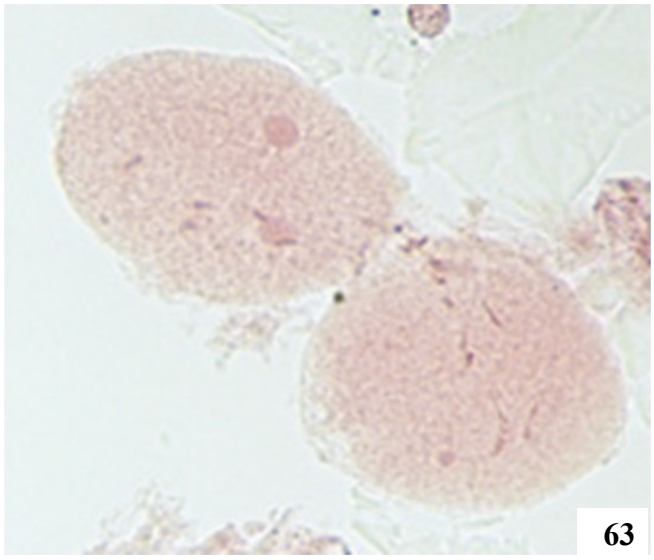
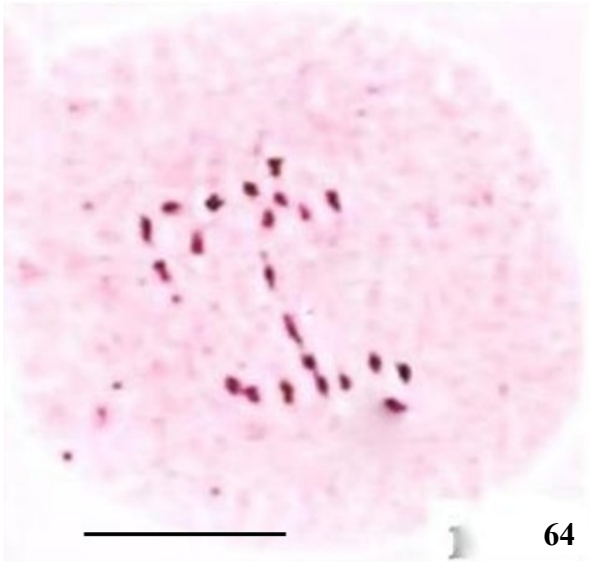


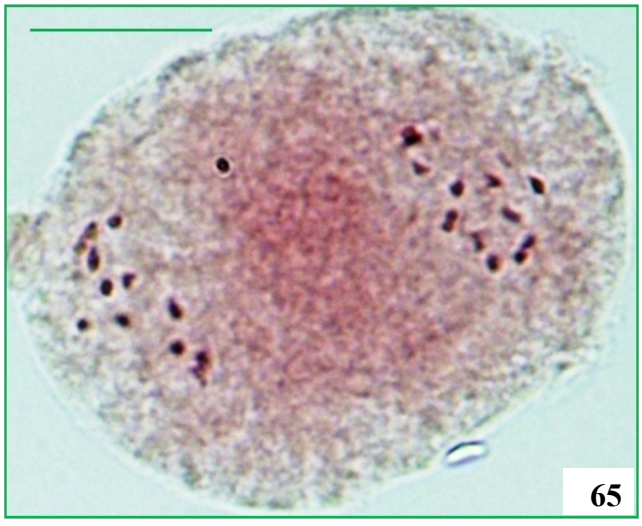
Fig: 55. PMC showing stickiness in *Cucumis melo* var. *momordica* L.
Fig: 56. Triad in *Cucumis melo* var. *momordica* L.
Fig: 57. Triad with micronuclei in *Cucumis melo* var. *momordica* L.
Fig: 58. PMC showing laggards at Anaphase in *Cucumis melo* var. *momordica* L.
Fig: 59-60. PMC showing 14 bivalents at Metaphase in *Momordica dioica* L.











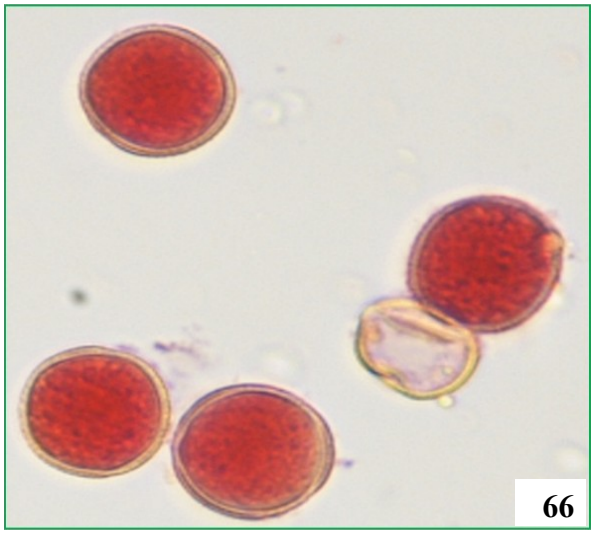
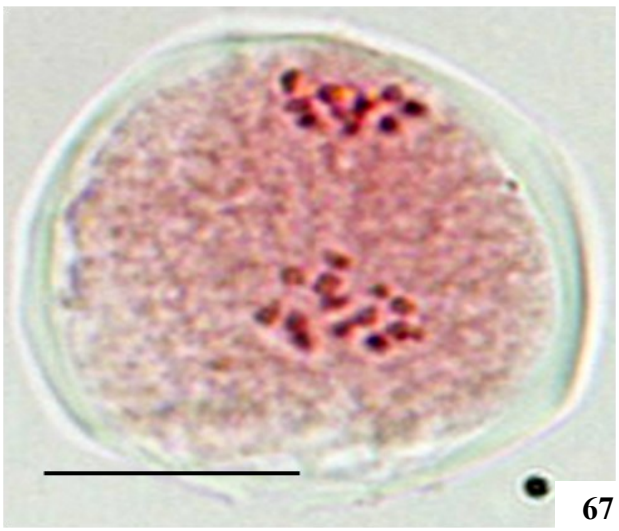
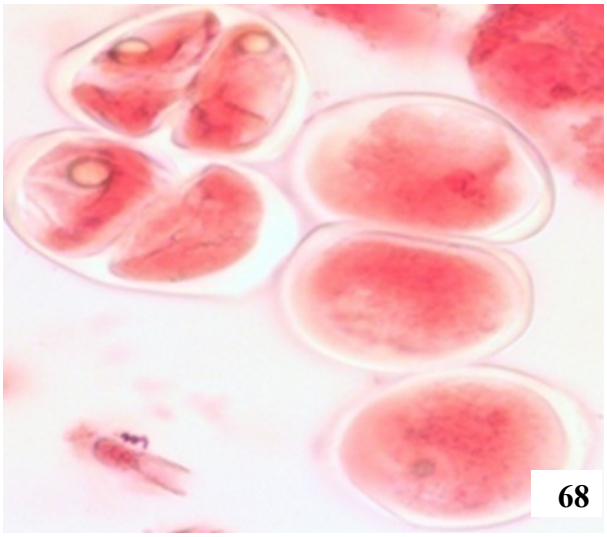
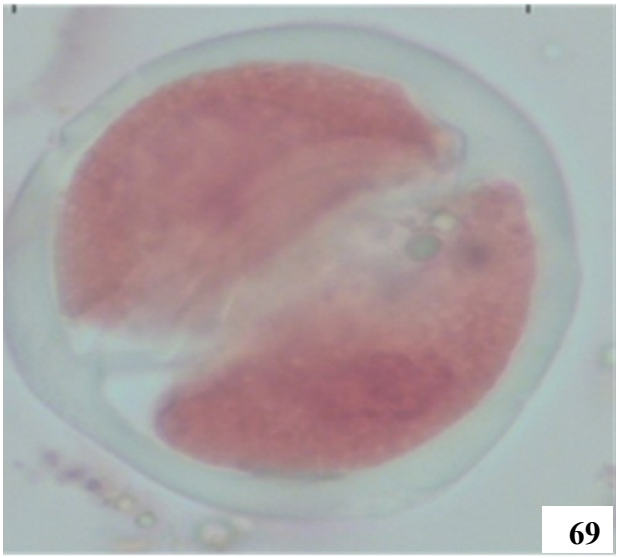
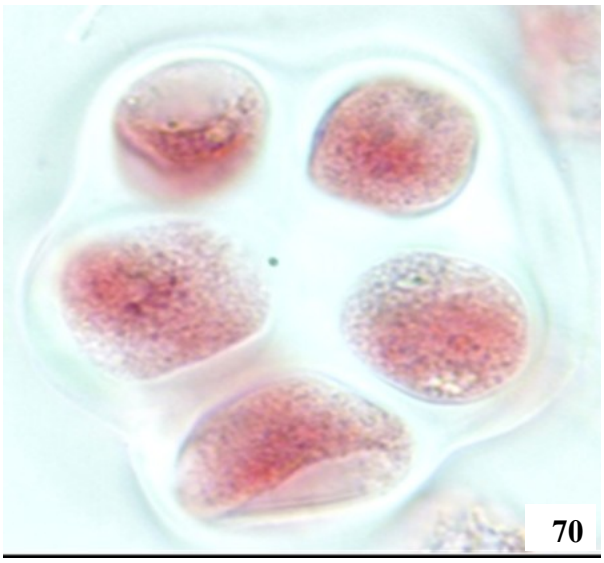


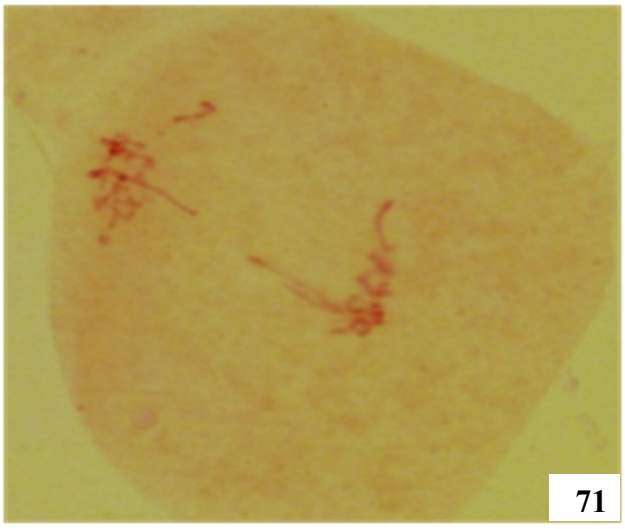
Fig: 61.PMC showing 14:14 distribution of chromosomes at Anaphase I in *Momordica dioica* L.
Fig: 62. Tetrad with micronuclei in *Momordica dioica* L.
Fig: 63. PMC showing cytomixis in *Momordica dioica* L.
Fig: 64. PMC showing laggard at Anaphase in *Momordica dioica* L.
Fig: 65. PMC showing 11:11 distribution of chromosomes at Anaphase I in *Momordica balsamina* L.
Fig: 66. Unequal sized apparently fertile (dark) and sterile (light) pollen grains in *Momordica balsamina* L.











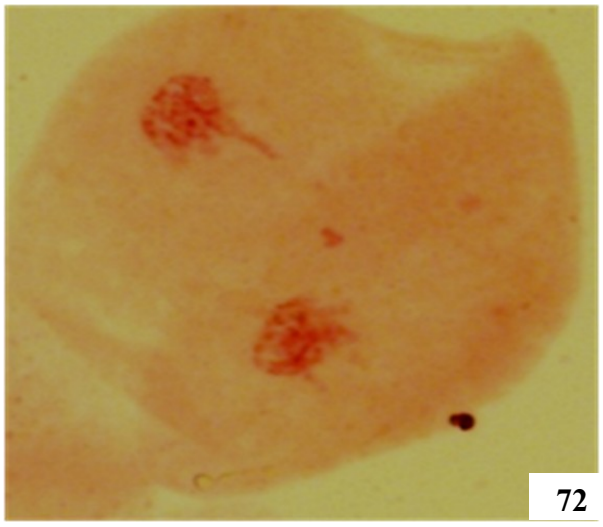


Fig: 67. PMC showing equal distribution of 11:11 chromosomes at Anaphase I in *Momordica charantia* L.
Fig: 68. Monads in *Momordica charantia* L.
Fig: 69. Dyad in *Momordica charantia* L.
Fig: 70. Polyad in *Momordica charantia* L.
Fig: 71-72. PMC showing laggards in *Momordica charantia* L.

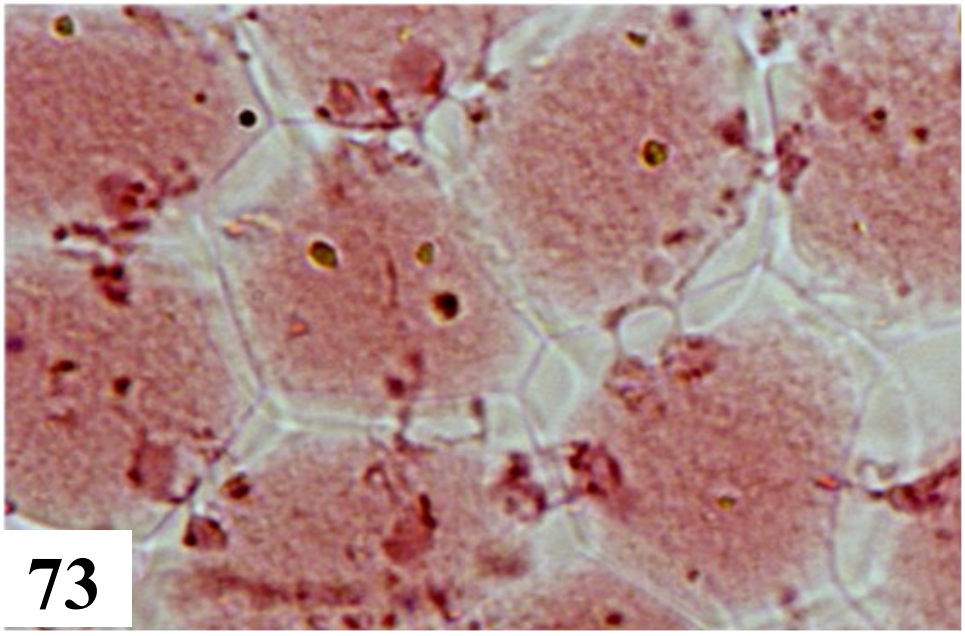


Fig: 73. PMC involved in chromatin transfer in *Momordica charantia* L.

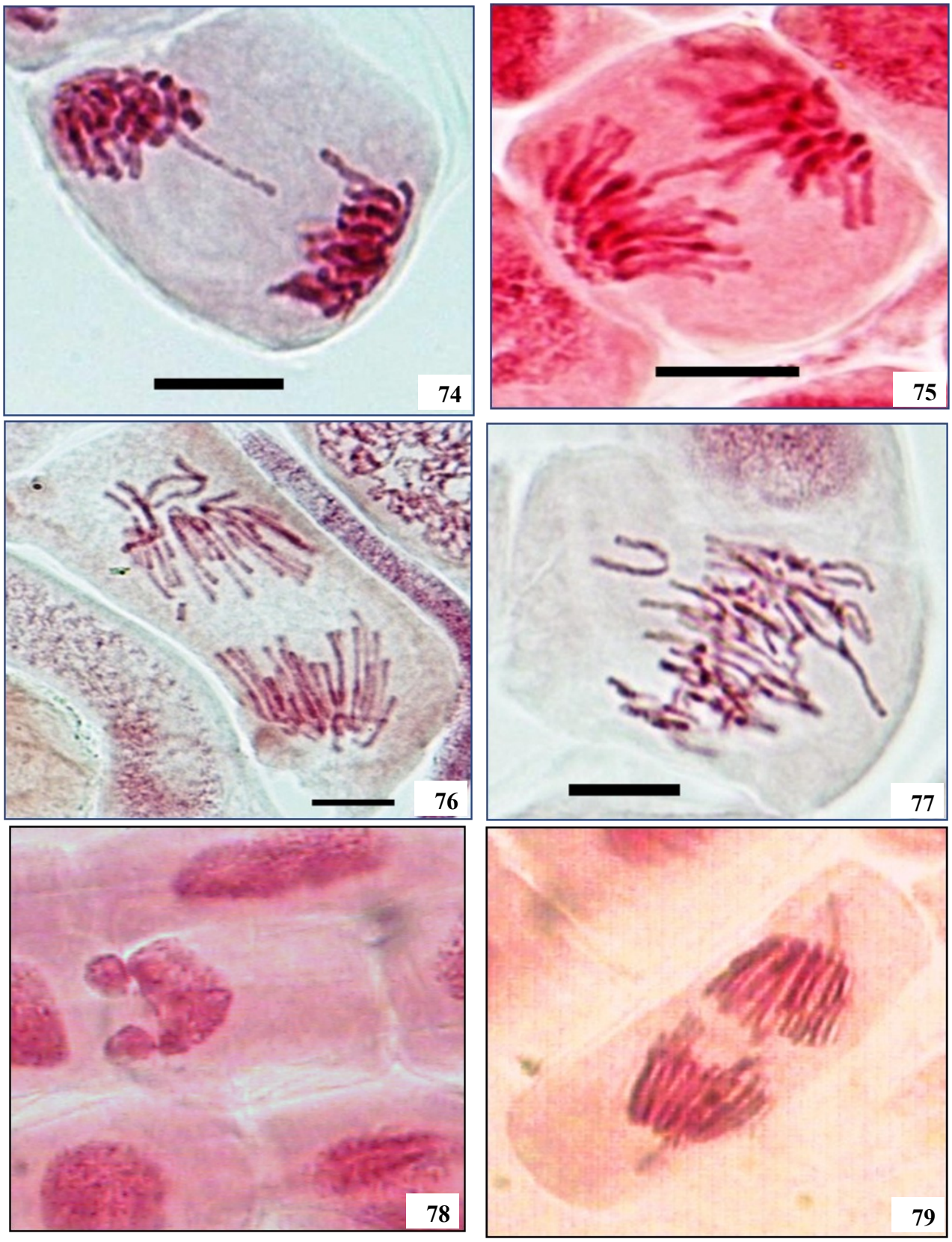


Fig: 74. Bridge at Telophase in methanol extract of *Cucumis melo* L.
Fig: 75. Bridge at Anaphase in aqueous extract of *Cucumis melo* L.
Fig: 76. Fragment at Anaphase in methanol extract of *Cucumis melo* L.
Fig: 77. Vagrant at Metaphase in extract of *Cucumis melo* L.
Fig: 78. Micronuclei at Prophase in methanol extract of *Cucumis melo* var. *agrestis* L. (Morphotype I)
Fig: 79. Vagrant at Anaphase in methanol *Cucumis melo* var. *agrestis* L. (Morphotype I)

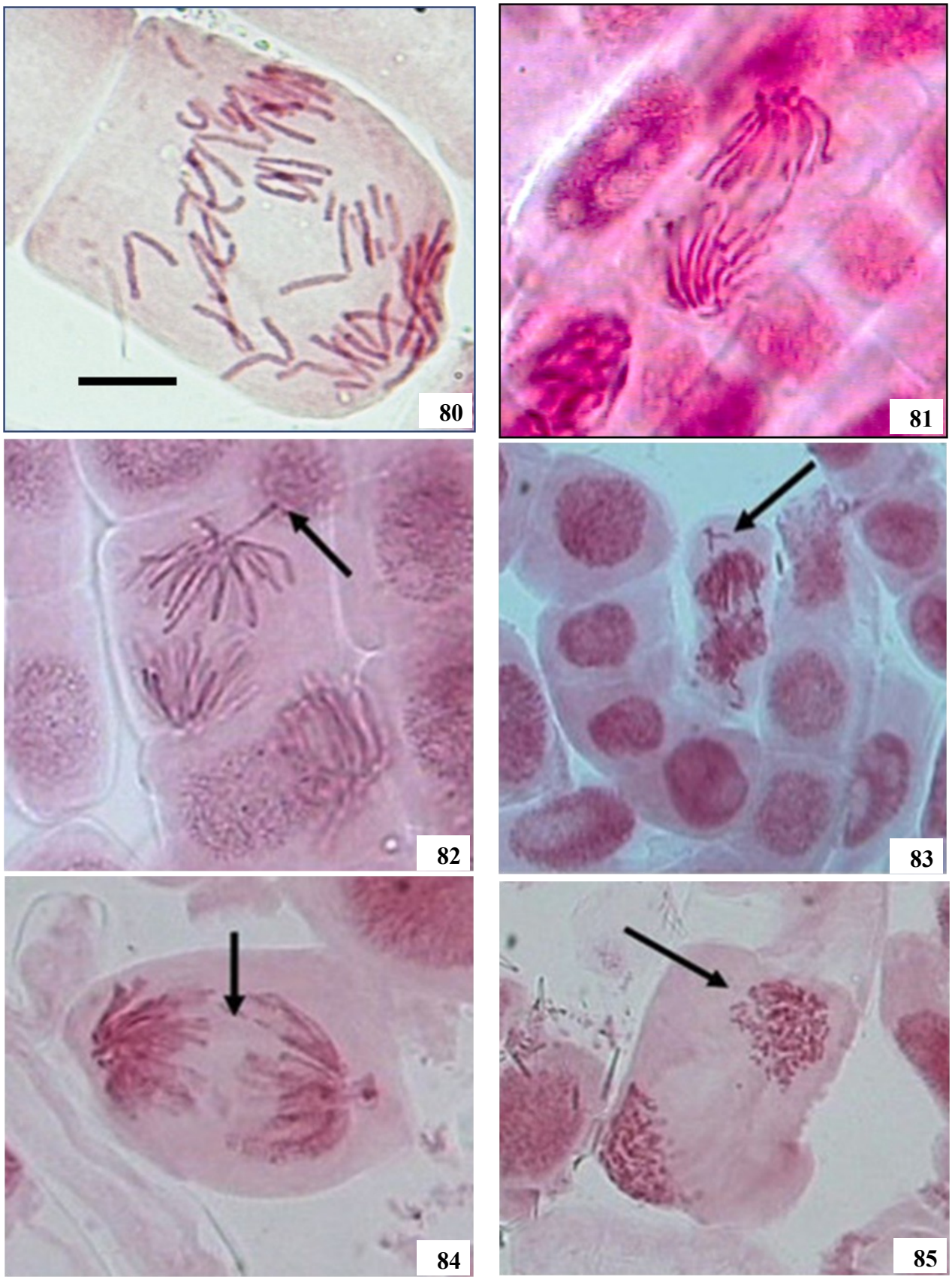


Fig: 80. Multipolarity at Anaphase in aqueous extract of *Cucumis melo* var. *agrestis* L. (Morphotype I)
Fig: 81. Vagrant at Anaphase in methanol extract of *Cucumis melo* var. *agrestis* L. (Morphotype II)
Fig: 82-83. Vagrant at Anaphase in methanol extract of *Cucumis melo* var. *agrestis* L. (Morphotype II)
Fig: 84. Bridge at Anaphase in aqueous extract of *Cucumis melo* var. *agrestis* L. (Morphotype II)
Fig: 85. Fragments at Telophase in acetone extract of *Cucumis melo* var. *agrestis* L. (Morphotype II)

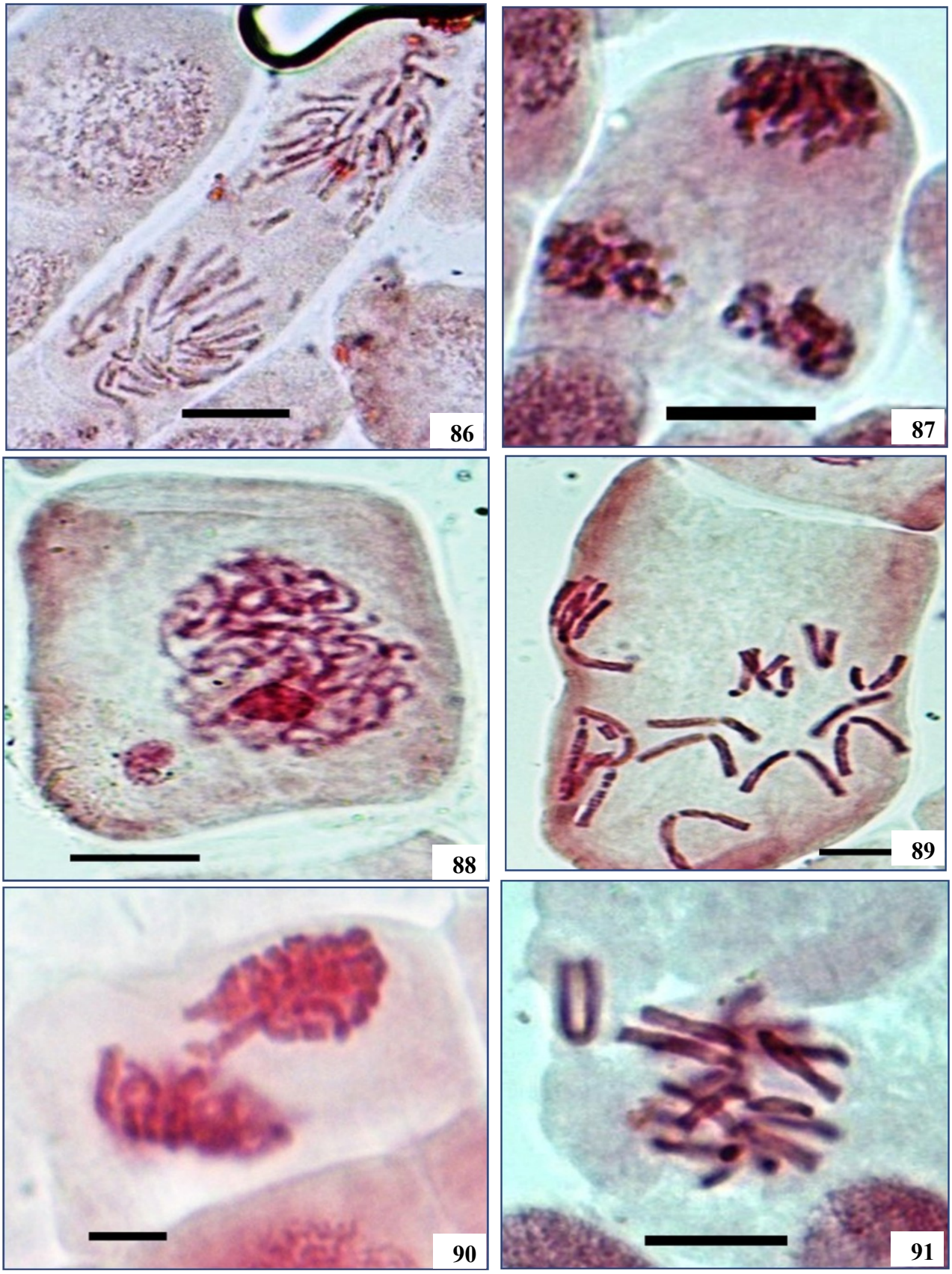
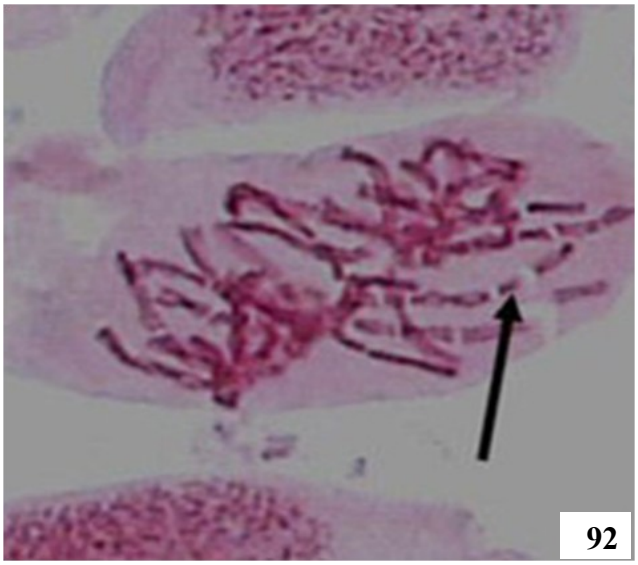
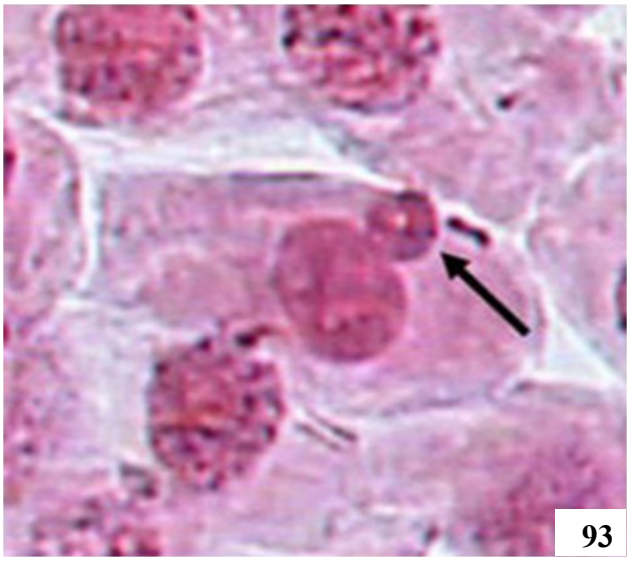
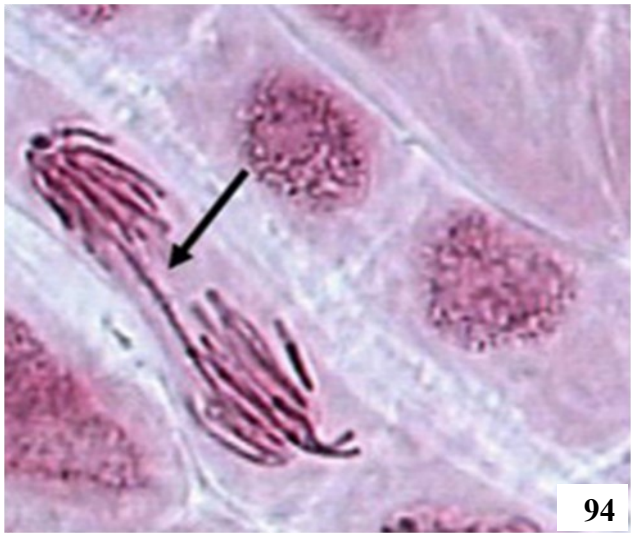
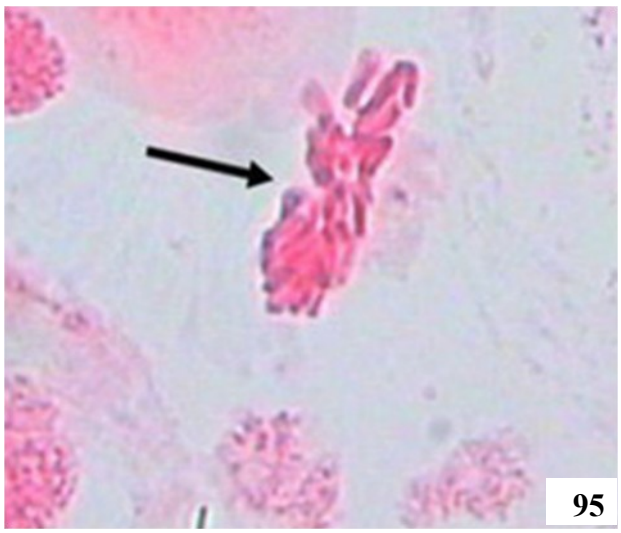


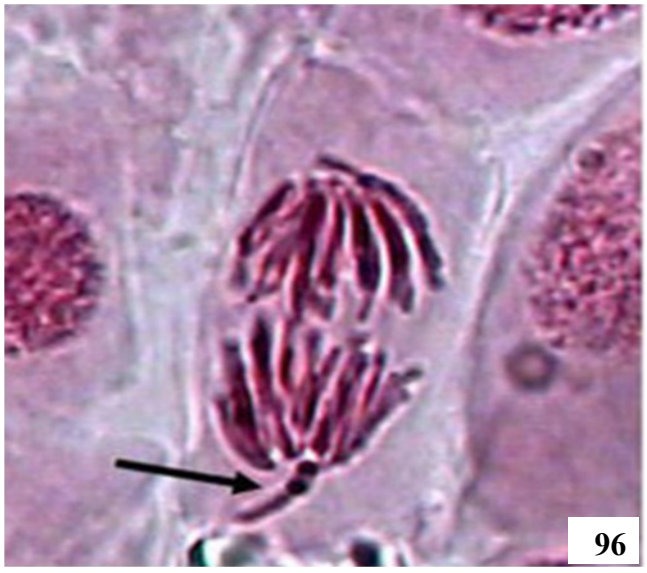
Fig: 86. Laggard at Anaphase in methanol extract of *Cucumis melo* var. *momordica* L.
Fig: 87. Multipolarity at Anaphase in aqueous extract of *Cucumis melo* var. *momordica* L.
Fig: 88. Micronuclei at Prophase in methanol extract of *Cucumis melo* var. *momordica* L.
Fig: 89. Fragments at Metaphase in acetone extract of *Cucumis melo* var. *momordica* L.
Fig: 90. Bridge at Telophase in methanol extract of *Momordica charantia* L.
Fig: 91. Vagrant at Metaphase in aqueous extract of *Momordica charantia* L.











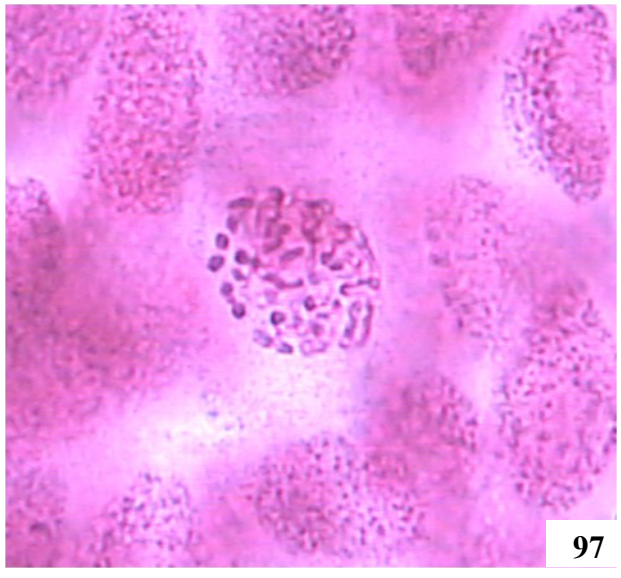


Fig: 92. Fragments at Metaphase in acetone extract of *Momordica charantia* L.
Fig: 93. Micronuclei at Prophase in methanol extract of *Momordica charantia* L.
Fig: 94. Bridge at Anaphase in methanol extract of *Momordica balsamina* L.
Fig: 95. Stickiness at Metaphase in aqueous extract of *Momordica balsamina* L.
Fig: 96. Vagrant at Anaphase in methanol extract of *Momordica balsamina* L.
Fig: 97. Fragment at Prophase in methanol extract of *Momordica dioica* L.

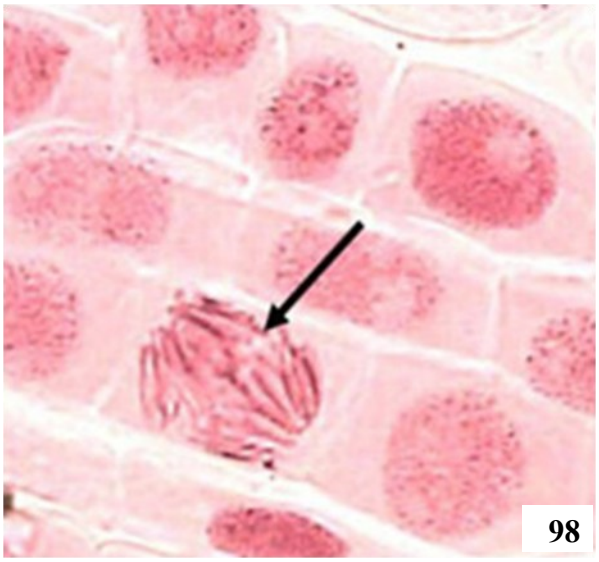


Fig: 98. Fragments at Anaphase in methanol extract of *Momordica dioica* L.

Papers published

1. Rupinderpal Kaur, Yumnam Devashree, Ravindra Kumar, Saurabh Gupta, Mohd. Saleem Wani & Vijay Singh (2021): Genotoxic effect of fruit extract of wild and cultivated cucurbits using *Allium cepa* assay, International Journal of Vegetable Science, DOI: 10.1080/19315260.2021.1958403.
2. Rupinderpal Kaur, Yumnam Devashree, Vijay Singh, Renu Sharma. Preliminary phytochemical screening of different solvent mediated extracts of genus *Cucumis* L. and *Momordica* L. of family Cucurbitaceae from Northern India. International Journal of Botany Studies.Volume 6; Issue 2; 2021; Page No. 175-179.

Papers presented in conferences

1. Rupinderpal Kaur, Yumnam Devashree, Vijay Singh, Renu Sharma. Comparison of phenolic, flavanoid contents and antioxidant activities of various extracts of selected plants of Genus *Cucumis* L. and *Momordica* L. of family Cucurbitaceae. virtual international conference on "Technologies for Environmental Sustainability and Smart Agriculture" organized by Centre of Excellence in Sustainable Technologies for Rural Development [CESTRD], Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology during September 18 and 19, 2020.
2. Rupinderpal Kaur, Yumnam Devashree and Vijay Singh. Cytotoxic and Genotoxic effect of fruit extract of 6 species of Family Cucurbitaceae in the roots of *Allium* assay L. A comparative analysis. Internationl conference on advancement in Engineering and Technology, held at Bhai Gurdas Institute of Enginnering and Technology, Sangrur. 20-21, March,2020.
3. Rupinderpal Kaur[1*], Yumnam Devashree [1] and Vijay Singh[2]. Cytotoxic and Genotoxic effect of fruit extract of 6 species of Family Cucurbitaceae in the roots of *Allium* assay L.: A comparative analysis. 3rd National conference on Innovations in Bioscience and Technology, held at Multani Mal Modi College, Patiala, Punjab. 7th March,2020.
4. Rupinderpal Kaur[1*], Yumnam Devashree[1] and Vijay Singh[2] Preliminary phytochemical screening of different solvent mediated extracts and Antimicrobial activity of genus *Momordica* L. of family Cucurbitaceae from Northern India. International herbinar "Recent trends in pharmacognosy-2020 by Mother Theresa Post Graduate and Research Institute of Health sciences (A Government of

Puducherry Institution) Department of Pharmacognosy, College of Pharmacy, Puducherry - 605 006 on 2nd October,2020. (FIRST POSITION).

5. Rupinderpal Kaur[1*], Yumnam Devashree[1] and Vijay Singh[2] (E-Poster) a paper entitled EPP-4: Preliminary phytochemical screening of different solvent mediated extracts and Antimicrobial activity of genus *Momordica* L. of family Cucurbitaceae from Northern India in the two day International E-Conference on New Horizons in "Biochemistry, Microbiology and Food Technology – 2020" (IECBMFT-2020) organized by Yogi Yemana University and Universiti Malayasia Kelantan, held on October 12th & 13th, 2020.