COMPARATIVE ASSESSMENT OF BIOCHEMICAL PROPERTIES OF DIFFERENT VARIETIES OF *MORINGA OLEIFERA* LAM. TO SCREEN THE ELITE VARIETY FOR CULTIVATION IN PUNJAB

A Thesis

Submitted in partial fulfillment of the requirements for the

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in

Botany

By

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Declaration

I hereby declare that the thesis entitled, **"Comparative Assessment of Biochemical Properties of *Moringa oleifera* Lam. Varieties to Screen the Elite Variety for Cultivation in Punjab"** submitted for doctor of philosophy in botany is entirely my original work and all ideas and references have been duly acknowledged. It does not contain any work for the award of any other degree or diploma at any university.

Date:

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Certificate

This is to certify that Ms. Bilques Farooq has completed the thesis entitled "**Comparative Assessment of Biochemical Properties of *Moringa oleifera* Lam. Varieties to Screen the Elite Variety for Cultivation in Punjab"** 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 the thesis has ever been submitted for any other degree or diploma at any university.

The thesis is fit for the submission and the partial fulfillment of the condition for the award of **DOCTOR OF PHILOSOPHY IN BOTANY**

Date: Signature of Advisor

Dr. Bhupendra Koul

Abstract

Moringa oleifera Lam., a versatile plant grown in the tropic and sub-tropic areas of the world is an excellent source of phytonutrients and phytochemicals which exhibits several biological properties such as anti-oxidant, anti-fungal, anti-bacterial, anti-inflammatory, anti-pyretic, anti-tumour, anti-cancer, anti-diabetic, anti-ulcer and anti-hypertensive properties. *M. oleifera* has begun to achieve more popularity as an innovative 'superfood' due to its great nutritional value. It has been utilised for generations to cure and prevent the diseases like, anemia, diabetes, arthritis, digestive, respiratory, heart, skin, and liver disorders. Other than these pharmacological properties, it also possesses plant growth enhancing properties. The ethanolic and aqueous leaf and seed extracts of *M. oleifera* varieties, such as 'ODC', 'Jaffna', 'Conventional', 'PKM-1' (Periyakulam-1), and 'PKM-2' (Periyakulam-2), has been utilised to analyse their free radical scavenging effect on 2, 2-diphenyl-2-picryl hydrazyl (DPPH) and 2, 2-azinobis,3-ethyl-benzothiazolin-6-sulfonic acid (ABTS). The DPPH and ABTS radical scavenging activities (based upon IC_{50} values) with leaves extracts was trended as: Conventional (24.45 µg/ml) > ODC (22.69 µg/ml) > PKM-2 (17.23 µg/ml) > PKM-1 (13.34 µg/ml) > Jaffna (11.46 µg/ml) and Conventional (34.71µg/ml) > ODC (31.55 µg/ml) > PKM-2 (30.52 µg/ml) > PKM-1 (25.98 µg/ml) > Jaffna (20.24 µg/ml) respectively. Correspondingly, the DPPH and ABTS activity of seeds extracts was: Conventional (30.88 µg/ml) > ODC (28.72 µg/ml) > PKM-2 (24.41 µg/ml) > PKM-1 (21.08 µg/ml) > Jaffna (20.72 µg/ml) and Conventional (37.60 µg/ml) > ODC (36.03 µg/ml) >PKM-2 (33.72 µg/ml) > PKM-1 (32.14µg/ml) > Jaffna (29.72 µg/ml) respectively. The results perceived with TPC (total phenolic content) and TFC (total flavonoid content) for both seeds and leaves extract was revealed the Jaffna with highest contents followed by PKM-1, PKM-2, ODC and Conventional. The antibacterial activity of the aqueous and ethanolic leaf and seed extracts of all five varieties on *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* was determined by using the disc-diffusion method. The results observed (for both seeds

and leaves extracts of five different varieties of *M. oleifera*) that Jaffna variety showed highest anti-bacterial activity followed by PKM-1, PKM-2, ODC and Conventional. Hence, the comparative study relates that the *M. oleifera* ethanolic seed and leaf extracts of Jaffna variety have strong antioxidant and antibacterial properties with a great TPC and TFC, as compared to the other four varieties. Thus, Jaffna variety can be explored more as a consistent source of natural antioxidants and bacteriostatic agent.

M. oleifera is considered as an essential source of food as it contains almost all important nutrients and has great medicinal, pharmaceutical, and industrial value as well. This study offers a comparative analysis of anticancer compounds (HPLC), antioxidant activity (FRAP) and different nutritional parameters of *M. oleifera* varieties (ODC, Jaffna, Conventional, PKM-1 and PKM-2). According to the resuts of present study Jaffna variety has the highest content of anticancer compounds (beta-sitosterol 0.244, quercetin 0.216, khaempherol 0.013%, and moringin 0.063% content), protein (0.94 and 0.69 µg/ml; young and mature stage respectively), sugar (0.39 and 0.51 µg/ml; young and mature stage respectively), chlorophyll (1.31, 1.18, 0.82 mg/g; plantlet, vegetative, and reproductive stage respectively), and antioxidant activity (9.47, 18.48, 29.39 and 35.73 µg/ml), followed by other four varieties. However, in modern times, people are acutely dependent on synthetic drugs and in order to return to the natural and safe health, it is highly recommended to promote the Jaffna variety of *M. oleifera* globally.

The current research also presents a comparative assessment of the foliar spray of different varieties of *M.oleifera* on the growth parameters of *Stevia rebaudiana* such as the stevioside, zeatin, chlorophyll a and b, mineral, phenolic and flavonoid content, carotenoids, total soluble sugars and proteins of *Stevia.* According to the results of the present study, Jaffna has expressively enhanced all the growth parameters (used in experiment) of *S. rebaudiana*. High Performance Liquid Chromatography (HPLC) was performed to determine the stevioside and zeatin content in treated and untreated *S. rebaudiana*. Jaffna (7.73%; 0.0063%) possess high content of both stevioside and zeatin followed by PKM-1 (6.93%; 0.0056%), PKM-2 (6.45%; 0.0051%), ODC

(4.14%; 0.0048%) Conventional (3.86%; 0.0042%) and Control or untreated (2.94%; 0.00088%) respectively. Flame photometer was also used to determine the mineral content in treated and untreated *S. rebaudiana*. Jaffna foliar spray showed highest mineral content (sodium: 0.95%, calcium: 0.89%, lithium: 0.62%, potassium: 0.44%) followed by PKM-1, PKM-2, ODC, Conventional, and control. As per the results are concerned among all the five varieties of *M.oleifera*, Jaffna has proved to be an effective plant growth enhancer and can be beneficial as a cost-effective and an eco-friendly biofertilizer and biostimulant for growth promotion of *S. rebaudiana*, a commercially important natural sweetner crop.

Since the dawn of human existence, the dreadful diseases began to arise which became the main cause of existential devastation. Among the deadliest diseases, Cancer has always been regarded as the main cause of increasing mortality rate throughout the universe without any appropriate cure. From the time immemorial, herbs have been satisfactory accepted as major source of immensely effective traditional drugs for the cure of several dreadful diseases. *M. oleifera* is very important medicinal pant, used to treat almost 300 diseases. In this study five different varieties of *M. oleifera* were used to test their cytotoxicity on HepG2 (human liver cell line) by using the MTT assay. The trend observed for cytotoxic activities of ethanolic leaf extracts on HepG2 cell line, based on the IC_{50} values, was Conventional (1.22 mg/ml) > ODC (0.90 mg/ml) > PKM-2 (0.65 mg/ml) > PKM-1 (0.35 mg/ml) > Jaffna (0.15 mg/ml). As evident from the results, the Jaffna leaf extract exhibited highest cytotoxic effect against HepG2 cell line. Thus, Jaffna variety could be regarded as a potential source for the development of anticancer agent.

By assessing these parameter it became possible to conclude that Jaffna variety has proved to be the most beneficial variety in terms of nutrition, anticancer bioactive compounds, and in anticancer activity. In the Jaffna variety, the experimental values of all the parameters appeared to be significantly higher than the other four varieties. Thus, Jaffna has proved to be the best variety for cultivation in Punjab. In addition, it has been proved that in order to rehabilitate the already affected areas from such

deadly diseases, cultivation of Jaffna is a must. This research makes a strong recommendation for the people of Punjab to maintain and upgrade their lifestyle with the regular consumption and cultivation of Jaffna variety.

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Table of Contents

List of Tables

List of Figures

Chapter 1

Introduction

The plant kingdom comprises of approximately 400,000 species which inhabit the planet Earth (Keddy, 2007). The plants are a source of oxygen, food, nutrients, cooking oils, essential oils, medicines, wood, fibers, fuels and shelter for all living organisms (Gallo et al,. 2019; Kellogg, 2019; Myers, 2019; Shogren et al., 2019; Salam and Quave, 2019). Nature has blessed us with an immense treasure of medicinal flora to cure most of the diseases. The usage of medicinal plants as a panacea to several diseases and disorders can be dated back to 1500 BC. Explicit information about the medicinal plants has been accrued from many centuries, based on specific medicinal systems which include: Unani, Ayurveda, and Siddha. Egyptian papyrus, Chinese writings and ancient unani manuscripts also described the use of medicinal plants. Evidence showed that, Indian Vaids, Unani Hakims, Mediterranean and European cultures have been using medicinal plants for over 4000 years. Indigenous cultures of Africa, America, Egypt, Iran and Rome used herbs in their healing sacraments.

The Indian herbal medicine is antiquity and deep-rooted in the tribal culture and folklore. The primeval use of flora has been archived inside the historical scriptures of Hindus like 'Sushruta Samhita' (800-700) BC, 'Charaka Samhita' (1000-800) BC, 'Rigveda' (4500-1600) BC, and others (Rana et al., 2014). Among historical civilisations, India has been regarded as a storehouse of medicinal plant (eight hundred herbal treatments were codified in AYUSH systems). The India forests are considered as the important repository of huge range of aromatic and medicinal plant that is widely used as raw materials for manufacture of medicines and perfumery products.

A huge number of plants are utilized in the preparation of medicines, as they contain bioactive compounds (atriopine, 4-(4-O-acetyl-α-Lrhamno pyranosyloxy) benzyl isothiocyanate quinine, β-sitosterol-3-O-β-D- glucopyranoside, niazirin, niazirinin, O-ethyl-4-(α-L-rhamnosyloxy) benzyl carbamate, niazimicin, 4-(α-L-rhamno pyranosyloxy) benzyl glucosinolate, niaziminin (niaziminin B and niaziminin A), β-sitosterol, moringine, pterygospermin, ascorbic acid, quercetin, kaempherol, iso-quercitin, kaempferitrin, 3-O-(6-O-oleoyl-β-D glucopyranosyl)-β-sitosterol and β-carotene) that have a great physiological or immunomodulatory impact on the human health.

Altogether, 28,187 species are identified as medicinal plants from which 74 % of the commercial drugs are extracted (Caceres et al., 1992; Bharali et al., 2003; Sadek et al., 2013; Salam and Quave, 2019; Solis-Salas et al., 2019; Metwally et al., 2017).

Nowadays, 80% world population, have faith on the traditional medicinal flora for the treatment of chronic diseases. Traditional medicine with its evolution through centuries has always fascinated the researchers worldwide for its applications in sustainment and betterment of human health. Common diseases like fevers, diarrhea, bronchial asthma, dysentery, constipation, coated tongue, low sperm count, weak penile erection, piles, hypertension, menstrual disorders and leucorrhoea can be effectively by the use of traditional medicine (Gurib-Fakim, 2006).

This system also ensures limited access to modern biomedicines, owing to their high cost and adverse effects. The aggregative conception of traditional medicine emphasizes on personalized remedy, early diagnosis, disease prevention, and health promotion (Jafari et al., 2014). Approximately 75% of Indian population relies on herbal medicines for their primary healthcare. The important hot-spots for medicinal plants are the Western Ghats, Andman and Nicobar Islands, and Eastern Himalayas. It has been proven in various researches that plants defend themselves against the pathogens by numerous defence responses which include production of membrane interacting proteins, antimicrobial peptides, lytic enzymes, and secondary metabolites (Waterman et al., 2019).

The medicinal plants can treat several diseases and exhibits several biological properties such as anti-fungal, anti-bacterial, anti-malarial, anti-viral, anti-cancer, anti-inflammatory, anti-helminthic, anti-tumor, anti-spasmodic, anti-rheumatic, anti-diabetic, anti-pyretic, anti-fertility, acaricidal, hepatoprotective, anti-parasitic, wormicidal, anti-hypertensive, trypanocidal, emmenagogue, trichomonacidal, diuretic, anti-arthritis, abortive, neuroprotective, immunomodulatory, menopause, dysmenorrhea and attention deficit hyperactivity disorder, analgesic, premenstrual syndrome, anti-ulcerogenic, anti-leishmanial, bile stimulant, anti-plasmodial, anti-nociceptive, anti-venom, anti-epileptic, anti-hyperlipidemic, anti-coccidal, and anti-cholesterolemic, cholagogue, anti-sclerosis, anti-convulsant, vasodilator, and febrifuge, disinfectant, deobstruents, balsamic, choleretic, depurative, emmenagogue, anti-leukaemia and vermifuges, , urine stimulant, anti-biotic, anti-migraine, insecticidal, anti-feedant, abortifacient, anti-herpes virus, anti-leprotic activity, anti-vitiligo activity. anti-psoriatic activity, anti-asthma

activity, anti-depressant activity, oestrogenic and estrogenic activity, immune-modulatory activity, anti-filarial activity, anti-obesity activity, anti-Alzheimer's activity etc., due to the manifestation of countless phytochemicals that fall under the chemical class of amides, alkaloids, saponins, flavonoids, glycosides, esters, terpenoids, tannins, and phenolic compounds, alkylaldehyde, benzofuran, benzopyran, fattyacids, aliphatichydrocarbons, sterols, isoflavone, chalcone, coumesterol, coumarins, dihydrofuran, meroterpene, phenylpropene, phenoliccinnamate etc. The medicinal plants and their preparations possess unique curative properties and are safe, effective, immunity booster, non-toxic, and cost-effective (Salam and Quave, 2019).

Cancer is one of the main cause of death globally and is responsible for an estimated 9.6 million deaths in 2018. The highest cancer occurrence rate has been found in Australia, New- Zealand, Ireland, Hungry, US, Belgium, France, Denmark, Norway, and Netherlands (WHO 2008; Dai and Mumper, 2010). In India, cancer is an underlying root of morbidity and mortality. Among the Indian states, Punjab is called as the 'cancer capital of India' where, 265,000 people in three regions (Malwa, Doaba, and Manjha) suffer from cancer (Burgess 2008). It is a debilitating disease wherein the patient suffers pain and loss of many physiological processes, caused due to various genetic and environmental factors. (Ferlay et al., 2015).

It is completely acceptable that "when we plant a tree, we plant a life". According to U.N, till the year 2050, the worldwide population may reach approximately 10 billion. Our food supplies will be under the great stress and the food demands will increase more than 60 %. In order to feed the teaming millions and to overcome the various biotic (pathogens, insect pests, weeds, herbivores, anthropogenic activities) and abiotic (water stress, soil salinity, temperature stress, heavy metal stress, air and radioactive pollution) constraints, it is crucial to raise new varieties, either through genetic engineering or through conventional breeding for sustainable agriculture and to ensure food and health security (Pental, 2019).

Moringa (family: Moringaceae) is a medicinal plants which has several nutritional, medicinal, and other economical properties. It consists of 13 species namely, *M. ovalifolia*, *M. drouhardi*, *M. peregrina*, *M. borziana*, *M. hilde-brandtii*, *M. ruspoliana*, *M. longituba*, *M. oleifera*, *M. pygmaea*, *M. concanensis*, *M. rivae*, *M. stenopetala*, and *M. arborea* found worldwide (Arora et al., 2013).

M. oleifera (Syn Miracle, Drumstick, Cabbage, Horseradish, and Benzoil tree, and 'Mother's best friend') is native to India, and is also found in Mexico, Niger, China, Haiti, Srilanka, and Pakistan (Alhakmani et al., 2013; Vongsak et al., 2014). It is a perennial, short to medium sized, drought resistant, straight stem tree which attains a height of 1.5–2 m, bears compound leaves (tripinnate) with green to dark green elliptical leaflets and branches grow in a unorganized way to form umbrella shaped canopy and grows well in dry-moist tropical and sub-tropical climates with 760-2500 mm and annual precipitation, 18-28 °C temperature. It grows in almost all soil types but better growth has been observed in clayed with a pH range of 4.5-8. Flowers are white to cream in color, lightly fragrant, borne on 10-25 cm long axillary drooping panicle inflorescence. Fruits are tri-lobed capsules called pods which are pendulous, triangular, both ends are tapering, 9 ribbed almost twenty seeds sowed in pith. They are almost round with brown semipermeable hull of seed having 3 papery wings (Anwar et al., 2007; Prabhu et al., 2011). The mode of pollination is geitonogamous and xenogamous.

Entire parts of the *M. oleifera* are edible and are having multipurpose properties. This plant is extremely valuable because of its enormous nutritional properties like, vitamin A and C (7565 IU; 51.8 mg), calcium (186 mg/100g), magnesium (148 mg/100g), phosphorus (112 mg/100g), potassium (338 mg/100g) and iron (4.1 mg/100g). More than twelve flavonoids including quercetin, kaempherol, pterygospermin, glucoside-glycosides of thiocarbamate and iso-thiocyanate class, moringine, niaziridin, glucoside malonates 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate, and 4-(α-L-rhamnopyranosyloxy) benzyl glucosinolate have been identified from Moringa plant. Among these, quercetin, kaempherol, β-sitosterol and moringine are used in cancer treatment. Thus, there is a propitious future by utilising the *Moringa* plant extracts as anti-cancer agent (Coppin et al., 2013).

Moringa plant has medicinal properties, few are listed as: anti-bacterial (Vinoth et al.,2012; Shailemo et al., 2016), anti-oxidant (Sravanthi and Roi, 2014), anti-pyretic and anti-fungal, (Peixoto et al., 2011), anti- inflammatory (warra 2014; Martinez Gonzalez et al., 2016), anti-spasmodic and anti-hypertensive (Das et al., 2012), anti-ulcer and anti-tumor (Choudhary et al., 2013) anti-epileptic, anti-diabetic, and diuretic (Niranjan et al., 2010; Jaiswal et al., 2013; Jung et al., 2015), cholesterol lowering (Ghasi et al., 2000) and cardiac activities (Metwally et al., 2017).

M. oleifera plays a vital role in water purification and biosorption (Camacho et al., 2016) and be able to used as biopesticide, growth enhancer, (Mahmood et al., 2010) biogas and biodiesel (Azad et al., 2015) production. Apart from these properties, it has industrial values as well (Anwar et al., 2007). Many industries are using it, especially for the cosmetic products such as Moringa -tea, capsules, shampoo, soap, anti-wrinkle cream, and oil (Thurber et al., 2009; Kumar et al., 2012). Further uses of Moringa plant is that it is very helpful for the production of milk to the lactating mothers and so in a way it helps to maintain the proper health of child.

Thus, entire Moringa plant has some or the other use and the extracts of roots, leaves, pods, stem-bark, root-bark, and seeds have medicinal properties, some are discussed below:

Moringa oleifera is reported to contain essential phytochemicals from different parts (seeds leaves, and pods) with estimate ability to provide twenty-five times more Fe than spinach, seventeen times more Ca than milk, fifteen times more K than banana, ten times Vitamin A than carrot, nine times more protein than yoghourt, and seven times more ascorbic than orange (Kruthi and Santhosh, 2016).

To enhance the yield and growth characteristics, besides the Conventional variety of *M. oleifera*, several additional varieties like Jaffna, PKM-2, PKM-1, and ODC, have also been advanced at the HRS (Horticultural Research Station), Tamil Nadu Agricultural University (TNAU), Periyakulam, India.

By raising Moringa in Punjab, the farmers who are caught in tedious patterns of paddy and wheat may get a beneficial choice to ensure their income. *Moringa* is a perennial tree which gets ready for harvest after every 4-5 months and continuously may keep helping the farmers in getting income for several years. Moreover, almost entire plant can be utilized in one mode or the other, even other routine vegetables or crops can be grown in between so as to help farmers

to make multiple profits. People living in the Malwa region of Punjab, has been generally victimized by ‘diabetes’ and ‘cancer’.

Innumerable studies have been done on anti-diabetic, anti-cancerous and many more properties of the Moringa plant. Incorporating its plant parts in our staple diet can help to fight against such wide spreading fatal diseases. Therefore, this study is to compare the anticancer potential of *M. oleifera* varieties like: Jaffna, Conventional, ODC, PKM-2, and PKM-1 leaf extract to HepG2 cell line.

Regular crops of Punjab are tremendously sensitive towards untimely rains and draughts, thereby, causing a risk of heavy losses to the farmers in case of climatic disturbances. On the contrary, *Moringa* tree is a draught resistant plant with sufficient strength to bear climatic changes. Thus, it can be an alternative support to the farmers in bad weathers. As mentioned before, *Moringa* plant parts have potential wide applications in developing herbal tea, health supplements, medicines, cooking oil, cosmetics etc. (Abdulkarim, 2007).
The foliar spray prepared from the seed and leaves of the Moringa has been reported to hasten the growth of multiple plants: legumes (Abohassan et al., 2018), sunflower (Iqbal, 2014), tomato (Culver and Fanuel, 2012; Mvumi et al., 2018), maize (Maswada et al., 2018), etc. by diminishing plant nutrition (macro-nutrient and micro- nutrient) inadequacies and also improves the quality of soil. Thus, Moringa is a propitious substitute source to pricy inorganic fertilizers which benefit the farmers. The present work also focuses on the influence of *M. oleifera* leaf extracts on growth and biochemical properties of *Stevia rebaudiana.*

Stevia rebaudiana a commercial crop, belonging to the family Asteraceae, and is an native to South America (Paraguay and Brazil), commonly known as candy leaf, honey leaf, and sweet leaf of Paraguay. This plant is a perennial herb, grown on sandy and red loam soil (pH 6.5-7.5) and temperature 43ºC. Stevia is popularly known as “artificial sweetener plant”, and is in great demand by consumers due to various benefits. Leaf is the economical part of the plant. Leaves are 70-400 times sweeter than the sucrose, with no carbohydrates or calories. It brings a hope to diabetic people (Elkins, 1997).

The production of more leaf biomass with large amount of bioactive compound is the primary aim of crop production. Thus an experiment was conducted to assess the foliar spray of MLEs on yield of stevioside, zeatin and mineral content of *Stevia*. *Stevia* is cultivated as a cash crop, requires little land and allow farmers to diversify their crops on smaller plots of farmlands for adding their income. So, it becomes necessary to obtain high yielding and good quality *Stevia* from that limited land only.

To achieve the goal, it requires the biostimulant biofertilizer and biopesticide to enhance the productivity of *Stevia*, as they are cost efficient and eco-friendly as compared to chemical fertilizers and chemical pesticides. Some disadvantages are always associated with chemical fertilizers such as, chemical fertilizers comprises of a less mineral content, which produces an imbalance in the plant body (Das et al., 2012).

Also, chemical fertilizers do not improve soil structure when applied without organic additions. sometimes nitrogen from fertilizers gets into water supplies and cause environmental pollution, chemicals application needs proper management as farmers may damage their health by applying fertilizers, pesticides, and herbicides in incorrect manner, further there is a serious problem of chemical pesticides resistance in the target organism but there is no such significant problems with biofertilizers, biopesticides, and biostimulants. Thus, the present work focuses on the potential of *M. oleifera* foliar spray on promoting the mineral zeatin and stevioside content of *S. rebaudiana.*

S. rebaudiana contains large number of secondary metabolites, thus, there leaves produce stevioside and rebaudiosides (zero-calorie ent-kaurene glycosides) which can be utilised as a natural sweetener in beverages and eatables. Steviosides while passing over the digestive process will not chemically shatter-down, which makes the *Stevia* nontoxic for the obese, diabetics, and phenylketonuria (PKU) people (Geuns, 2003; Chatsudthipong and Muanprasat, 2009). *Stevia* leaves contain the phytohormones zeatin (cytokinin) derived from adenine which enhances the growth of buds (lateral and terminal) by cell division (Schafer et al., 2015).

This research can be the boom for the farmers as Moringa leaf extract can be utilized as a potent alternative of expensive chemical fertilizers as well as used as bio-organic fertilizer, biopesticides and biostimulant not only for *Stevia rebaudiana* but also for various crops such as

cash crops as well as food crops, because of its high nutritive value, high productivity, easy preparation, antioxidant effect, eco-friendly nature and low cost.

A comparison of the anti-oxidant, anti-bacterial, anti-cancer activities and growth promoting properties five different varieties of *M. oleifera* intend to reveal the elite variety to be recommended for cultivation in Punjab, for fulfilling the afore-mentioned objectives. Briefly, it can be said that *Moringa* is definitely one of the most important herbal plants which has numerous properties like anti-oxidant, anti-bacterial, anti-cancer and be able to be a strong candidate to enhance the growth characteristics of other plants too. This plant can be an alternate source of income for the farmers as it can grow in the scarcity of water. So, even if a region is hit by the drought, the farmer would not face any problem as this plant will ensure their earnings. Cancer is a very common problem in the Malwa region of Punjab, so growing this plant can be very beneficial for that particular region as this plant has anti-cancerous properties which can be another important topic to be researched on.

Chapter 2

Review of literature

The studies on traditional medicines and medicinal plants have always captivated the herbalists worldwide. The universal conception of traditional medicine emphasises on four points: (1) Health promotion. (2) Disease prevention and (3) Early diagnosis and (4) Personalized treatment. Moreover, the herbal therapies ensure limited access to modern biomedicines, owing to their high cost and side-effects (Patwardhan, 2014).

It has been estimated that more than 60% of synthetic drugs are extracted from the plant and 70% of Indian (1.1 billion) population are use non-allopathic medicines because of the following reasons (1) easy availability (2) low production cost and (3) mildness of the herbal formulations (Mishra et al., 2011; Martinez Gonzalez et al., 2016). Consequently, suitable yeild and sustainable conservation of the 'medicinal plant germplasm' is a necessity, so as to restore the community from numerous illnesses. At the same time accurate standardization of herbal drugs is obligatory in order to upsurge their efficacy towards particular illness such as cancer, diabetes, brain dysfunction etc. (Metwally et al., 2017).

Moringa is one such blessful plants with scientifically confirmed nutritional, medicinal industrial and economical properties. *M.oleifera* belongs to the plant family Moringaceae which includes 13 species (*M. oleifera*, *M. arborea*, *M. concanensis*, *M. drouhardi*, *M. longituba*, *M. hildebrandtii*, *M. pygmaea*, *M. ruspoliana*, *M. ovalifolia*, *M. peregrina*, *M. borziana*, *M. rivae*, and *M. stenopetala*) (Arora et al., 2013). *M. oleifera* is also known by some different names some are given as: drumstick, horseradish, cabbage, benzoil, mother's best friend and miracle tree. This plant is native to India and moreover grown in other parts of the world like Pakistan, China, Srilanka, Niger, Mexico, Haiti, and so on (Alhakmani et al., 2013; Vongsak et al., 2014).

It is a perennial, short to medium sized, drought resistant, straight stem tree which attains a height of 1.5-2.0 m and bears compound (tripinnate) leaves with green to dark green leaflets (elliptical).

Its branches grow in an unsystematic manner to form umbrella shaped canopy. It grows well in a (moist to dry) tropical and subtropical climates with 760-2500 mm of an annual precipitation, at temperatures ranging from 18-28˚C. *Moringa* can grow in almost all soil types but better growth has been observed in heavy clay with a pH range of 4.5-8.0.

Flowers are white to cream color, lightly fragrant, borne on 10-25 cm long axillary drooping panicle inflorescence. Fruits are tri-lobed capsules called pods which are pendulous, triangular, tapering at both ends, nine-ribbed with nearly twenty seeds embedded in the pith. Seeds are almost round, with brown semipermeable seed hull, bearing three papery wings (Anwar et al., 2007).

In order to improve the growth features and yield, besides with the 'Conventional' variety several other varieties like PKM-1', and 'PKM-2', 'Jaffna', and 'ODC' have also been established in India at the HRS (Horticultural Research Station), Tamil Nadu Agricultural University (TNAU), Periyakulam. Jaffna bears soft and tasty fruits (pods). This variety is extensively grown in southern parts of India. The flowers and pods are routinely used in making several culinary preparations. Flowering starts in the month of February-March and pods are harvested in June to July.

PKM-1 variety was developed from pure-line selection which is seed propagated, trees grow in medium or dwarf, growing up to 2-3 m in height. Pods are long (60-70 cm) with girth of 6.3 cm, weighing of 120 g and bears fruits 220-250 per tree. The yield is estimated upto 50-54 tonnes per hectare and is appropriate for ratoon crop. It has les occurrence of insect pest and diseases. This variety is appropriate for different soil types (freely drained) in the tropical plains. 'PKM-2' is a hybrid variety with high production of fruits and oil. It can grow in different types of soils. Its pods are better in taste than other hybrid varieties (Bhatnagar and Krishna, 2013).

ODC is also an improved variety, pollinated openly and suitable for the wild production. It contains a good amount of vitamin C and vitamin A and also contains a high nutritional value. Its seeds have antibiotic, anti-inflammatory properties and are very effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* which are responsible for the skin-infections.

It is also deployed for water purification (Rajangan et al., 2001; Kumar et al., 2012; Kumar et al., 2014). *M. oleifera* possess a blend of nutritional components such as vitamins (A and C), calcium, Magnesium, Phosphorus, potassium, and iron (Oduro, 2008; Mahmood et al., 2010; Saini et al., 2016; Oyeyinka et al., 2016).

More than twelve flavonoids including quercetin, kaempherol glucoside of thiocarbamate and isothiocyanate class, moringyne, pterygospermin, niaziridin, 4-(α-L-rhamnopyranosyloxy) benzyl isothiocyanate, 4-(α-L-rhamnopyranosyloxy) benzyl glucoside malonates and also glucosinolate have been identified from *M. oleifera*. Amongst these bioactive compound kaempherol and quercetin are used to combat cancers. Thus, there is an auspicious future to customize the *Moringa* plant extracts as anti-cancer agent (Vasanth et al., 2014; Jung et al., 2015; Al Asmari et al., 2015).

It is also notorious for other medicinal properties like anti-bacterial (Peixoto et al., 2011; Vinoth et al., 2012; Al- Husnan et al 2016; Shailemo et al 2016), anti-pyretic (Patel et al., 2014) anti-malarial (Prabhu et al., 2011), anti-inflammatory, (Warra, 2014; Martinez- Gonzalez et al., 2016). *M. oleifera* showed strong scavenging activity on DPPH, superoxide, inhibition of lipid peroxidation and nitric oxide radical (Sreelatha et al., 2009; Al hakmani et al 2013; Sravanthi and Roi, 2014; Wang et al., 2017), Hepatoprotective (Das, et al., 2012), anti-ulcer (Verma et al., 2012; Choudhary et al., 2013), anti-diabetic (Niranjan et al., 2010), and cholesterol lowering and cardiac activities (Metwally et al., 2017).

M. oleifera plays a vital role in water purification and biosorption (Camacho et al., 2016), and can be deployed for plant growth enhancement, (Mahmood et al., 2010), biodiesel and biogas fabrication (Azda et al., 2015). Countless industries are developing cosmetic stuffs such as *Moringa*-tea, *Moringa*-capsules, *Moringa*-soap, *Moringa*-oil, *Moringa*-shampoo, *Moringa*-anti wrinkle cream, etc. (Anwar et al., 2007; Thurber et al., 2009; Oyeyinka; Razis et al., 2014; Chasi, 2015). Dyes extracted from the bark of Moringa cultivars possess mild antimicrobial properties.

This property can be used to promote textile industry to produce organic clothing and special safe fabrics for new borns and infants. It has been testified that intake of *Moringa* leaf powder has helped the lactating mothers in improving the quality and nutrient content of the milk to maintain the proper health of their child (Bhatnaga and Gopalakrishna 2013). *M. oleifera* possess ample amounts of antioxidants that inhibit the assembly of free- radicals and shield the human body against different dreadful diseases (Sreelatha et al., 2011). There has been a rise in the number of life threatening infections initiated by the pathogenic micro-organisms which is becoming the main cause of mortality in the developing countries (Shailemo et al., 2016). Large numbers of reports are existing which describe the antibacterial activity of Moringa (Al-Husnan et al., 2016).

M. oleifera comprises of polysaccharide arabinogalactan (MOP-1) which exhibits the best antioxidant potential like prevention of hydrogen atom abstraction, decomposition of peroxides, free radical scavenging, hindrance of chain initiation, and sinking the capacity and binding of transistional metal in catalyst (He, 2018). The hydrogel was prepared from the *M. oleifera* gum polysaccrides by radiation induced crosslinking for the gentle discharge of model drug ciprofloxacin which is used as antibiotic for the cure of innumerable infections caused by different strains of bacteria, in the human being (Singh and Kumar, 2018). The extent of antioxidants and antibacterial

properties vary with the plant parts and the type of solvents used for extracting the active components.

By growing *Moringa* in Punjab, the farmers who are confined in repetitious cycles of wheat and paddy, may get a profitable alternative to ensure the earnings. *Moringa* is a perennial tree which gets ready for harvest after every 4-5 months and continuously may keep helping the farmers in getting income for several years. Moreover, almost entire parts of the plant can be utilized in one mode or the other, even other routine vegetables or crops can be grown in between so as to help farmers to make multiple profits. Population in Punjab specially, in the region of Malwa has been mainly persecuted by diseases like cancer and diabetes. Several researches have confirmed anti-cancerous and anti-diabetic potential of *M. oleifera*. Incorporating its plant parts in our staple diet can help to fight against such wide spreading fatal diseases. Regular crops of Punjab are tremendously sensitive towards untimely rains and draughts ,thereby, causing a risk of heavy losses to the farmers in case of climatic disturbances.

On the contrary, *Moringa* tree is a draught resistant plant with sufficient strength to bear climatic changes. Thus, it can be an alternative support to the farmers in bad weathers. As mentioned before, *Moringa* plant parts have potential wide applications in developing herbal tea, health supplements, medicines, cooking oil, cosmetics etc. (Abdulkarim, 2007; Oyeyinka, 2016; Muhammad et al., 2016). Multifarious products from the similar plant may lead to search many economic endeavors for entrepreneur startups of Punjab. Pulverized seeds of Moringa can be used to purify contaminated water with minimal cost, thus helping rural and poor communities to afford clean drinking water. This thesis focuses on the antibacterial, antioxidant, anticancer, and plant growth promoting characteristics of *M. oleifera*.

2.1. Worldwide distribution of *Moringa oleifera*

M. oleifera is a reputed member of family Moringaceae. It is native of North West India (sub-Himalayan regions) and South America. It is also grown in many countries like- Central and South America, South East Asia, Arabia, West Indies, Islands East and West Africa, Caribbean and Pacific (Alhakmani, et al., 2013; Vongsak, et al., 2014).

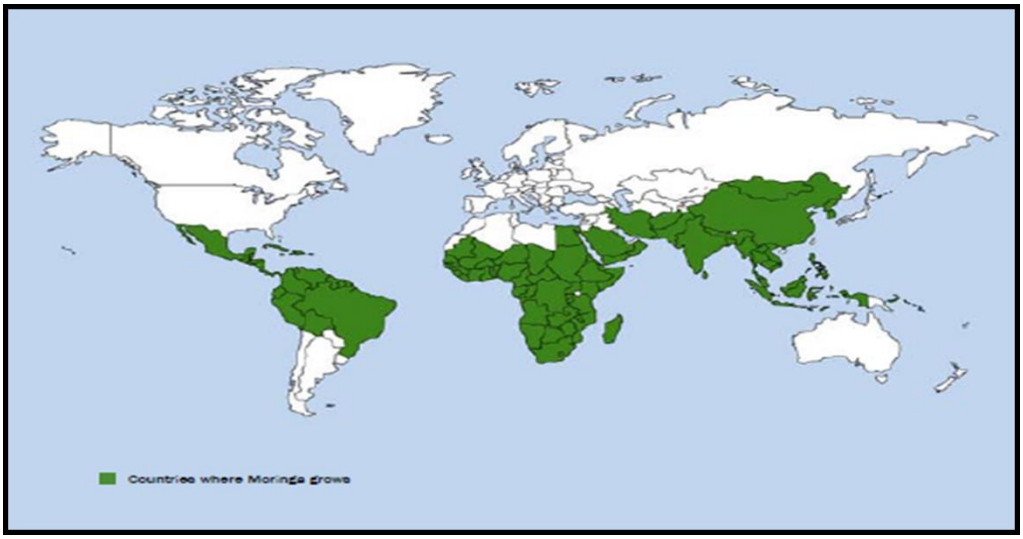


Figure 1: Worldwide distribution of *Moringa oleifera*

About 33 species have been reported and among these 13 species are well-known and found worldwide. The most common species are *M. oleifera*, *M. aptera*, and *M. concanensis* (Stohs and Hartman, 2015). *M. oleifera* is a reputed member of this family and is a multipurpose softwood-tree. Different varieties of *M. oleifera* have been developed in India, so as to improve the characteristic features and yield. The different varieties are ODC, Jaffna, Conventional, PKM-1, and PKM-2. These varieties possess improved pod, and leaves production and are expansively grown in southern region of India (Radovich, 2011).

2.2. Taxonomic position

Kingdom - Plantae
Division - Magnoliophyta
Class- Magnoliopsida
Order- Capparales
Family- Moringaceae
Genus- Moringa
Species- oleifera

2.3. Botanical description

Moringa or drumstick belongs to the Moringaceae family. It is perennial, evergreen, and short to medium sized tree. It is also draught resistant tree. Its stem is straight which attain a height of 1.5-2 meter. Leaves are bipinnate or tripinnate compound with light green to dark green 1-2 cm elliptical leaflets. Its branches grow in disorganized manner to form umbrella shaped canopy. Its flowers are bisexual and yellowish-white in colour. *Moringa* fruits referred as pods. It is generally green in colour but when it get mature it turn into brown in colour. It possesses tuberous root. Moringa leaves are 45 cm long which can be bipinnate or tripinnate. They are alternate and spirally arranged. Flowers of Moringa are little aromatic, yellowish to whitish in colour, slender having stalks of hairy texture having long axillary dropping panicle inflorescence. Fruits are dangling trilateral pods having nine ridges which are longitudinal in nature. The seeds of Moringa are round which are 1 cm in diameter with brown semipermeable seed hull and three whitish papery wings on the sides (Anwar et al., 2003). It is perennial, short or medium sized, drought resistant, straight tree having height of 10 to 12 m. It basically has open crown of drooping branches which are fragile in nature (Vinoth et al., 2012). The mode of pollination is geitonogamous and xenogamous .The xenogamous is more prominent than geitonogamous mode. Bees are the pollinators of which Xylocopa (major pollinator) and Amegilla carry the pollen on the head or thorax to accomplish the nototribic pollination.

2.4. *Moringa oleifera* varieties

Different varieties of *M. oleifera* have been developed Botanical description of ODC, Jaffna, conventional, PKM-1, and PKM-2, and are mentioned below in table 1.

Table 1: Botanical description of the *Moringa oleifera* varieties used in the present study.

Variety	Type	Plant height	Leaves	Flower	Pods	Seed germination (%)	Yield	Reference
PKM-1	Pure line selection, prepared by continuous selfing for six generations introduced from Eppothumvendran of Tirunelveli region	4-6 m	Tripinnate	white or creamy-white	Long: 75 cm Girth: 6.3 cm; Weight: 150 g	55.77	220 fruits/tree	Gopalakrishnan et al., (2016); Katoriya et al., (2019)
PKM-2	Hybrid variety improved over PKM-1, introduced from Eppothumvendran local and Arasaradi local	6-7m	Tripinnate	white or creamy-white	Long: 126 cm; Girth: 8.3 cm' Weight: 280g		220 fruits/tree	Lalas and Tsaikins, (2008)
ODC	Indigenous perennial variety introduced from ottdanchatra, place near Maduria, called as drumstick hub of Tamil Nadu	8-10 m	Tripinnate	white or creamy-white	Long: 2-2.5 feet; Girth: 2.5 inch; Weight: 70 -80 g		200 fruits/tree	
Jaffna	Yazphanam type of Moringa introduced from Sri Lanka	4-6 m	Tripinnate	white or creamy-white	Long: 60-90 cm; with soft flesh and delicious taste		600 pods/tree	Bhatnagar and Krishna, (2013)
Conventional	Natural type of Moringa	10-12 m	Tripinnate	white or creamy-white	Long: 60 cm; Girth: 6.2 cm; Weight: 95-100g		300 pods/tree.	Booth and Wickens, (1988)

2.5. Nutritional properties

Moringa plant parts, such as leaves, flowers and pods are consumed as they contain abundant nutrients such as manganese, phosphorus, iron, β-carotene, vitamin B and C etc. It is very beneficial for the human health benefits. Commonly people use the Moringa leaves in culinary preparations and they crush the leaves and prepare different types of foods such as soups, juice, sauces etc. *Moringa* fruits are also used as vegetable and they contain high vitamin C and B content.

Many parts of it are edible such as immature seed pods, leaves, mature seeds and they are potent reservoir of calcium, iron, proteins and ascorbic acid (Moyo et al., 2011). *Moringa* plant is extremely valuable because of its countless nutritional properties such as vitamins, calcium (2009.00 mg/ml), iron (28.29 mg/ml), copper, manganese, zinc, selenium, and magnesium (Oyeyinka et al., 2016).

Growth hormone called as zeatin derived from the leaves, can be used as a commendable foliar spray and can rise the crop production by 25%-30% (Fuglie, 1999). Tiny dry leaves of the miracle tree really shows their miracle as they are rich in iron (almost 25 times more than spinach), vitamin C (10 times more than red grapes and oranges), potassium (10 times more than banana), fibre (4 times more than oats), protein (3 times more than egg), vitamin E (3 times more than spinach), calcium (10 times more than milk), iron (3 times more than almonds), polyphenol (8 times more than red wine), protein (2 times more than milk). Moringa plant parts comprises of different nutrients that are listed in table 2.

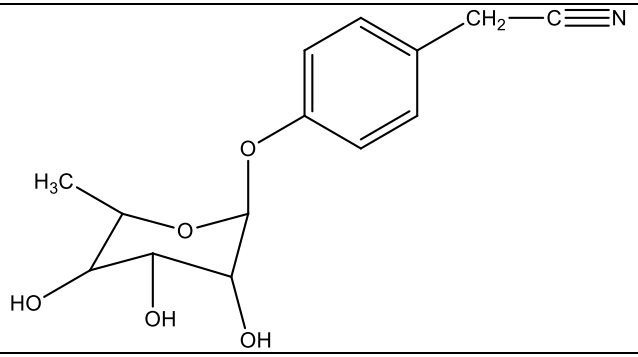
Table 2: Nutritional composition of leaves seeds and pods of *Moringa oleifera* (per hundred gram)

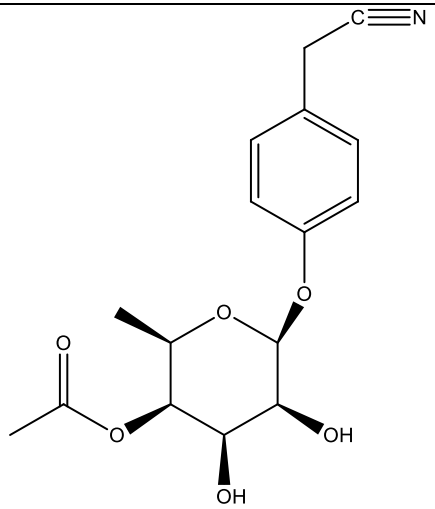
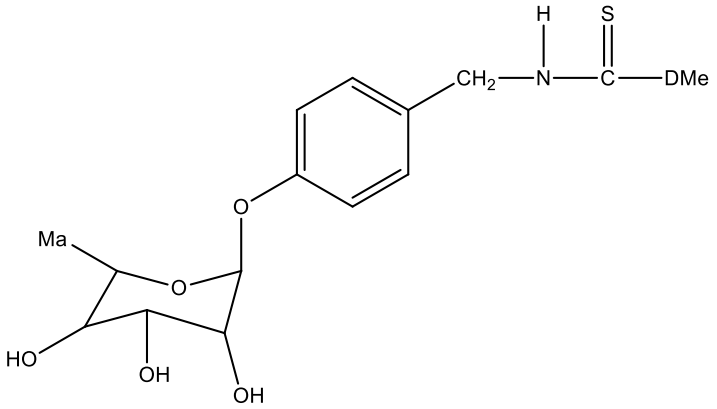
Nutrients	Dry leaves	Fresh leaves	Leaf powder	Seeds	Pods
Calories (cal)	328	93	208	-	27
Proteins (g)	29.7	6.5	27.9	35.37	2.8
Fats(g)	5.4	1.9	2.5	38.57	0.2
Carbohydrates	41.1	12.9	35.2	8.65	3.8
Fibres(g)	12.4	0.8	19.6	2.89	4.7
VitaminB1	2.02	0.07	2.60	0.04	0.06
VitaminB2 (mg)	21.8	0.04	20.6	0.03	0.09
VitaminB3 (mg)	7.8	0.9	8.1	0.3	0.1
Vitamin C (mg)	15.3	230	17.5	4.4	130
Vitamin E (mg)	10.6	450	119	721.67	-
Calcium (mg)	2184	450	2004	47	31
Magnesium (mg)	449	44	361	695	25
Phosphorus (mg)	251	69	202	79	120
Potassium (mg)	1286	269	1354	-	249
Copper (mg)	0.47	0.08	0.57	5.10	3.2
Iron (mg)	25.9	0.83	28.2	-	5.1
Sulphur (mg)	-	-	870	0.05	136
β-carotenoid(mg)	39.68	-	-	-	-
Lutein(mg)	102	6.94	-	-	-
Total phenol (mg)	6160	-	-	-	-

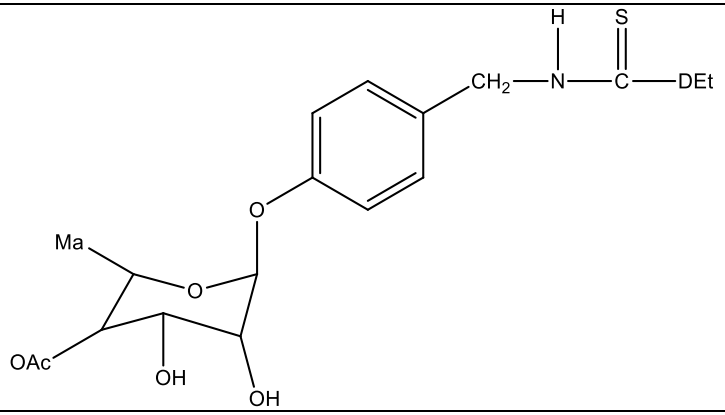
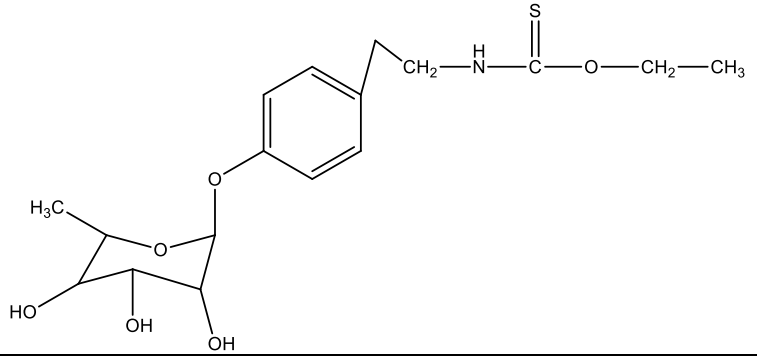
2.6. Bioactive compounds

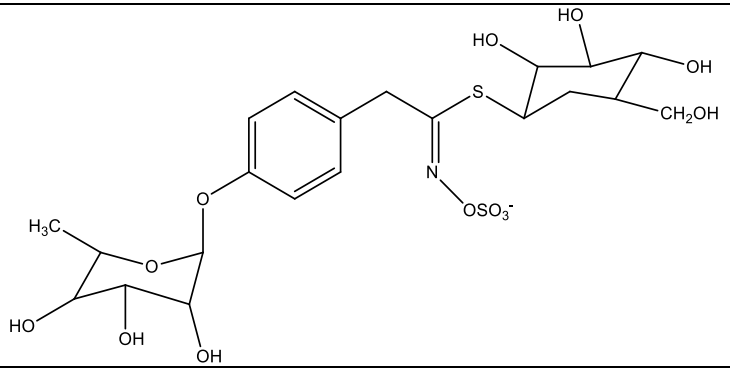
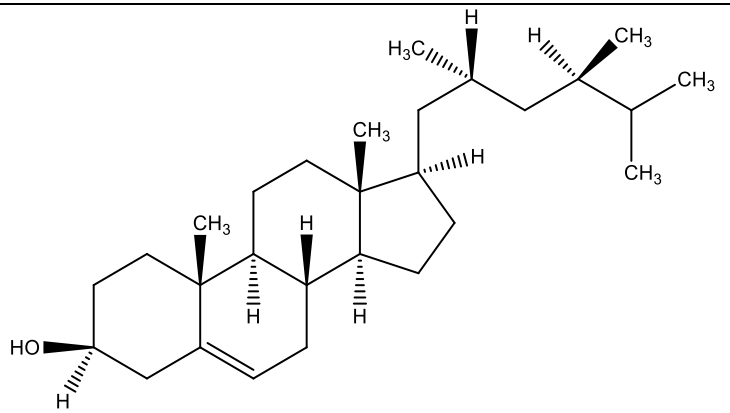
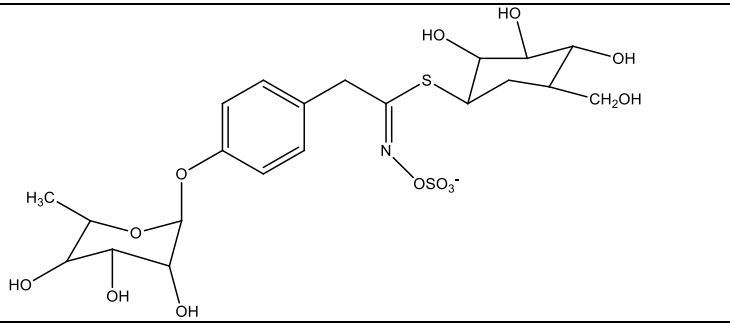
Apart from the vitamins and minerals *Moringa* leaves contain a several minerals, amino acids and fatty acids and it also contains various anti-oxidant compounds like ascorbic acid, flavonoids, phenolic, carotenoids, glucosinolates, tocopherols, oxalates and tannins enlisted in table 3.

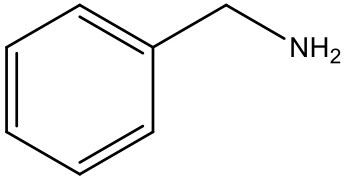
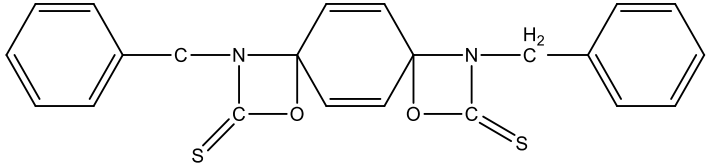
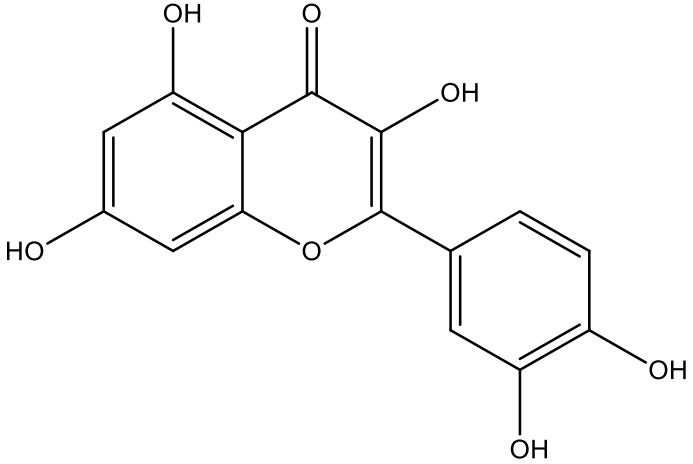
Table 3: Phytochemical constituents reported in various parts of *Moringa oleifera*

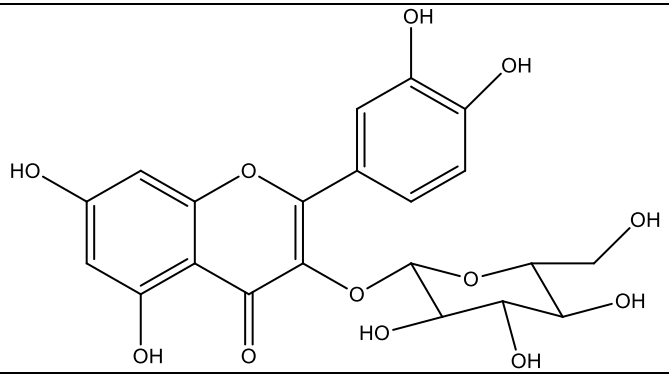
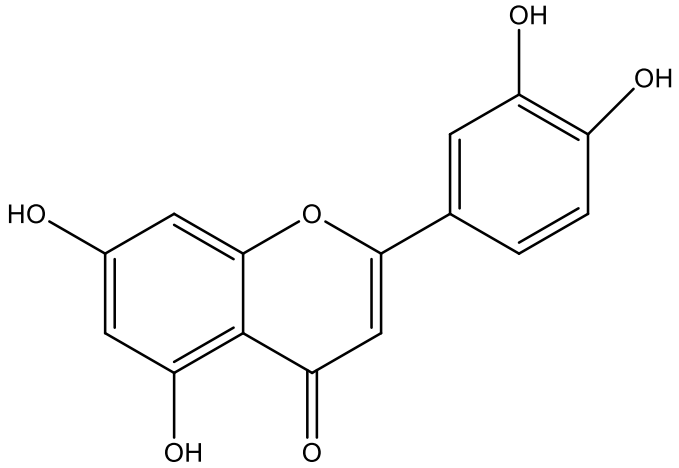
Plant part	Bioactive compound	Biological activity	Chemical structure	Reference(s)
Leaves	Niazirin	Hypotensive activity		Bennett et al. (2003), Kumar et al. (2012), Anwar et al. (2007), Farooq et al. (2012)

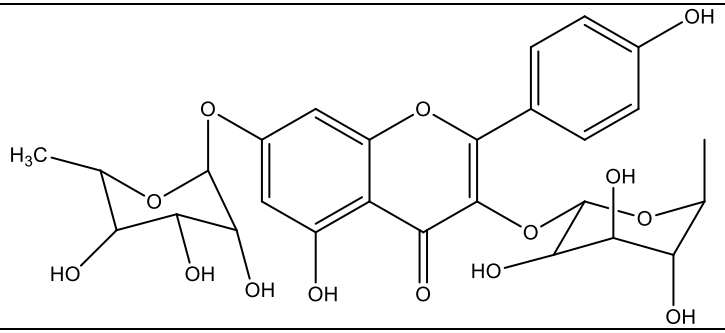
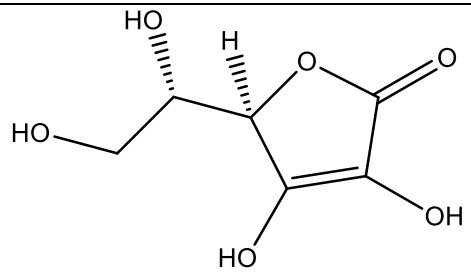
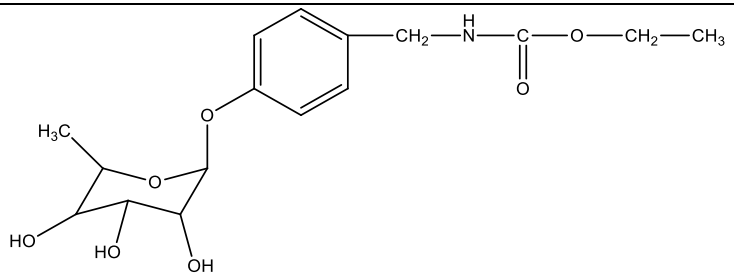
	Niazirinin			
	4-(4′-O-acetyl-α-Lrhamnopyranosyloxy) benzyl isothiocyanate	Antibacterial, Antitumor, Anticancer, Antiulcer, Antispasmodic, Antihyperthyroidic, Estrogenic, Hypoglycaemic, Hypocholesterolemic,Purgative and Abortifacient		

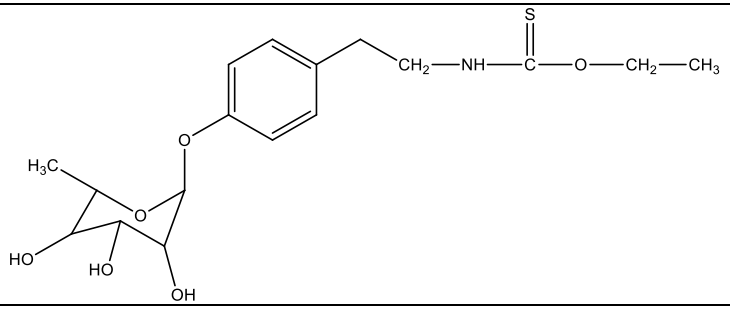
		properties		
	Niaziminin A and B	Antitumor and Hypotensive activity		
	Niazimicin	Antispasmodic, Anticancer, Antibacterial, Antitumour		

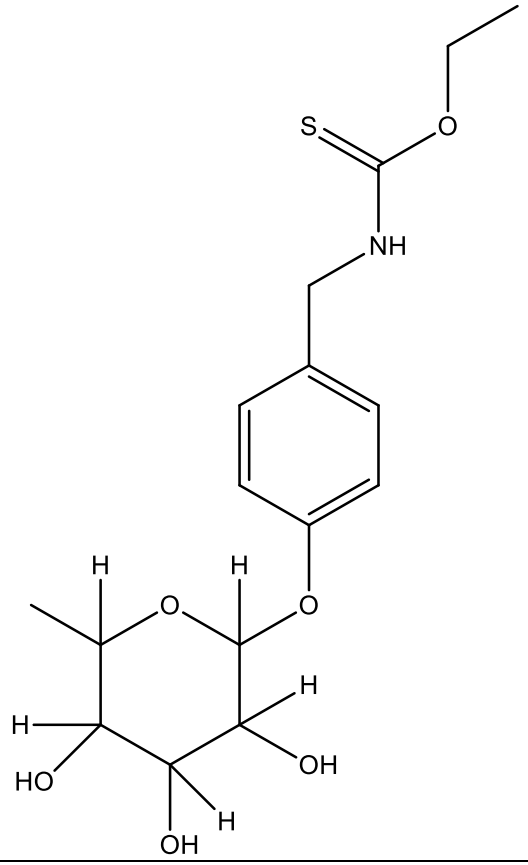
Bark	4-(α Lrhamnopyranosyloxy)benzyl glucosinolate	Hypotensive, Anticancer, Antibacterial, Antifungal activity		Anwar et al. (2007)
Stem	β-sitosterol	Anticancer and hypotensive activity		Anwar et al. (2007), Ruckmani et al. (1998), Kumar et al. (2010), Anwar et al. (2007)
	4-(α-Lrhamnopyranosyloxy)benzyl glucosinolate	Hypotensive, Anticancer, Antibacterial and Antifungal activity		

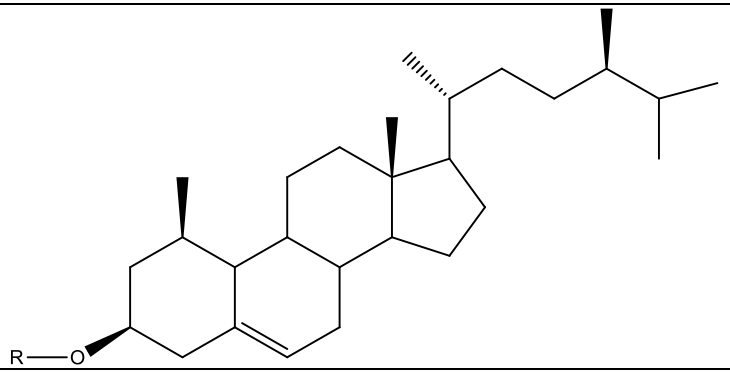
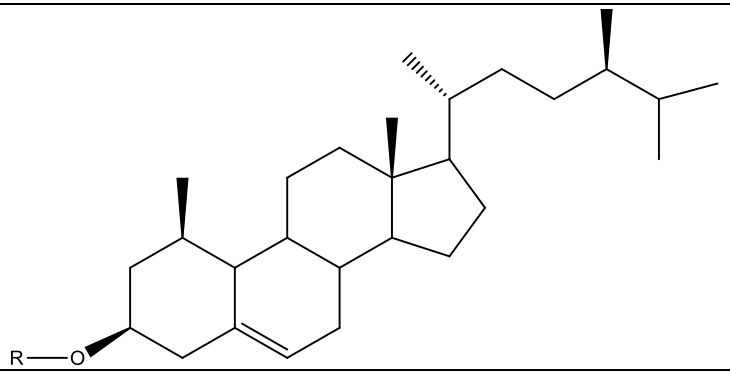
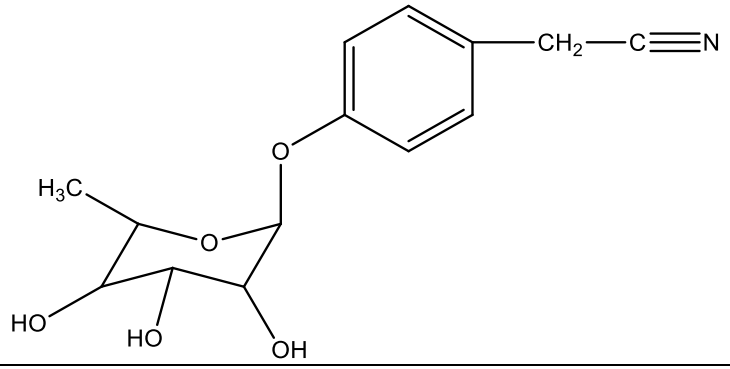
Roots	Moringine	Cardiac stimulant, hypolipidimic bronchodilator activity		
	Pterygosperm in	Antimicrobial, antibacterial and Anti-fungal properties		
Flower	Quercetin	Antioxidant and Hepatoprotective activity		Ruckmani et al. (1998), Anwar et al. 2007)

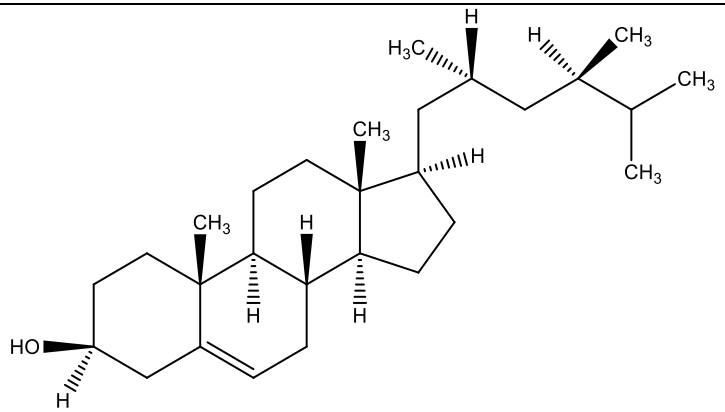
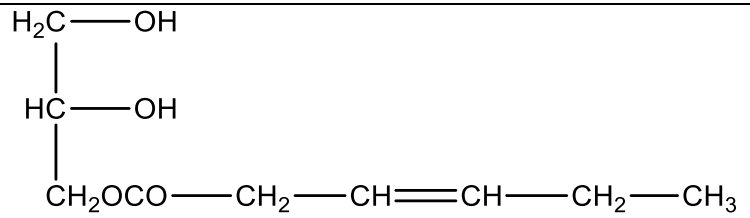
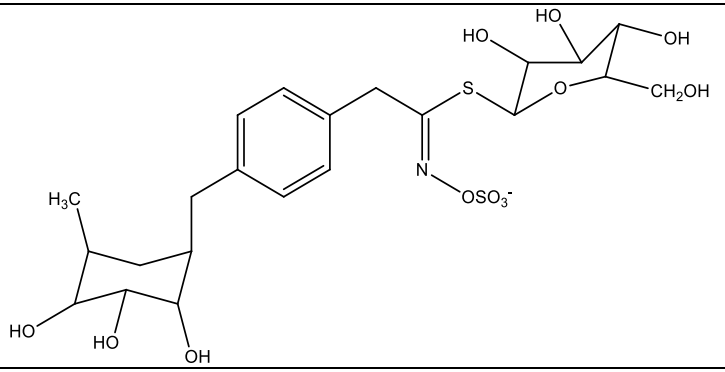
	Isoquercitin	Antioxidant activity		
	Kaempherol	Antioxidant activity		

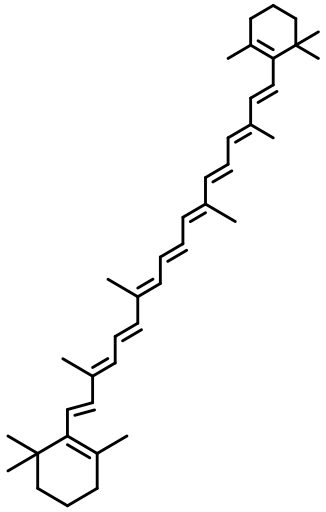
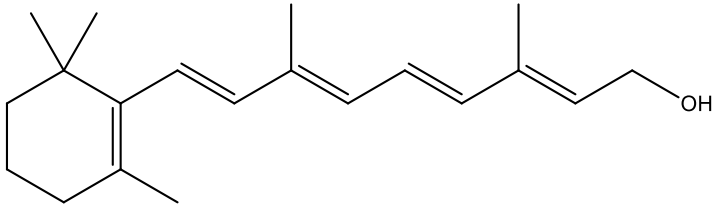
	Kaempferitrin	Anti-inflammatory anti-oxidant, and anti-microbial and		
	Ascorbic acid	Antioxidant activity		
	O-ethyl-4-(α-Lrhamnosyloxy)benzyl carbamate	Antitumor activity		

Pods/Seeds	4(α- L-rhamnosyloxy)-benzyl isothiocyanate	Antitumor, Antimicrobial, Hypotensive, Anticancer and Antibacterial activity		Kumar et al. (2010), Anwar et al. (2007)

	Niazimicin	Antispasmodic, Anticancer, and Antitumour		

	3-O-(6′-O-oleoyl-β-Dglucopyranosyl)-β-sitosterol	Antitumor and Anticancer property		
	β-sitosterol-3-O-β-Dglucopyranoside	Anticancer property		
	Niazirin	Hypotensive activity		

	β-sitosterol	Anticancer property		
	Glycerol-1-(9octadecanoate)	Anticancer property		
	4-(α-Lrhamnopyranosyloxy) benzyl glucosinolate	Hypotensive, Anticancer; Antibacterial and Antifungal activity		

	β-carotene.	Antioxidant activity		
	Vitamin A	Prevents maternal mortality, blindness, enhances immunity to fight infections enhances lactation (breast milk);		

2.7. Medicinal properties

Each and every part of the *Moringa* possesses medicinal properties. Leaves used as antipyretic, antioxidant, hepatic tonic, diuretic, etc. and it is also used for thyroids disorders, diarrhoea, dysentery (Kodia et al., 2014). Stem used as rubefacient, vesicant and it is also used to protect the eyes from the eye diseases. It is used for common cold, digestion etc. Root has anti-inflammatory, rubefacient, antilithic properties and it is very beneficial for the common cold, fever, asthma, inflammation, kidney pain etc. (Fahey et al., 2001; Gupta et al., 2013).

M. oleifera comprises of polysaccharide arabinogalactan (MOP-1) which exhibits the best antioxidant potential like prevention of hydrogen atom abstraction, sinking the capacity and binding of transistional metal in catalyst, free scavenging, and decomposition of peroxides (He, 2018). The hydrogel was prepared from the *M. oleifera* gum polysaccrides by crosslinking induced by radiation, for the gentle release of model drug (ciprofloxacin an antibiotic) for the cure of countless bacterial infections in the humans (Singh and Kumar, 2018). The extent of antioxidants and anti-bacterial properties vary with the plant parts and the type of solvents used for extracting the active components.

Table 4: Medicinal properties of Moringa extracts.

Plant part extract	Medicinal uses	Bioactive compound
Leaf juice	Antibacterial activity, Antidiabetic, Prevent cardiovascular disorder, Antihypertensive	Thiocarbamate glycosides, Niazinin A, Niazinin B
Aqueous leaf extract	Antioxidant activity, Antitumor activity, Antihyperthyroidism, Antibacterial activity	4(a-L- rhamnosyloxy)- benzyl isothiocyanate, O-EthyM-(a-L-rhamnosyloxy) benzyl carbamate, 3-0-(6'-O-oleoyl-P-D-glucopyranosyl)- P-sitosterol, Niazinin, Niazimicin, Moringine
Methanolic leaf extract	Antibacterial activity, Antiulcerogenic activity, Radioprotective, activity to bone marrow, Hepatoprotective effect	
Ethanolic leaf extract	Antihypertensive effect, Antibacterial activity	
Aqueous seed extract	Biosorbent and turbidity reductant, Hypotensive effect, Metabolizing essential enzymes, Antimicrobial activity	Campesterol, P-sitosterol, 3-0-(6 ’ -0-oleoyl-P-D-glucopyranosyl)- pi-sitosterol, Stigmasterol, 4(a-L-rhamnosyloxy)-benzyl isothiocyanate, Clerosterol, Moringine
Ethanolic seed extract	Antimicrobial activity, Skin papillomagenesis, Flocculant,and pH correctant	
Aqueous flower extract	Diuretic and hypotensive activity,	Quercetin,

	Hepatoprotective effect	Pterygospermin
Ethanolic flower extract	Fungicidal, Antibacterial activity Anthelmintic activity	
Ethanolic extract of pod	Antispasmodic, Hepatoprotective effect, Diuretic	Pterygospermin, 4-a-L-rhamnosyloxy-benzyl isothiocyanate
Ethanolic and Chloroform bark extract	Antimicrobial activity	S-ethylthioformate, P-sitostenone, Moringine, 4-hydroxymellin, Aglycone of deoxyniazimicin (N-benzyl)
Gum purified and degraded extract	Diuretic, Hypotensive	L-mannose (degraded), Galactose, L-galactose, L-arabinose, Glucoronic acid

2.8. *Moringa* cultivation

In tropical region ploughing is important for high planting. But in low type of planting its dig to the soil and sow the seeds. For Moringa cultivation, the soil should be loamy, sandy loam type and the soil pH is 5-9. July-October months are suitable for the plantation (Alhakmani et al., 2013).

2.9. Moringa tissue culture

Germination percentage of *Moringa* seed is not good so that the propagation of *Moringa* can be from seed or cutting parts. For vegetative propagation the length of cutting should be 1 m length and at least 4 cm in diameter (Vongsak et al., 2014).

2.9. Other uses

Apart from the medicinal properties *Moringa* used as a vegetable like soup, honey, in sambar, pizza etc. flowers are used as a salad and in tea making also. Gums are used in leather tanning. *Moringa* used as plant growth enhancer, biosorption and water purification, biodiesel, biopesticides and in biogas also.

2.10. Future prospects of *Moringa*

M. oleifera is the most widely cultivated tree and entire parts of its are very useful in the medicinal field (Rosenberg, 1992; Farber, 1994; Chitra et al., 2002). *M. oleifera* flowers have been utilized for medicinal as well as nutritional value and is consumed as a salad (Farber, 1994; Sharma et al., 2011). A huge number of investigations have been undertaken so far to verify its vital properties and health benefits. *M. oleifera* has unending scope for research and medicinal properties. Sreelatha et al., 2009, analysed the anti-oxidant potential and TPC of tender leaves and mature leaves of *Moringa*. Among the two types of leaves (mature and tender), the mature leaves were contain highest TPC and anti-oxidant activity.

Tsaknis et al., 2001, extracted *Moringa* oil from the seeds of *M. oleifera* (variety Mbololo) from Kenya and compared it with expensive virgin olive oil. They measured the viscosity, acidity, density, acidity, iodine value, color, smoke point, saponification value, tocopherols and high levels of unsaturated fatty acids, oleic acid followed by palmitic acid. High stability was shown by *Moringa* seed oil to oxidative rancidity. *Moringa* seed oil is economically better choice as it was found to be equivalent to virgin olive oil. Gopalakrishnan et al. (2016), described that the *M. oleifera* is a miracle tree and has a blend of nutrient, anti-oxidant, anti-bacterial, anti-cancer and other multipurpose properties for the human health benefits.

Sravanthi et al. (2014), described the medicinal properties of *Moringa* plant which is found in world wide. This study was conducted for evaluation of anti-oxidant (flavonoids, flavones, phenols, β-carotene, and free radical scavenging activity) in *Moringa* by different types of assay: DPPH, ABTS, FRAP assay and with the help of catalase, glutathione reductase to evaluate the enzymatic activities. Leaves showed higher phenol content and maximum anti-oxidant activity found in FRAP assy. Qwele et al. (2013), evaluate the scavenging activity of different solvent extracts of *Moringa* leaves with sunflower oil. They used methanolic and acetone extracts of leaves with refined and bleached sunflower oil. It was concluded that the anti-oxidant property of *Moringa* is due to efficient content of flavonoids, phenols and tochopherol.

Mishra et al. (2011), conducted a study that provides important information about *M. oleifera* phytochemicals properties, pharmacological properties and medicinal uses in anxiety, anaemia, blackheads, asthma, bronchitis, chest congestion, impurities of blood, cholera, joint pain, headaches, cough, diarrhoea, infection of ear and eye, abnormal blood pressure, fever, and tuberculosis. Faizi et al. (1994), reported the extraction of niazirinin and niazirin (nitrile; gycosides) and [4-(4'-O-acetyl-alpha-L-rhamnosyloxy) benzyl] isothiocynate, niazirinin A and niazirinin B (mustard oil gycosides). As a mixture from remaining extract, both niazirin A and niazirinin B have been previously obtained but niazirinin is a new compound while from this source 4-[(4'-O-acetyl-alpha-L-rhamnosyloxy) isothiocynate] is novel. By using spectroscopic method like appropriate 2D nmr experiments and chemicals reactions, structural determination was obtained.

Shailemo et al. (2016), from the dicholoromethane extract and methenolic crude crude extract obtained from the capsules of *Moringa* antibacterial activity was reported. From column chromatography of capsules of *Moringa* the same activity was determined by agar well diffusion. No activity was detected against *E. coli*, *S. aureus*, *P. areginosa*, and *K. pnemoniae* by the methanolic crude extract. Antibacterial activity was observed by isolated parts from column chromatography and purified dichloromethane extract.

Anwar et al. (2005), analysed *Moringa* seed oil grown in temperate regions. They use hexane as solvent; the oil content ranged from 38-42%. The protein, fibres, and ash content were 26.50-32.00%, 5.80-9.29%, and 5.60-7.505% respectively. Tocopherols (δ, γ, and α) were found to be sufficient amount along with high levels of oleic acid followed by behenic, palmitic, arachidic, and stearic acid up to levels (7.00, 7.50, 5.99, and 4.21% respectively).

Siddhuraju et al. (2003), analysed the anti-oxidant activities of different solvent extracts of phenolic ingredients from various agro-climatic source of *Moringa*. The main compounds of phenols were established to belong to the flavonoid groups such as kaemopherol and quercetin. *Moringa* leaves are famous as a great source of natural anti-oxidants. Health stimulating secondary metabolites ('glucosinolates, phenols and flavonoids') was also found in Moringa leaves for human health benefits.

Pari and Kumar (2003), analyse the hepato-protective influence of *M. oleifera* leaf ethanolic extracts on liver damage prompted by anti-tubercular drugs [Rifampicin (RMP), Isoniazid (INH), and Pyrazinamide (PZA)] in rats. Leaves extract of *Moringa* exhibited a substantial protective action to the liver. The findings of this study indicated that the treatment with the leaf extracts of *M. oleifera* gives the protective effect from hepatic damage.

Santosh et al. (2005), identified natural coagulation in *M. oleifera* for water treatment. Seed extracts were explored the presences of lecithin, trypsin inhibitor, tannin and anti-oxidant activity. Tannins or trypsin inhibitor were found absent. The anti-oxidant component reduced DPPH (1, 1-diphenyl-2-picrylhydrazyl radical) was found to be tender than catechin and was found to be thermostable.

Jayaprakasam et al. (2005), studied insulin excretion by bioactive compounds (anthocyanins and anthocyanidins) present in the fruits of *Moringa*, which have been found to be involved in a declined in 'coronary heart disease' and in anti-diabetic measures. The 'Pelargonidin-3-galactoside' is one of the foremost anthocyanins, its aglycone, and pelargonidin, results by a 1.4 fold, enhances in insulin production at 4 mM (glucose concentration). The result confirmed its role in the cure of diabetes.

Ghasi et al. (2000), described anti-diabetic effects of *M.* oleifera leaf extract on glucose acceptance in Wistar rats and Goto-Kakizaki. *Moringa* induced substantially decrease in the blood glucose levels of Wistar rats. The overall action of *Moringa* leaf extracts was found higher in Goto-Kakizaki rats as compare to the Wistar rats.

Shanker et al. (2007), used reverse phase HPLC method to estimate bioactive compounds like; niazirin, and nitril 'glycosides-niaziridin' and in the pods, bark, and leaves of *M.* oleifera. Niaziridin (0.015% and 0.039%) and niazirin (0.038% and 0.033%) were found to be present in leaf and pod respectively. Niaziridin and niazirin absent in bark, but, moderately higher in amount of niazirin in leaves than the pods, while niaziridin content was established almost 3 times more in the pod than the leaf.

Parvathy et al. (2007), explored cytotoxic effect of various extracts of *M. oleifera* leaves on 'cell lines' (human multiple myeloma). *Moringa* methanolic leaf extracts exhibited least viability at higher dose. Heredia et al. (2008), studied azo dye removal from seed extracts of *M. oleifera*. The study was mainly fixated on testing the exclusion of an azo dye [Chicago sky Blue 6B (CSB)]. Less CSB %age removal is achieved as compare to the control.

Agrawal et al. (2008), accompanied clinical trials to confirm the role of *M. oleifera* in curing bronchial asthma. It is stated that produce worthy clinical response in persons, suffering from chronic rheumatism, upper respiratory tract infection, asthma, and other allergic disorders. An alkaloid extracted from *Moringa* plant called moringine closely resembles ephedrine in exploit and is found beneficial in the treatment of asthma by causing relaxation of bronchioles.

Karadi et al. (2008), found that anti-urolithiatic activity from the aqueous, methanolic and ethanolic extract of *M. oleifera* root bark. Kidney retention and urinary excretion levels of calcium, oxalate, and phosphate were significantly lowered by these extracts. Moreover *Moringa* extracts significantly reduced the increased serum levels of urea nitrogen, and creatinine, ureic acid. Heredia et al. (2009), tested *Moringa* seed extract for carmine indigo dye. The kinetics of high capability of this coagulant agent to manage water wastes from dye stuff has been exposed. *M. oleifera* has been found to work well in flocculation and coagulation process and it attains a normal removal (almost 80%). Temperature has a negative influence and coagulant process does not affect by the pH. By accumulative the initial dye concentration, lower dye %age removal is obtained.

Jaiswal et al. (2009), described anti-diabetic properties of *M. oleifera* aqueous leaf extracts on glycaemic control, urine sugar, haemoglobin, urine protein, total protein, and body weight. Rastogi et al. (2009), described that anthelmintic property of ethanolic *M. oleifera* extracts against Indian earthworm *Pheritima posthuma*. Results stated in terms of the paralysis and the for the death time of worms under various concentration of *Moringa* extracts. Distilled water was used as control group where 'piperazine citrate' (10 mg/ml) was taken as a reference standard.

Amagloh et al. (2009), investigated the role of mature dried *Moringa* seeds in water purification. Completely randomized planned with different loading dose, (4, 6, 8, 10, and 12g/l) of processed *Moringa* seed powder and aluminium sulphate were used as coagulants. The results obtained from 12 grms/litre treatment of *Moringa* and 10and 12 grms/litre alum treatments were quite acceptable as per WHO guidelines.

Thurber et al. (2009), described the significant role of *M. oleifera* in the treatment of malnutrition, healing and other disease due to its nutritional rich leaves with high protein quality. The "diffusion of innovation theory" describe the growth and adoption of *Moringa* as dietary supplement but at the same time calls for scientific consensus on its nutritional benefits.

Patel et al. (2010), reviewed medicinal and economic importance of *Moringa*. The study focused on various parameters including medicinal uses, phytochemical

composition laterally with pharmacological properties of various parts of *Moringa*. Broad spectrum activities were shown by this plant, so further studies on other models and extensive clinical trials are required to confirm the findings.

Kasolo et al. (2010) in Ugandan rural communities, they studied the uses and phytochemicals of *M. oleifera* leaves. The powder of *Moringa* leaves was used to treat adequate malnutrition in children at health facilities. To established medicinal uses of *Moringa* was used to treat several common diseases by the local communities of Uganda. Presence of phytochemicals like steroids, triterpinoids, flavonoids, tannins, anthraquinones, alkaloids, saponins, and reducing sugar in the leaf extracts directs the preventive and curative properties of *Moringa* leaf.

Amalog et al. (2010), studied phytochemicals in altered tissues from reproductive and vegetative parts of *M. oleifera* grown in Ghana. Acetyl-rhamnose and rhamnose substituted glucosinolates were appeared in entire tissues, in addition to gucosides, rutinosides and malonylglucosides. Fatty acid profiling established that leaves were rich in palmitic acid (16:0) and linolenic acid (18:3). Seeds were found rich in oleic acid (18:1), roots were found rich in oleic and palmitic acid whereas stems were found rich in 'palmitic acid, potassium, magnesium and calcium' were found in entire tissues whereas traces of selenium was found in whole seeds only.

Prabhu (2011), estimate the pupicidal and larvicidal capability of methanolic extracts from *M. oleifera's* methanolic seed extracts against malaria vector 'Anophelese stephensi'. The phytochemicals derived from its seed extracts were found to be active mosquito vector control and the plant extracts can be utilised in pest management programs.

Luqman et al. (2012), investigated that the influence of *Moringa's* extracts (fruit and leaf) on markers of 'oxidative-stresses', correlation with anti-oxidant properties practice in vivo and in vitro assays and its toxicity evaluation. The fruit ethanolic extract showed 'strong reducing power' 'highest phenolic content', and 'free radical scavenging activity'. The anti-oxidant property of leaf and fruit ethanolic extracts was greater in vitro assay, as compared to aqueous extract of both the parts, which exhibited higher potential in vivo. Result support the potent anti-oxidant activity of *M.*

oleifera which add-ons one more positive character to its notorious pharmacological status.

Moyo et al. (2011), studied nutritional value of *Moringa* leaves in South Africa ecotype. The dried leaves supported to possess crude proteins (30.3%), calcium (3.65%), phosphorus (0.3%), sodium (0.164%), sulphur (0.63%), potassium (1.5%), copper (8.25%), zinc (13.03 mg/kg), magnesium (0.5%), magnese (86.8) and selenium (363) mg/kg. 17 fatty acid s were also detected including Linolinic acid (44.5%). Beta carotene content was observed to be 18.5 mg/100g whereas vitamin A content was 77 mg/100g in dried leaves. Thus a desirable nutritional balance of proteins, vitamins, minerals, fatty acids has been found in Moringa leaves.

According to many reports the leaf extract of *Moringa* had capability to induced apoptosis and having anti proliferative property on many cell lines (Sreelatha et al., 2011), and also enhances the cytotoxicity on pancreatic cancer cells during chemotherapy (Berkovich et al., 2013). So, furthermore research can be done on different type of cancer cell line as its main aim is to induce apoptosis to those growing cancer cells as *Moringa* shows effectiveness to cancer cell lines.

More than twelve flavonoids (thiocarbamate and isothiocyanate, quercetin, and kaempherol) have been identified from *M. oleifera*. Among these twelve flavonoids, kaempherol and quercetin are used to cure a dreadful disease called as cancer. Thus, there is a hopeful future for the use of M. oleifera extracts as anti-cancer agent (Al Asmari et al., 2015)

Atawodi et al. (2010), studied *Moringa* for its therapeutic, anti-cancerous, anti-oxidant properties and confirmed its chemoprotective potential against cancer. Its different parts were analysed for polyphenol content *in vitro*. Its methanolic extract contained different bioactive compounds such as rutin, quercetin, chlorogenic acid, kaemopherol rhamnoglucoside, moreover, in the root and stem barks, more than a few procyanidin peaks were found. The extraordinary anti-oxidant effects were tested for different parts of *Moringa* offers validation for their broad therapeutic usage in traditional medicine in diverse continents.

Bose (2007) studied possible role of roots of *M. oleifera* in cancer ('epithelial ovarian cancer'). A compound which causes the 'epithelial ovarian cancer' has been assumed and the part of FSHR has been demonstrated also. The results verified the role of *M. oleifera*. In the dealing of 'epithelial ovarian cancer' is worth exploring.

2.11. Growth promoting activity of *Moringa oleifera*

Moringa leaf, stem, seed extract are used as foliar spray and accelerates the growth of numerous plants such as sunflower (Iqbal, 2014), wheat (Yasmeen et al., 2013), maize (Sana et al 2015), tomato (Culver and Fanuel, 2012) etc by improving plant nutrient deficiencies and elevating their soil. Thus, become potent alternative to expensive inorganic fertilizer and benefited to farmers.

Different scientists have researched on its nutritional value, its phytochemistry, and its medicinal values are also examined for human benefits so that it can be used as herbal medicines and can be a potent alternative of chemical drugs that are usually associated with some side-effects. Also, its ability of water purification has been evaluated by many scientists, also its ability to act as biopesticide has been appraised by many scientists.

This review focuses on its effect on other plants rather than human or animal, thus present research, describes the effect of plant growth promoting characteristic of *Moringa* on other plant such as *Stevia rebaudiana.*

Different scientist had worked on this aspect of *Moringa* in different ways on different plants. However, this will be the first time that this study will evaluate the effects of growth promoting features of five different varieties of *M. oleifera* on plant *Stevia*.

Table 5: Reports associated with the plant growth promoting characteristics of *Moringa oleifera*.

Plant	Investigation	Methodology	Finding	Citation
Bitter Gourd cv. CO	*Moringa oleifera* leaf extract, $CaCl_2$, KNO_3, and panchakavya (seed priming agent)	'Emergence % of bitter gourd cv. CO seeds'	100 per cent achievement in pre-germination of bitter gourd seeds when priming was done in panchakavya.	Thirusenduraselvi and Jerlin (2007)
Maize seedlings (R-33330	Foliar spray of *Moringa oleifera* leaf extract, benzylamino purine (BAP) on Maize (drought stressed seedlings R-3333)	Chlorophyll and phenolic content, relative water content estimation	MLE increased all the biochemical parameters	Ali et al. (2011)
Hybrid maize seeds cv Dekalb-5219	*Moringa oleifera* leaf extract, BAP (benzyl aminopurine), ascorbate, $CaCl_2$ (seed priming agent)	Chlorophyll and phenolic content estimation	Seeds of plants primed with MLE showed maximum improvement in seedling emergence and seedling vigour followed by ascorbate priming.	Basra et al.(2011)
Pisum sativum				Yasmeen (2011)

(pea)	Foliar spray of MOLE and BAP	Phenolic and chlorophyll and content, relative water content estimation	MLE treatments were effective in enhancing germination and seedling growth attributes of pea plant as compared to other treatment	
Moringa oleifera leaf extract, kinetin, KCl as seed priming agent	*M. oleifera* leaf extracts, kinetin, KCl (seed priming agent)	Priming with *M. oleifera* leaf extracts was more productive in inducing chilling tolerance in maize as compared to other treatments.	Chlorophyll and total soluble sugar estimation	Afzal et al. (2012)
Brassica napus	Foliar spray of *M.oleifera* leaf extract	Root, shoot and dry weight, and plant height	Highest height, root weight, , dry, and crop production in the greenhouse and field experiments were found with MLE treatment	Culver et al. (2012)
Solanum lycopersicum (Tomato)	Foliar spray of *Moringa oleifera* leaf extract	Plant height, Dry matter (DM), and root dry matter weight	Moringa extract improved all the yield parameters of the tomato	Culver and Fanuel (2012)

			plant	
Spring maize (FH-810)	*Moringa oleifera* leaf extract, salicylic acid and $CaCl_2$ (seed priming agent)	Emergence time, cell membrane permeability and relative water content estimation	Priming of seed with SA and $CaCl_2$ improve temperature stress resistance of spring planted maize as equated to other priming treatments.	Rehman et al. (2012)
Triticm aestivm (wheat)	Foliar spray of *Moringa* leaf extract, H_2O_2 and BAP on wheat (salt stressed)	Chlorophyll content evaluation, SOD, CAT, POD assay	MLE application improved the grain weight (18.5 %) and kernel yield (18.5 %)	Yasmeen et al. (2012)
Triticum aestivum (wheat)	Foliar spray of *Moringa* leaf extract on wheat (late sown)	Yield parameters: biological yield, grain weight, harvest index, and grain yield	grain weight, biological yield, grain yield, and harvest index, increased by 11.73%, 7.00%, 11.70%, and 5.00% in 1000 respectively	Yasmeen et al. (2013)
Phaseolus vulgaris L.(Common	MOLE as seed priming agent in salt stressed	Chlorophyll, Sugars, Proline, Leaf mineral,	Presoaking treatment	Rady et al.(2013)

Bean)	*Phaseolus vulgaris*	Glycine betaine, ascorbate, glutathione content estimation	improved growth, yield and antioxidants, and detoxified the stress generated by NaCl (100 mM)	
*Phaseolus vulgaris L.(*Common Bean*)*	Foliar spray of *Moringa oleifera* leaf extracts in *Phaseolus* (salt and cadmium stressed)	Photosynthetic pigments and protein content estimation	MLE foliar spray diminish the stress created by NaCl and CdCl2 and increase the Growth and yield	Howladar (2014)
Triticum aestivum(wheat)	*Moringa oleifera* leaf extract, $CaCl_2$ and distilled water as seed priming agent	Chlorophyll ,flavanoid, phenolic, ascorbic acid content estimation	MLE improved germination and seedling growth wheat over the control.	Yasmeen et al. (2013)
*Triticum astivum*cv.Sehar -2006 (wheat)	Foliar spray of *Moringa oleifera* leaf extracts and BAP on wheat plant (drought stressed)	Estimation of chlorophyll, ascorbic acid, protein content and SOD, POD, and CAT assay	*Moringa* leaf extracts mitigate the damaging effects of drought by improving antioxidant levels and secondary metabolites as	Yasmeen et al. (2013)

			compared to BAP treatment	
Brassica napus (Canola)	Foliar spray and seed priming agent of *Moringa oleifera* and *Brassica* leaf extract	Leaf area, biological yield, seed weight, seed yield, and harvest index	Maximum seed yield (2942 kg /ha), biological yield (13721 kg/ ha) and harvest index (21.44%) were testified in treatment of combination of both the leaf extracts.	Iqbal (2014)
Spring maize	*Moringa oleifera* leaf extract, ascorbic acid (ASA) ,SA and H_20_2 (seed priming)	Estimation of germination rate, index, length of root and shoot and seedling dry weights	seeds treated with MLE+H give better performance followed by SWE+ASA+SA+ H, SWE+ASA, and ASA+SA+H	Imran et al. (2013)
Allium cepa (onion)	Foliar spray of *Moringa oleifera* leaf extract of different concentrations	Leaf number and plant height estimation	All the parameters showed increase in case of highest concentration MOLE treated plant.	Mohammed et al. (2013)

Helianthus annuus L. (hybrid Hyson-33)	Foliar spray of *M.oleifera* leaf extract of various concentrations	Plant height, biological and economic yield, leaf area index, head diameter, no of achene per plant, and harvest index.	20% *Moringa* leaf extract sprayed at 40 DAS improved all agronomic factors and gave the highest economic yield (2.96 t/ha)	Iqbal (2014)
Paraserianthes falcataria (Falcata)	MOLE as foliar spray (priming agent)	Dry weight, height, root-shoot ratio, and fresh shoot weight	All factors increased on MLE application	Gilbero et al. (2014)
Sesamum indicum (Sesame)	Foliar spray of MLE	Height, fresh and dry shoot weight and root-shoot ratio	3 % *Moringa* extract along with 90 kg N ha-1 are reported to improve grain yield	Mahumma and Mohammad (2014)
Maize	Foliar spray of *Moringa oleifera* shoots extract and nitrogen	Leaf area index	Mixture of both the stimulators gave highest yield	Mahumman et al. (2014)
Solanum lycopersicum (Tomato)	Foliar spray of *Moringa oleifera* leaf extract and benzyl aminopurine	Protein, chlorophyll, phenolic, lycopene content and CAT, SOD,	Foliar-applied MLE produced maximum	Yasmeen et al. (2014)

		and POD assay estimation	flowering and branches, heaviest fruits per plant, large no of flowers and also enhanced studied parameters in comparison with synthetic BAP.	
Triticum aestivum (wheat)	Foliar spray of *M.oleifera*, rice, sunflower canola, and sorghum water extracts	Leaf area indices, harvest index, yield, grain yield, and CGR	Application of 2% MOLE as a foliar spray gives 40 times higher grain yield of wheat than control and other treatments.	Afzal and Iqbal (2015)
Maize hybrid 32-F10	Foliar spray of *M.oleifera* leaf extract and kinetin solution	Chlorophyll, height and number of grain, starch, protein, oil and leaf content estimation	Foliar spray of MLE showed best results compared to kinetin solution	(Bakhtavar et al. 2015)
Cynodondactyln (Turf Grass)	Foliar spray of *M. oleifera* , seaweed, sunflower, and sorghum, on *Turf Grass* (heat stressed)	Height, shoot weight and root length, leaf area index, and tiller estimation	*Moringa* and seaweed extracts proved most effective in improving all	Bashir and Qadri (2015)

			selected parameters	
Capsicum annum (Sweet bell pepper)	Foliar spray of Moringa and fertilizer (organo-bio degrable)	Estimation of height, leaf number, fruit weight, and number	mixture of *Moringa* Leaf Extract and fertilizer increased the yield of studied parameters	Dunsin et al. (2015)
Sorghum bicolor	Foliar spray of *M.oleifera* and *M. stenopetala* leaf juice	Plant height, and grain yield, above ground and root biomass , number of tillers per plant estimation	No significant difference observed yield parameters with either of the foliar as compared to control	Gessesse et al. (2015)
Glycine max (forage soybean)	MOLE, tap water and urea, (seed priming agent)	Germination rate, weight, length of root and shoot estimation	*Moringa* leaf extract priming improved the studied parameters.	Iqbal et al. (2015)
Phaseolus vulgaris(Beans)	*Moringa oleifera* leaf extract and salicylic acid	Leaf pigment, relative water content, electrolyte proline, sugars, ascorbic acid,	Foliar spray with MLE + Seed saturated in SA treatment found to	Rady and Mohamed (2015)

		leakage, leaf mineral content estimation	be the best	
Solanum lycopersicum (tomato)	Foliar spray of drumstick fermented leaf juice (DFLJ) with Jeevamrit solution and Humic acid	Number of leaves, flowers, root length and shoot length and biomass estimation	Increase in studied parameters by Jeevamrit+ drumstick Fermented Leaf Juice + consortium + Humic acid than control.	Rajamani et al. (2015)
Pyrus (pear)	*Moringa oleifera*, garlic extract and licorice extract	Chlorophyll, leaf minerals, sugars, ascorbic acid content estimation	All the studies parameters improved by MOLE	El-Hameid et al. (2015)
Sommondsiachin ensis (Jojoba)	Foliar spray of *Moringa* leaf and putrescine	Chlorophyll ,flavanoid, phenolic, amino acid and tannin, content estimation	All studied parameters were showed increased in all the contents by *Moringa oleifera* leaf extract compared to putrescine	Taha et al. (2015)
Zea mays (maize)	*Moringa oleifera* leaf	Leaf number, area,	MLE, kinetin,	Chattha et al. (2015)

	extract, humic acid, kinetin, Salicylic acid, ascorbic acid, Neem seed powder extract	height and biological yield, starch and total phenolic content	humic acid, and ascorbic acid improved quality growth yield and of maize while salicylic acid and neem seed powdered extract. was not so much effective in action	
Phaseolus vulgaris (common bean)	Moringa, IAA, vitamins E and C, gibberllic acid, *Trichoderma harzianum*, thyme and lemon grass oils	Height, diameter of stem, leaf no., proline phenolic content and APX, SOD, and CAT activity estimation	100% suppression of the disease was recorded with a treatment of combination of IAA, GA and *T. harzianum* 105 cfu/ml.	Abdel-Kader et al. (2016)
Cultivar CIM-573 of cotton and Bt cotton	Foliar spray of *Moringa oleifera* leaf extract and mepiquate chloride	Number of bolls per plant estimation	No. of bolls per plant increased by *Moringa* leaf extract application.	Yasmeen et al. (2016)
Spinacia oleracea (spinach)	Foliar spray of *Moringa oleifera* leaf extract, BAP	Malondialdehyde (MDA), chlorophyll ,	MOLE treatment enhanced	Aslam et al. (2016)

	and HA as foliar spray	phenolic, protein, content and DPPH free radical scavenging assay	phenolic, antioxidants and other biochemicals.	
Raphanus sativus (Raddish)	*Moringa*, sorghum mulberry, and brassica leaf extract	Phenolic and carotenoids content, bioactive compounds, radical scavenging capacity (RSC), dietary fiber, moisture, crude protein estimation	MOLE increased the phenolic content (54.51–182.71 mg GAE/g f.w) and bioactive compounds (37.65 ± 0.94%), as compared to other plant leaf aqueous extracts	Ashraf et al. (2016)
Maize hybrid SB-11.	Foliar spray of dry (MDLE) and fresh (MFLE) Leaf and Flower (MFE) on maize (heat stressed)	H_2O_2, phenolic and vitamin content analysis	MDLE was the most effective followed by MFLE and MFE.	Batool et al. (2016)
Super sweet - 100 cherry tomato	*Moringa* leaf extract, trans-zeatin (t-Z) and BAP	Shoot number, root length, and height protein, sugars, lycopene, and proline content estimation	MLE enhanced tomato plant biomass, yield, and quality of fruits and other factors as	Basra (2016)

			compared to other treatment	
Cucumis sativus (cucumber)	Fresh and stored *Moringa oleifera* leaf extract and coconut water (CW) (priming agent)	Emergence time and final emergence, root length, seedling fresh and dry weight	MLE treatment reduced the mean emergence time and improving the final emergence % and CW treatment give maximum root length and seedling fresh and dry weight	Dunsin et al. (2016)
Parkia biglobosa	Foliar spray of *Moringa*, coconut water, and honey	Number of rooted cuttings, length of longest root	Moringa expressively increased total number of roots, and their roots	Dusin et al. (2016)
Alstonia scholaris	*Moringa oleifera* leaf extract on *Alstonia scholaris* (cadmium stressed)	No. of leaves, height, root length, and stem diameter, carbohydrate, nutrient content and pigment and estimation	Considered parameters were increased with the MOLE treatment and overcome the dangerous and destructive of effect of different	Hashish et al. (2016)

			concentrations of cadmium.	
Brassica napus (Canola)	leaf extracts of *Moringa oleifera and Brassica napus*	Weight, leaf area indices, yield of seed, and harvest index estimation	Highest yield recorded when treated with three sprays of *Moringa* and *Brassica* water extracts mixture	Iqbal et al. (2016,)
Triticum aestivum (Wheat)	Foliar spray of MOLE	biological yield (straw and grain), yield efficiency, 1000 grain weight, and protein content estimation	Plants sprayed with 4% of *Moringa* extract showed increase in all selected parameters	Merwad et al. (2016)
maize (Hybrid)	*Moringa oleifera*, maize rice and sorghum extracts	Number of grain, height, protein, starch, chlorophyll and oil contents	*Moringa* and sorghum extracts in combination enhanced maize yield compared to other sprays	Mohamed et al. (2016)
Pisum sativum	MLE and Magnetized Water as seed priming agent	Root and shoot growth, total soluble sugars and α-amylase assay	Combination of magnetised water and *Moringa* leaf	Noor et al. (2016)

			extract improved germination capacity, stand establishment and seedling vigor compared with the control	
Helianthus annuus (Sakha53)	*Moringa oleifera* leaf extract (seed priming agent)	Protein, chlorophyll, phenolic, and POD, SOD, CAT activity estimation	foliar spray of MLE improved the growth and yield of sunflower (salt stressed)	Taha (2016)
Hybrid maize Dekalb-6789	*Moringa oleifera* leaf extract, Salicylic acid (SA) Sorghum water extract (SWE) and thiourea (T) on hybrid maize	Chlorophyll, relative water content and phenolic content estimation	a *Moringa oleifera* leaf extract, Salicylic acid (SA) enriched TPC, RWC and chlorophyll contents as compared to others	Waqas et al. (2017)
Sorghum bicolor L.	Foliar spray of *Moringa oleifera* leaf extract	Hormonal and mineral content estimation	Productivity increased by	Abusuwar and Abohasan (2017)

			MOLE foliar spray under harsh environment of salinity and aridity.	
Spring maize (hybrids PS525: heat tolerant)	MOLE, triacontanol, kinetin, and silver nitrate	Chlorophyll, cell membrane permeability and relative water contents estimation	Maximum positive results were obtained in hybrid (PS525) by foliar application of tri-acontanol and by MOLE	Ali et al. (2017)
Lagerstoemia indica	MOLE, seaweed, and Humic acid on *Lagerstroemia indica L.* seedlings (salt stressed)	Plant height, stem diameter, leaf number, and branches, root length, and dry weight Phenolic, and proline, content and CAT, SOD, and APX activity estimation	*Moringa* leaves extract (MOLE) improved vegetative growth, and chemical compositions of treated plant	Soliman and Shanan, (2017)
Trigonellafoenum-graecum (Fenugreek)	MOLE on Fenugreek plant (salt stressed)	Height of plant , number of leaves, diameter of stem, proline. Phenolic content and APX, SOD, CAT and activity estimation	MLE increased all the studied parameters	Latef et al. (2017)

Triticum asetivum(wheat)	Foliar spray of seeds powder of *Moringa oleifera* and *Moringa peregrena* on wheat (cadmium stressed)	root and shoot length, and weight, protein and chlorophyll content estimation	*Moringa* defatted seeds pre-treatments, diminished cadmium toxicity by 3 and 2-folds in shoots and roots, respectively	Hassanein et al. (2017)
Tomato *(Solanum lycopersicum)*	MLE were sprayed on *Alternaria solani* infected (IN) and non-infected (NIN) tomato (Solanum lycopersicum)	Determination of lamina thickness, stomatal density, stomatal size and yield of Tomato	MLE increased lamina thickness, stomatal density, size of stomata and production of Tomato	Mvumi et al. (2018)
Alfalfa, Clitoria, and *Mung bean*	MLE as foliar spray	Number and weight of pods, seed dry weight	MLE increases the production of all studied parameter	Abohassan et al. (2018)

In this research, the effect of 'plant growth promoting' characteristic of different varieties of *M. oleifera* leaf extract as a foliar spray will have appraised on *Stevia rebaudiana* plant in greenhouse experiment setup.

2.12. *Stevia rebaudiana* and its benefits

Stevia rebaudiana (perennial bush) belongs to Asteraceae family, native to specific areas of South America. Its leaves create 'zero calorie ent-kaurene' glycosides such as stevioside and rebaudiosides which are sought after as non-nutritive and high intensity sweetener in eatables and drinks by the people experiencing diabetes and obesity. The expanding interest in herbal treatment for diabetes requests for extraordinary cultivating of *S. rebaudiana* to expand the generation of its low-calories glycosides.

According to the severa studies, there are many glycosides, particularly stevioside, significantly relies upon the aggregate biomass created, which thus relies upon horticultural performances for development of *Stevia* plants. In quest for high creation of glycosides, specialists have embraced current agro-procedures (Geuns 2003; Das et al. 2010), water administration (Fronza and Folengatti 2003) and fertilizers applications. Likewise, tissue culture methods and development of *Stevia* plants in bioreactors have additionally been tried. Moreover, in the overheaded practices, the fundamental inadequacies are the great expenses and little in-field practicability.

Further, chemical fertilisers organized a few mineral nutrients and generate an imbalance in the complete mineral pattern of the plant body by delaying the uptake of supplementary useful nutrients. Subsequently, the proficient alternative for these techniques that recommended are the use of arbuscular mycorhiza and phosphate solubilising microbes.

In this study, *M. oleifera* leaf extract has been used as biofertilizer or biostimulant for defensible cultivation of *S. rebaudiana.* This will be the first time when plant extract has been used for growth promotion of *Stevia*.

Stevia rebaudiana is an herb that is used extensively in various areas of the world as a non-caloric sugar substitute. As *Stevia rebaudiana* are in demand by consumers due to various benefits, *Stevia* farming has been increased. *Stevia* farming offers a profitable crop for

thousands of farmers in South America, Asia, and Africa. It is cultivated as a cash crop on minor scale of farmlands for added income. *Stevia* requires little land and permits farmers to spread their crops, As *Stevia* is normally grown on little plots of land, so it become necessary to obtain high yielding and good quality *Stevia* from that limited land only.

Thus, it requires the biostimulant, biofertilizer and biopesticide to enhance the productivity of *Stevia* as they are cost efficient and ecofriendly as compared to chemical fertilizers and chemical pesticides because some disadvantages are always associated with them such as chemical fertilizers have rare mineral nutrients and can produce an imbalance in the mineral procedure of the plant by obstructing the uptake of valuable nutrients (Das et al., 2010), also, chemical fertilizers do not improve soil structure when applied without organic additions, sometimes nitrogen from fertilizers gets into water supplies and cause environmental pollution, chemicals application needs proper management as farmers may damage their health by applying fertilizers, pesticides and herbicides in incorrect manner, further there is a serious problem of chemical pesticides resistance in the target organism but there is no such significant problems with biofertilizers, biopesticides and biostimulants. Thus provides supplemental income to the "cash" crops and generate the improved *Stevia rebaudiana* plant with increased content of stevioside and rebaudiosides.

2.12.1. Distribution of *Stevia rebaudiana*

Stevia rebaudiana is native to Paraguay and Brazil. This plant belongs to family Asteraceae. It is also identified as compositae. It is grown well in Rajasthan, Maharashtra, Kerela, Orissa and also in Punjab (Lemus-Mondaca et al., 2011). This plant has made agricultural impact in various countries all the world includes Japan, Canada, China, Korean, USA, Mexico, Russia, Australia, Taiwan, Abkhazia ,Thailand (Yadav et al., 2011).

2.12.2. Habitat

Stevia is popularly known as "artificial sweetener plant". It can grow in the temperature of 6ºC-43ºC.The average temperature of growth of plant is 23 ºC (Brandle et al., 1992). The soil pH ranges from 6.5-7.5 and grown on fine drained red and sandy loam soil (Lemus-Mondaca et al., 2011).

2.12.3. Botanical description

It is a perennial herb. It is having extensive root system in which roots are fibrous and filiform. The stem is brittle and subligneous. The leaves are small, lanceolate, and elliptical having blunt-tipped lamina. They are having the alternate leaf arrangement. They are sweet to taste which are non-calorific .There are white tiny florets which are perfect. They borne in the little corymbs of 2 to 6 florets and they are arranged in loose panicle. The seeds are about 3mm in diameter and borne in achenes. It is propagated by stem cuttings (Brandle et al., 1992).

2.12.4. Chemical constituents

It is used basically for its leaves which is source of sweet-ent-kaurene diterpenoid glycosides (Karimi et al., 2015).The major glycoside is Stevioside. It sweetness of this glycoside is relative sucrose is 210. Other glycosides are rebaudioside A, Rebaudioside C and its sweetness is relative to sucrose are 243 and 30 respectively (Brandle et al., 1992)).

This research can be the boon for farmers as *Moringa* leaf extract can be utilized as a powerful alternative of expensive chemical fertilizers as well as used as bio-organic fertilizer, biopesticides and biostimulant not only for *Stevia rebaudiana* but also for various crops such as cash crops as well as food crops due to its high nutritive value, extraordinary productivity, antioxidant effect, low cost, easy preparation, and eco-friendly nature.

Chapter 3

Objectives

- To compare the antioxidant activities of different varieties of *Moringa oleifera* Lam.
- To analyse the antibacterial potential of different varieties of *M. oleifera* extracts.
- To study the anticancer potential of *M. oleifera* extracts on specific cancer cell line.
- To analyse the plant growth promoting activity of *M. oleifera* varieties.

Chapter 4

Methods and material

5. Material and Methods

5.1 Material

- In the present study all the chemicals were purchased from Qualigens, India, Sigma, USA, and Hi Media, India.
- Organic solvents were procured from Qualigens, India and Merck, Germany.
- As per the instructions given by manufacturer's the chemicals were stored at room temperature, 4ºC or at -20ºC.

5.1.1 Glassware and plastic ware

- All the glassware was procured from Rivera, India and Borosil, India.
- The plastic ware were procured from Axygen, USA and Eppendorf, Germany and Tarsons, India.

5.1.2 Water quality

- For preparing culture media, buffers, stock solutions, seed sterilization and all molecular biology work autoclaved deionized water was used. A broad list of chemicals and other accessories used in the present study has been shown below in Table 6.

Table 6: List of chemicals, glassware and plasticware used in the present study

Sigma	Product code	Himedia	Product code
2,2- diphenyl -1- picryl hydrazyl (DPPH)	29128	Boric acid (H_3BO_3)	MB007
2, 2-azinobis,3-ethyl-benzothiazolin-6-sulfonic acid (ABTS)	A9941	Disodium EDTA (Na_2EDTA)	RM3915
Folin-Ciocalteu reagent(FCR)	38220090	Ferrous ammonium sulphate ($FeSO_4.7H_2O$)	RM1377
Streptomycin ($C_{21}H_{39}N_7O_{12}$)	85886	Nutrient broth	M002
Zeatin, 6-(4-hydroxy-3-methyl-*cis*-but-2-enylamino) purine	Z0750	Potassium chloride (KCl)	RM698
Agar (C_{12} H_{18} O_9)n	A7002	Sodium carbonate (Na_2CO_3)	RM851
Bovine serum albumin (BSA)	A2153	Sodium carbonate (Na_2CO_3)	RM849
Dimethyl sulphoxide (DMSO)($C_2H_6O_5$)	B4869	Autoclavable bags (Hi Dispo bag)	PW038
Ethylenediamine-N,N,N',N'-tetraaceticacid (EDTA)	E5134	Potassium iodide (KI)	RM1086
Phosphate buffered saline (PBS)	P4417	Qualigens	Product code
Glucose ($C_6H_{12}O_6$)	G5500	Acetone (CH_3COCH_3)	32007
Sodium chloride (NaCl)	S7653	Methanol (CH_3OH)	32407
Sodium dodecyl sulphate (SDS) ($C_{12}H_{25}O_4S$)	L3771	Ethanol (C_2H_5OH)	1009830511
Sodium hydroxide (NaOH)	S8045	Tarsons	Product code
Sodium hypochlorite	7681529	Centrifuge tubes (15 ml)	546021
Ascorbic acid ($C_6H_8O_6$)	50817	Centrifuge tubes (50 ml)	546041
Sodium phosphate (Na_2Po_4)	7558794	Measuring beaker with handle (2000 ml)	431080
Potassium ferricyanide ($K_3[Fe(CN)_6]$)	LC19040	Measuring cylinder (50 ml)	345030
Trichloroaetic acid (TCA)	RM6274	Measuring cylinder (500 ml)	345060
Ferric chloride ($FeCl_3$)	034901	Micro test plate (96 well)	941196
Sodium carbonate (Na_2Co_3)	2836 20 00	Micro tip box for 0.2–10 µl tips	524051
Gallic acid ((HO)3C6H2CO2H)	G0011	Micro tip box for 2–200 µl tips	524050
Aluminum chloride (AlCl3)	MFCD00003422	Petri dishes (100×20 mm)	960030
Potassium acetate ($C_2H_3KO_2$)	2915 29 00	Rack for microtube (24 places)	241020

Quercitin (C15H10O7)	Q4951	Round magnetic string bar (8×50 mm)	4114
Phenol (C6H5OH)	W322340	Test tube basket with cover (23 ×23 ×23)	180030
Sulphuric acid (H_2So_4)	05427ES	Test tube stand (18 places)	203070
Lowry reagent	L 3540	Wash bottle	561110
Sodium potassium tartarate (KOCOCH(OH)CH(OH)COONa · 4H2O)	S2377	Axygen	Product code
Citric acid (HOC(COOH)(CH2COOH)2)	251275	Micro tubes (1.5 ml)	MCT-150-C
Perchloric acid ($HClO_4$)	V800294	Micro tubes (2 ml)	MCT-200-C
Copper sulphate ($CuSo_4$ $6H_2O$)	203165	Petri plate (90×15 mm)	AXY-PTD-90
Se-powdered	229865	Pipette tips (0.5–10 µl)	T-300
Mercury oxide (HgO)	05427ES	Pipette tips (100–1000 µl)	T-1000-B
Potassium sulfate (K_2So_4)	P0772	Pipette tips (2–200 µl)	T-200-Y
Ammonia (NH_3)	294993	Borosil	Product code
Sodium hydroxide (NaOH)	100594	Beaker (500 ml)	1000D24
Ammonium pursurate (C8H8N6O6)	56085	Beaker (1000 ml)	1000D29
Potassium dichromate ($K_2 Cr_2 O_7$)	231906	Conical flask, 150 ml	4980018
Orthophosphoric acid (H_3PO_4)	05427ES	Conical flask, 2000 ml	4980030
Diphenylamine indicator ($C_{12}H_{11}N$)	33149 MSDS	Culture tubes (25×150)	9820U08
Ferrous ammonium sulphate $(NH_4)_2Fe(SO_4)_2$)	05427ES	Funnel (50 mm)	6140065
Hydrogen chloride (HCl)	294837	Measuring cylinder (50 ml)	3027012
Barium chloride crystals ($BaCl_2$)	2337881	Measuring cylinder (250 ml)	3027021
Vanadate molybdate	38220000	Screw cap tubes (30 ml)	9910010
Anthrone (C14H10O)	2019940	Eppendorf	Product code
Glucose ($C_6H_{12}O_6$)	G7021	Pipette 0.5–10µl	3120000020
Stevioside ($C_{38} H_{60} H_{18}$)	S020802	Pipette 10–100µl	3120000046
Acetonitrile (C_2H_3N)	AX0152	Pipette 20–200µl	3120000054
Acetic acid ($C_2H_4O_2$)	AX0074	Pipette 100–1000µl	3120000062
Butylated hydroxytoluene (BHT)	12837	Miscellaneous	Description

Dulbecco's Modified Eagle's Medium (DMEM)	5796D	0.22 μ nylon syringe filter	Millipore, USA, SLGS025NB
Fetal bovine serum (FBS)	A2153	3MM filter sheets	Whatman, USA, 3030917
3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide (MTT)	M5655	Forceps	HiMedia, A824
Phosphate buffered saline (PBS)	P4417	Soilrite (soil conditioning mixture)	Keltech Energies Ltd.,Vishwasnagar, Karnataka, India
Dimethyl sulphoxide (DMSO) ($C_2H_6O_5$)	B4869	Scalpel blade	No.24, Swan, Japan
Kaempherol ($C_{15}H_{10}O_6$)	96353	Gloves	Kimberly-Clark purple nitrile, Canada, (product code-55083)
Beta sitosterol ($C_{29}H_{50}O$)	43623	Parafilm	Pechiney Plastic Packaging, IL, Chicago
Moringin ($C_{14}H_{17}NO_5S$)	CFN89445	Spirit lamps	HiMedia, LA275

Precautions for handling of toxic chemicals have been summarized in Table 7.

Table 7: Handling of toxic chemicals

Chemical	Hazard	Precaution
Acetone	Slight toxicity; At very high vapour concentrations, may depress the central nervous system	Do not inhale any chemical in use
DPPH	Direct exposure to DPPH by skin absorption and inhalation can cause allergy, asthma or breathing symptoms	Wear mask and gloves during its use
ABTS	Exposure to ABTS can cause headache and dizziness	Use protective equipment during its use
MTT dye	Harmful if came in contact by skin or by inhalation and may cause eye, skin, and respiratory allergies.	Wear protective gloves and clothing during its use
Dimethyl sulphoxide	Harmful if came in contact by skin or by inhalation and may cause eye, skin, and respiratory allergies.; Also combustible	Wear mask and gloves during its use; Store it in a tightly closed container
Ethanol	Mild irritation in the skin and eyes; vomiting and nausea by ingestion	Do not inhale
Na_2PO4	Exposure may cause eye, skin and respiratory irritations	Wear gloves while using it
Ethlene diamine tetra acetic acid	EDTA exhibits low acute toxicity; Oral can cause developmental and reproductive effects	Use of gloves
Folin-Ciocalteu reagent	Folin-Ciocalteu reagent is highly toxic and cause eye damage and severe skin burns	Wear gloves while using it.
potassium ferricyanide	Concentrated potassium ferricyanide is harmful and can cause skin and eye irritation	Use of resistant gloves, made up of nitrile rubber when handling the compound
Copper sulphate	Hazardous if in contact with skin, eye, and ingestion	Wear gloves while using it; close the cap tightly after use
Aluminium chloride	Inhaling aluminium chloride can cause eye, nose and throat irritation and also can cause skin burn	Protective gloves and well-ventilated areas are suggested
Potassium dichromate	It is harmful if inhaled it can cause skin and eye irritation	Wear gloves while using it
Iron chloride	It is considered a toxicant, causing irritation to the nasal passage and respiratory tract upon inhalation, irritation to the skin, vomiting and can cause serious eye damage	Protective gloves and well-ventilated areas are suggested for its use
Mercuric oxide	Mercuric oxide is highly toxic, not only acutely but as a cumulative poison	Wear gloves during working and wash hands after

		handling
Methanol	highest toxicity in humans, can cause permanent blindness by destroying the optic nerve	Do not inhale; Wear gloves
Phenol solution	Hazardous if in contact with skin, eye, and ingestion	Use appropriate gloves and glasses; Keep container tightly closed to avoid spilage; Do not reuse the gloves after handling phenol solution
Sodium hydroxide	Can causes chemical burning and permanent blindness if contact with eyes	Wear gloves during use
sodium carbonate	sodium carbonate inhalation can cause breathing problem and suffocation, severe eye burns and skin burns	Do not breathe it and wear specialised gloves while handling

5.2. Methods

The study was started by the collection of seeds of several varieties of *M. oleifera* Lam. such as ODC, Conventional, Jaffna, PKM-2, and PKM-1, from the Tamil Nadu Agricultural University (TNAU) Tamil Nadu, India. Meanwhile, Stevia plantlets age of one month (variety: GVS-16) were obtained from 'Green Valley Stevia', Nawanshahr, Punjab. The Stevia plantlets were maintained in pots (10-inch) filled with soil and soilrite in the ratio of 1:1 w/w and maintained in a greenhouse ['LPU-Agriculture field, Lovely professional university, Phagwara Punjab']. Seeds of five different varieties of *M. oleifera* were surface-sterilized by ethanol for 60 s (80%) followed by sodium hypochlorite for 10 min (20%). After ten washes with the help of deionized water, seeds were germinated in soilrite, in the dark for two days and in the light for one week. After the occurrence of first pair of leaf, the seedlings were relocated to 10-inch pots filled with soil and soilrite in the ratio of 1:1 w/w and maintained in the LPU-poly house as shown in the pictute below:

Figure 2: Five different varieties of *Moringa oleifera* in LPU poly-house

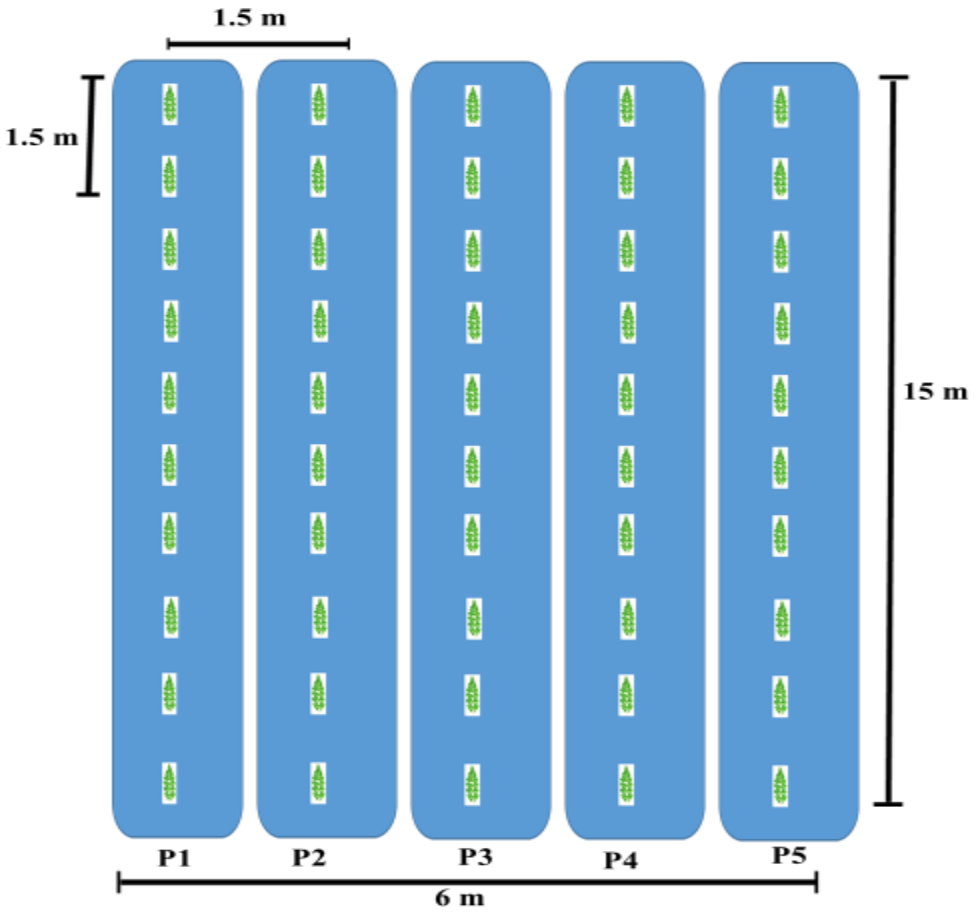


Figure 3: Experimental field layout for *Moringa oleifera* varieties: P1= PKM-1, P2= PKM-2, P3= ODC, P4= Jaffna, P5= Conventional

Preparation of leaf extracts

After one month leaves were harvested, and shade dried for one week. Dried leaves were turned into the powder form. Then the leaf powder was suspended in ethanol solvent and kept in rotary shaker (5 h) for extraction. Filtration of extraction was done by filter paper (Whatman No. 1). The ethanol was evaporated in rotary evaporator (40-45°C), (Superfit, India), centrifugation was done (8000 x g, 20 min) and supernatant was stored (4˚C) for future use (Shi, et al 2019).

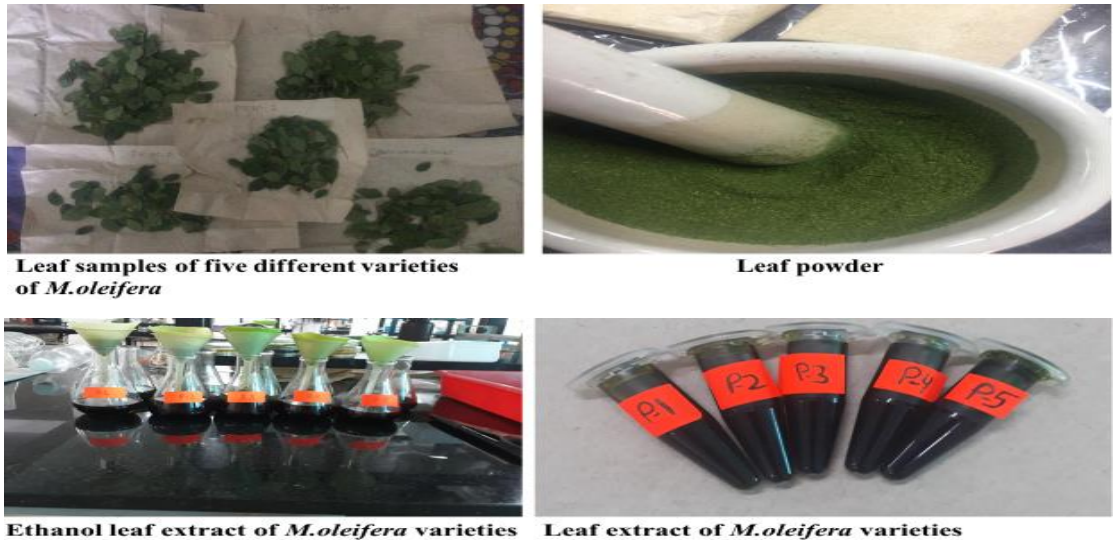


Figure 4: Extract preparation of five different varieties of *Moringa oleifera*

Preparation of foliar spray

From five-month old plants leaves were harvested and followed by shade drying for almost one week. 15 g of dried leaf powder was suspended in ethanol (100 ml of 80%) and retained in rotatory shaker (5 h) for extraction followed by filtration of the extract through filter paper 'Whatman No. 1'. The filtrate was then evaporated with the help of rotatory shaker set (40 °C, for 1 h). The thick mass (~15 mg) was then re-suspended in distilled water to a final concentration of 6% (v/v), to be used as foliar spray under green-house environment (ShM et al., 2017). During the experiment, no fertilizers were applied to the plants.

5.2.1. Antioxidant assay

The antioxidant activity of the five different varieties of leaf and seed extracts of *M. oleifera* (ODC, PKM-2, PKM-2, Jaffna, and Conventional) was determined by DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay (Brand-william, et al., 1995) and ABTS (2, 2-azinobis, 3-ethyl-benzothiazolin-6-sulfonic acid) assay (Re, et al., 1999).

[A] DPPH scavenging activity

DPPH is a form of free-radical founded on electron-transfer mechanism. It yields violet color in methanol. The solution chances pale yellow to colorless in the presence of antioxidant particles. A 2ml of DPPH (0.0394g in 100ml of methanol) was added to the different concentrations (10-100 µg/ml) of *M. oleifera* leaves and seed extracts (ODC, Jaffna, Conventional, PKM-1, and PKM-2). The extracts were incubated for in dark (30 min) and the absorbance was recorded at 520 nm with the help of UV/VIS spectrophotometer (LAMBDA 950, Perkin Elmer, USA). Ascorbic acid was used as standard (Atawodi, 2010). The scavenging percentage was calculated according to the formula given below:

$$\%\ \text{scavenging activity} = \frac{[A_0\ (\text{control}) - A_1\ (\text{sample})]}{A_0\ (\text{control})} \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of sample.

[B] ABTS radical scavenging assay

The principle of ABTS (2, 2-azinobis,3-ethyl-benzothiazolin-6-sulfonic acid) is grounded on the generation of ABTS radical. In the presence of potassium per-sulphate it gives a blue green complex, which get compressed by antioxidants. A 2ml of ABTS (0.384g in 100ml) was added to the different concentrations (10-40 µg/ml) of leaf and seed extracts of *M. oleifera* varieties (ODC, Conventional, Jaffna, PKM-1, and PKM-2). The extracts were incubated at room temperature. After 30 min readings were taken at the absorbance of 745 mn (Charoensin, 2014). Ascorbic acid was taken as standard. The inhibition percentage was calculated by using the following formula given below:

$$\% \text{ scavenging activity} = \frac{A_0 \text{ (control)} - A1 \text{ (sample)}}{A_0 \text{ (control)}} \times 100$$

Where A_1 is the absorbance of the sample and A_0 is the absorbance of the control.

[C] FRAP (Ferric- reducing antioxidant power) activity

Antioxidants are the reducing agents which donate the electrons to the free radicles, generated by various injuries in DNA, cells, tissues and organ system. They are abundantly found in plants, vegetables, fruits, beverages etc. having beneficial role in lowering the risk of cancer, neurodegenerative disorders and heart disease. FRAP is colorimetric assay provides a sensitive, highly adaptable, easy and quick way for measuring antioxidant capacity of different biological samples.

This assay uses antioxidants (polyphenols; flavonoids; vitamins and enzymes like glutathione peroxidase and superoxide dismutase.) as reductants in a redox reaction [Ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion] at low pH results in the formation of coloured ferrous from colourless ferric complex (Vijayalakshmi and Ruckmani 2016). Weigh 5g of leaf powder of five different variety of *M. oleifera* and dissolved in 100 ml of ethanol in conical flasks.

The flask were kept on rotatory shaker set at 200rpm, at 30ºC for 48h. Extract was evaporated in evaporator shaker and was collected for further use in well labelled vials. In 250µl of leaf extract of each variety of *M. oleifera* with different concentrations (10-40 µl) 625 µl of Na_2PO_4 (0.2M, pH 6.6) buffer and 625 µl of potassium ferricyanide (1% ,w/v) was added and then incubate at 50ºC for 20min. The mixture was allowed to cool and then 625 µl TCA was added. Then the centrifugation was done at 2000 rpm for 10min. To the supernatant 625 µl of distilled water and 125 µl $FeCl_3$ 1% (w/v) were added, and absorbance was taken at 700nm (Hammi et al., 2016).

5.2.2. Total phenolic content (TPC)

The total phenolic content was calculated by Folin-Ciocalteu method (Folin and Ciocalteu, 1927). Phenol reacts with Folin-Ciocalteu reagent (FCR), which holds phosphomolybdic acid, to yield a blue colored complex in alkaline medium (at 37ºC). The value of absorbance of the reduced FCR can be detected spectrophotometrically (at a range of 690 to 710 nm). A 2.5 ml of FCR was added to *M.oleifera* leaf and seeds extract followed by the adding of 2 ml of sodium carbonate (Na_2CO_3) after 10 min of incubation at room temperature. The absorbance was measured at wavelength of 745 nm. The TPC in extracts were stated in terms of Gallic acid equivalent (GE) µg/ml.

5.2.3. Total flavonoid content (TFC)

Aluminium chloride ($AlCl_3$) colorimetric technique was used to define the total flavonoid content (Ghafar et al., 2017). Leaf and seed extracts (0.5 ml) were mixed with methanol (70%) in15ml) followed by the adding of 10% aluminium chloride (0.1 ml), potassium acetate (0.1 ml) and distilled water (2.5 ml) followed by incubation for 1h at room temperature. The absorbance was measured at 415 nm. For respectively analysis, the samples were prepared in triplicate and the mean value of absorbance was achieved. The standard solution of quercetin was prepared by following the similar procedure and the calibration line was assembled. TFC in the extracts were stated as quercetin equivalent (QE) µg/ml.

5.2.4. Estimation of Sugar content

Phenol-sulphuric acid reaction (DuBoi's method) is the elementary and most reliable method for the quantitative estimation of sugar content in oligosaccharides, polysaccharides, glycolipids, glycoprotein and proteoglycans. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural and in the presence of phenol it will convert into yellow brown colour. Glucose standard (0.1 mg/ml) with different dilutions (0.2- 0.8 µl) were prepared to develop the standard curve. In the leaf extracts (2ml each), phenol (1ml) and sulphuric acid (5ml) were added, placed in the water bath (25- 30°C, 20 m), and absorbance was measured at 490 nm (Fu et al. 2017).

5.2.5. Estimation of Protein content

Lowry's method is a colorimetric assay, used for protein estimation wherein, the protein sample is pre-treated with Cu ions in alkali solution, followed by addition of Folin-Ciocalteau reagent (phosphomolybdate + phosphotungstate). The phenol groups of amino acids (tyrosine and tryptophan) reacts with the Folin-Ciocalteau reagent to produce blue colour complex whose intensity can be measured spectrophotometrically at 660 nm (Folin and Ciocalteu, 1927).

Thus, the intensity of color indicates the amount of these amino acids present in protein sample. In this study, the soluble protein content was measured by the procedure of Lowry et al. (1951). The phenolics and other pigments were removed by the procedure of Mattoo et al. (1986).

Three aliquots of the leaf extracts (200 µl) of each of the five varieties (young and mature growth stages) were taken in a test-tube and the volume were adjusted to 1 ml with deionised water. Freshly prepared Lowry's reagent [4% $CUSO_4 \cdot 5H_2O$ (w/v) + 2% sodium carbonate (w/v) + 1% SDS (w/v), 0.4% NaOH (w/v) and 0.16 % sodium potassium tartrate (w/v)] was added and the reaction mixture was incubated for 60 min at room temperature prior to the addition of Folin-Ciocalteau reagent.

The absorbance was recorded at 660 nm using a UV/VIS spectrophotometer. Bovine serum albumin (BSA) was used as a standard protein.

5.2.6. Estimation of chlorophyll content

Chlorophyll (Chl) is an extremely significant biomolecule, present in the leaves and stems of plant. It acts as a photoreceptor which reflects the green and absorbs red and blue wavelengths of light and plays a key role in the processes of photosynthesis. The basic structure of chlorophyll is a porphyrin ring that co-ordinate to a central atom magnesium (Mg^{2+}). Two different types of chlorophyll (Chl A and Chl B) are slightly different in their structural composition at a side-chain (–CH3 in A, –CHO in B). The estimation of chlorophyll content was determined by the IUPAC standardised method of Arnon (1949).

Leaf extract of five different varieties of *M. oleifera* were homogenised (80% acetone), filtered, measured at 663 and 645 nm, and then the chlorophyll a, b and total chlorophyll content was calculated by using the formula given below:

Chlorophyll a (mg/g FW)= $[(12.7 \times A_{663}) - (2.6 \times A_{645})] \times$ml acetone/ mg leaf tissue

Chlorophyll b (mg/g FW)= $[(22.9 \times A_{645}) - (4.68 \times A_{663})] \times$ml acetone/ mg leaf tissue

Total chlorophyll content = Chl a+ Chl b

Where A_{663} and A_{645} units of absorbance at 663 and 645 respectively. Readings were recorded in triplicates by using the spectrophotometer.

5.2.7. Estimation of mineral content

To estimate the mineral contents, 1 g shade-dried leaves of each of the five varieties was powdered separately and the leaf-powder was transferred into separate Erlenmeyer flasks (100 ml capacity) and were digested in triacid containing nitric, perchloric and sulphuric acids (9:3:1 v/v), for estimation of phosphorus (P) and potassium (K), and in diacids containing nitric and perchloric acids (9:4), for estimation of calcium (Ca), sulphur (S), nitrogen (N), sodium (Na), and carbon (C).

Thereafter, 3g of catalyst mixture [$CuSO_4.6H_2O$ (20g) + Se-powder (1g) + HgO (3g) + K_2SO_4 (48g)] plus 10ml of H_2SO_4 (concentrated) was added to the flask and incubated overnight. The Erlenmeyer flask was placed in Kjeldahl digestion unit, set at 100°C for 20 min, to digest the mixture at low flame. After digestion, the flasks were removed from Kjeldahl unit and the volume was adjusted to 100ml with distilled water.

[A] Nitrogen

The analysis of nitrogen was done by the Kjeldahl method (Jones, 1991). An aliquot of 10ml of the aforementioned sample was transferred into a 100 mL distillation flask and 10ml of 40% NaOH were added to it. Boric acid was mixed with indicator solution (3 drops) and was kept at the receiving end of condenser outlet, so that outlet dips in Boric acid. Distillation was carried out by passing the steam into a distillation flask and the color of solution (boric acid + indicator) changes from reddish-purple to green and after some time all the ammonia (NH_3) was released from the distillation of sample. After distillation, the titration of the sample was done against 0.01N H_2SO_4 until green color changed into purple. Blank (without plant sample) was also used to check the contamination.

[B] Calcium

Calcium estimation was done by titration with EDTA (Richards, 1954). A 20ml aliquot of the sample was transferred into 100ml Erlenmeyer flask and diluted with 20ml of distilled water, followed by the addition of sodium hydroxide (2N, 2ml), and ammonium purpurate indicator (50mg). Then, titration was done with EDTA (0.01N). The color was changed from red to purple. Blank (without plant sample) was also used in the same way.

[C] Carbon

Carbon estimation was done by following the protocol given by Nelson and Sommers (1996). Leaf powder (1gm) of each variety of *M. oleifera* was transferred into 500ml conical flask, containing $K_2Cr_2O_7$ (1N, 10ml), followed by addition of H_2SO_4 (20ml concentrated) to it. The mixture was allowed to rest for 30 min followed by addition of

orthophosphoric acid (10ml), and diphenylamine indicator (1ml). Finally, the titration was done against (0.05N) ferrous ammonium sulphate (139g of ferrous ammonium sulphate and 15ml of concentrated H_2SO_4).

[D] Sodium and potassium

Estimation of Na and K was done by flame photometer, following the protocol given by Gloterman et al. (1978) and Sahrawat et al. (2002). Aliquots of leaf extracts (1ml) of five different varieties of *M. oleifera* were transferred into the volumetric flasks (50ml), and a final volume of 50ml was made with the distilled water. Finally, the solution was fed to the flame photometer, which has already been adjusted with the standard solutions (1000ppm stock solution and 5, 10, 15, 20 and 25 ppm working solution) of sodium (NaCl) and potassium (KCl). Flame photometer readings of standards were plotted to get a standard curve.

[E] Sulphur

Estimation of sulphur was done by following the protocol of Mottershead (1971). Leaf extracts (1ml each) of five varieties of *M. oleifera* was transferred into the volumetric flasks (50ml). The final volume was adjusted to 50ml with distilled water and the transmittance was read spectrophotometrically at 470nm. Meanwhile, 5, 10, 15, 20 and 25ml of 100 ppm sulphur standard was prepared in 250 ml volumetric flasks. Then 25ml of buffer salt solution was added to each flask and finally, the volume was adjusted to the mark with deionized water. 10ml of solution from each flask was transferred to volumetric flasks having 50ml capacity, followed by addition of 1ml HCl (6N) and 0.5 g of barium chloride crystals. The flasks were left undisturbed. The final volume was adjusted to 50ml with distilled water. Transmittance was read at 470nm and the standard curve was drawn.

[F] Phosphorous

Estimation of phosphorous was done by Vanadate-molybdate method of Piper (1966). Aliquot of five different leaf extract samples were transferred to 25ml volumetric flasks. Few drops of 2,4-dinitrophenol indicator and Na_2CO_3 (4N) were added to it, till

yellow color disappears. The pH of 4.8 was maintained by adding HCl (6N, 2ml). Thereafter, 5ml vanadate molybdate reagent was added and then final volume was adjusted to the mark. After 30 min, color starts to develop. Transmittance was read at 470nm. Meanwhile, 5, 10, 15, 20 and 25ml of 100 ppm of the phosphorous standard was transferred to the 25 ml volumetric flasks and the same procedure was followed as mentioned above. Transmittance was read at 470nm and the standard curve was drawn.

5.2.8. High performance liquid chromatography (HPLC) analysis of Moringa

HPLC is used for the separation and estimation of bioactive compound according to their molecular weights and polarity. When any extract (mixture of compound) is passed through HPLC column, it get separated into its compounds before it exit from the column and with the help of chromatograms we can calculate the desired bioactive compounds (Herbal Health Research Consortium Pvt. Ltd village khyala khudra , ram trith road, Amritsar). HPLC (High Performance Liquid Chromatography) of five different varieties of *Moringa oleifera* (PKM-1, PKM-2, ODC. Jaffna and Conventional) was done to estimate the concentration of different compounds (β-sitosterol, quercetin, khaempherol, and moringin) present in it. This was analysed by using an Agilent- 1260 system from Karlsruhe, Germany, Agilent. The system was equipped with quaternary pump, VWD UV detector, column C_{18} (4.6×250 mm I.D., 5 µm particle size) and an auto sampler. Data was recorded and analysed using Azur 5.0 software (Shi et al., 2019).

Formula

$$\frac{\text{Area of sample}}{\text{Area of standard}} \times \frac{\text{Weight of standard}}{\text{Weight of sample}} \times \frac{\text{Dilution of sample}}{\text{Dilution of standard}} \times \%\ \text{Purity of standard} = \text{Bioactive compound}\ \%$$

5.2.9. Bacterial disc-diffusion assay

All the bacterial strains used in this study are listed in Table 8.

Table 8: Bacterial strains used in the present study

Bacteria	Strain	Description	Source
Pseudomonas aeruginosa	MTCC 2453	Gram-negative pathogen, causes severe acute and chronic infections at urinary tract, skin and the respiratory tract	Lab collection
Bacillus subtilis	MTCC 168	Gram-positive, causes meningitis, endocarditis, and infections of ears, eyes, wounds, urinary tract, respiratory tract, and gastrointestinal tract	Lab collection
Escherichia coli	MTCC 443	Gram-negative, causes adult kidney failure, fever bleeding, confusion, and seizures	Lab collection
Staphylococcus aureus	MTCC 3160	Gram-positive, furuncles, abscesses (boils), and cellulitis	Lab collection

The bacterial cultures of different strains like: *Pseudomonas aeruginosa* (MTCC 2453), *Bacillus subtilis* (MTCC 168), *Escherichia coli* (MTCC 443) and *Staphylococcus aureus* (MTCC 3160) used in the current study were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. The bacterial culture (15 µl) was added to 5 ml nutrient broth (NB), followed by incubation on a rotatory shaker set (200 rpm for 24 h at 37˚C). For *B. subtilis* culture, the growth temperature was retained at 28˚C. After 24 h, 50 µl of bacterial culture was added to 25 ml of nutrient broth (NB) and was permitted to shake for 24 h. Broth cultures of 200 µl each were spread on NB plates under lab environment and reserved in a bacterial incubator for 24 h at 37˚C. Aqueous and ethanolic extracts of different concentrations of (25, 50, 75 and 100µg) of the five varieties were covered onto the sterilized Whatman no.1 filter paper discs. A 10 µg/disc streptomycin was used as positive control and distilled water as negative control (Bauer et al., 1959).

5.2.10. Plant growth promoting activity

Aqueous leaf extracts of five different varieties of *M.oleifera* (ODC, Conventional Jaffna, PKM-1, and PKM-2) were sprayed on single groups of *Stevia* plants (one month old) potted in mixture of soilrite and soil in the ratio of 1:1. Each group include twenty healthy plants (Ten for treatment + ten as control). The foliar spray of *M.oleifera* varieties was scheduled on 3rd, 6th, 9th, 12th, 15th, 18th and 21st day (7 treatments) of planting. The physiological and morphological characteristics were studied after a week of the 7th treatment. For dry weight investigation the plants were kept in an oven (set at 80ºC for four hours), followed by weighing after the treatment (Yasmeen et al., 2014).

The root and shoot length, number of leaves, chlorophyll a & b, carotenoids, plant dry weight, total soluble sugar, total phenolic and flavonoid contents, total protein content, and DPPH free radical scavenging activity, were calculated thrice, after every week.

5.2.10.1. Estimation of photosynthetic pigments

Quantification of the carotenoids and chlorophyll was performed by the spectrophotometric method described by Arnon, (1949). A 0.25 g of leaf tissue was crushed in 1.5ml of acetone

followed by centrifugation1 (0,000 x g for 10 min). The absorbance of the supernatant was measured at 470, 645, and 663 nm. The chlorophyll a, chlorophyll b, and carotenoid content was estimated by following formulae:

$$\text{Chlorophyll a (mg/g)} = \frac{[(12.7 \times A_{663}) - (2.6 \times A_{645})]}{\text{mg leaf tissue}} \times \text{ml acetone}$$

$$\text{Chlorophyll b (mg/g)} = \frac{[(22.9 \times A_{645}) - (4.68 \times A_{663})]}{\text{mg leaf tissue}} \times \text{ml acetone}$$

5.2.10.2. Estimation of total soluble sugars (TSS)

Anthrone test was achieved for the quantitative evaluation of sugars. A 0.25 g of leaves were homogenized in 15 ml of 96% ethanol, followed by centrifugation (2500 × g for 15 min) (Irigoyen et al.,1992). The supernatant was stowed at 4ºC. 4ml anthrone was added to 1 ml ethanolic extract. The final mixture was incubated for 10 min in boiling water bath. Absorbance was recorded with the help of spectrophometre at 660 nm. Glucose was used as standard.

5.2.10.3. Estimation of total soluble protein (TSP)

The phenolic collection of amino acids like tryptophan and tyrosine residue in protein produce blue colour (660 nm) with Folin-Ciocalteau reagent (Folin et al, 1927). Thus, the strength of color relies upon the amount of these amino acids present proteins. In the current study, the soluble protein content was estimated by Lowry's method (1951). A 0.25 g of leaves were crushed in 15 ml potassium phosphate buffer, pH 7.8, followed by centrifugation (1000 rpm for 20 min at 4ºC). By the procedure of Mattoo et al., 1986. the phenolics and other pigments were removed. Bovine serum albumin (BSA) was used as standard.

5.2.10.4. High performance liquid chromatography (HPLC) analysis of *stevia*

The estimation of stevioside and zeatin content of *Stevia* leaves was completed by using HPLC machine (1260 infinity Agilent Technologies, Palo Alto, CA, USA), provided with an auto-sampler, photodiode detector, and quaternary pump, at room temperature. C18 column was taken (25cm x 4.6mm I.D., 5µm, Germany) for separation. Acetonitrile and water in the ratio of 50:50 v/v were used as a mobile phase for stevioside while methanol-water in the ratio of 40:60

acidified with 5% acetic acid was taken for zeatin and the flow rate was maintained at 1 ml/min. The detector was maintained at 210 and 280 nm of wavelengths respectively.

Treated *Stevia* leaves were shade dried for 5 days and crushed into a fine powder. 50 mg of each of the powdered samples were dissolved in 10 ml of methanol, followed by the filtration with the help of Whatman filter paper no. 1. Filtrate was saved on a rotary evaporator (45ºC, 50 rev min^{-1} and 70 mbar vacuum pressures) for evaporation of solvent methanol. The residue were suspended in 100 ml of acetonitrile: water (80:20) followed by filtration through nylon membrane having pore size of 0.45µm and collection in HPLC grade vials for investigation (Kabiri et al., 2017). Stevioside standard (Sigma, USA) was prepared by dissolving 10 mg in 50 ml of acetonitrile: water in the ratio of 80:20. The stevioside content was estimated by means of the formula:

$$\frac{\text{Area of sample}}{\text{Area of standard}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \text{Purity of standard} = \text{Stevioside \% in sample}$$

The aliquots obtained for each sample were labelled as follows: OSS (ODC sprayed *Stevia* leaves), CSS (Conventional sprayed *Stevia* leaves), WSS (Control: water sprayed *Stevia* leaves), JSS (Jaffna sprayed *Stevia* leaves), P1SS (PKM-1 sprayed *Stevia* leaves), P2SS (PKM-2 sprayed *Stevia* leaves). Zeatin content of treated and untreated *Stevia* were also estimated by HPLC, by using a method described by Olivella, (2001). 1 g of leaves of each treated *Stevia* plant were crushed in 30 ml of acetic acid- methanol (0.2 M) and butylated hydroxytoluene solution in the ratio of 80:20 and stowed in deep freezer (set at -17 ^{o}C) then homogenized and followed by centrifugation (5000 rpm for 10 min at 5 ^{o}C). The pH of the supernatant was mantained to 3 and was stowed in a vacuum dried chamber for almost 1 hour to eradicate traces of solvent. After that it was diluted with the help of methanol-water in the ratio of 40:60 acidified with acetic acid (5%) followed by filtration through the membrane syringe filters having pore size of 0.45µm and assortment in HPLC grade vials. Zeatin of 5 mg (Sigma, USA) dissolved in 10 ml methanol-water in the ratio of 40:60, acidified with 5% acetic acid, was taken as a standard. The Zeatin content in the leaf samples was estimated by using the formula given below:

$$\frac{\text{Area of sample}}{\text{Area of standard}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \text{Purity of standard} = \text{Zeatin \% in sample}$$

The aliquots acquired for each sample were labelled as follows: OSS (ODC sprayed *Stevia* leaves), CSS (Conventional sprayed *Stevia* leaves), WSS (Control: water sprayed *Stevia* leaves), JSS (Jaffna sprayed *Stevia* leaves), P1SS (PKM-1 sprayed *Stevia* leaves), and P2SS (PKM-2 sprayed *Stevia* leaves).

5.2.10.4. Flame photometer analysis

For the study of minerals (Ca, Na, K, and Li), leaves of treated and untreated *Stevia* were digested in triacid (nitric acid, perchloric acid and sulphuric acid) in the ratio of 9:3:1 v/v. Volume of the extracts were diluted to100ml (Jackson, 1973; Black, et al., 2008). The minerals were estimated with the help of flame photometer and the content was calculated by means of the formula given below:

$$\text{Mineral content (\%)} = \text{Sample reading} \times \frac{\text{Dilution factor}}{10{,}000}$$

5.2.11. MTT assay

The human liver cell line (HepG2) was bought from NCCS (National centre for cell science), Pune, India and culture was maintained at Lovely Professional University, Punjab, India. The cells were developed in DMEM media, supplemented with 10% fetal bovine serum (Gibco, USA) in CO_2 incubator at 37° C. Cytotoxicity evaluation was carried out on HepG2 cell line, through MTT assay according to the protocol provided by American type culture collection (ATCC) (Cory et al., 1991; Wilkening et al., 2003). Cells were inoculated at the density of 20,000 cells in a 96-well plate (Thermo delta surface) and were maintained in a humidified conditions (5% CO_2 and 64% air) at 37°C. These cells were treated with ethanolic leaf extract of various concentrations like; 0.25, 0.5, 1, 2, 3, 4, and 5mg/ml. Then 50µl of MTT solution [5mg MTT dye dissolved in 1ml phosphate buffered saline (PBS)] was poured in each well and the plate was incubated at 37ºC for 4 h, followed by addition of DMSO (15µl) in each well. The absorbance was recorded using an ELISA reader at 570 nm. Percentage cytotoxicity was calculated from the absorbance values using the formula given below:

$$\% \text{ cell cytotoxicity } = 100 - \left(\frac{\text{OD of sample} - \text{OD of blank}}{\text{OD of control} - \text{OD of blank}}\right) \times 100$$

5.2.12. Statistical analysis

Each experimentation was repeated thrice and in replicates. The findings were represented as means ± standard error (SE). The experimental data was subjected to investigation of variance (ANOVA) using IBM SPSS Statistics 22 software, USA and comparisons of means were completed with least significant difference (LSD) post hoc test (at 5% level of probability).

Chapter 5

Results and discussion

6. Results and discussion

6.1. Morphological study: Morphological characteristic features of all different varieties of *M. oleifera* are listed in figure 3 and table 9.

Figure 5: *Moringa oleifera* varieties growing in the agriculture field of LPU.

Table 9: Morphological features of *Moringa oleifera* varieties

Characters	**Jaffna**	**PKM-1**	**PKM-2**	**ODC**	**Conventional**
Collection	Tamil Nadu Agricultural University, Tamil Nadu, India.	Tamil Nadu Agricultural University, Tamil Nadu, India.	Tamil Nadu Agricultural University, Tamil Nadu, India.	Tamil Nadu Agricultural University, Tamil Nadu, India.	Tamil Nadu Agricultural University, Tamil Nadu, India.
Method of Propagation	seeds	seeds	seeds	seeds	seeds
Seed germination (%)	65	85	78	90	58
Spacing (m)	1.5×1.5	1.5×1.5	1.5×1.5	1.5×1.5	1.5×1.5
Duration	Perennial	Annual	Annual	Perennial	Perennial
Plant height (ft.)	3.40	5.60	6.50	4.50	2.55
Plant spread (mm)	54.44	85.34	78.42	66.48	35.81
No. of primary branches	39.44	53.75	48.70	30.53	17.32
No. of leaves	120.34	364.45	300.76	190.62	78.45
Shape of leaves	Tripinnate, imparipinnate	Tripinnate, imparipinnate	Tripinnate, imparipinnate	Tripinnate, imparipinnate	Tripinnate, imparipinnate
Stem colour	Grayish white	Grayish white	Grayish white	Grayish white	Grayish white

6.2. Antioxidant activity of different varieties of *Moringa oleifera*

6.2.1. DPPH and ABTS scavenging assay

DPPH scavenging assay was performed with leaves and seeds of five varieties of *M.oleifera*. IC_{50} is a parameter that is used to analyse the antioxidant activity. Lesser the IC_{50} value, greater is the antioxidant potential. In this study, IC_{50} was calculated by using Labortary tool IC_{50}/IC_{90} calculation module. The trend of DPPH radical scavenging activity of leaves and seeds based upon IC_{50} is shown in table 10. In both cases, there is a statistically significant difference among the values obtained for the five varieties, ($P<0.05$) as determined by one way ANOVA. Thus, it is observed that in both the cases (with leaves and seeds) Jaffna is the most active DPPH radical scavenger followed by PKM-1, PKM-2, ODC and Conventional variety. The DPPH scavenging activity of Jaffna was double than that of the conventional variety which is least active DPPH radical scavenger. The results are in consonance with the findings of Das et al., (2012), Qwele et al., (2013), Sravanthi and Roa, (2014), and Mrudula et al., (2014).

Similarly, the ABTS radical scavenging activity of different varieties of *M. oleifera* leaves and seeds were ranked upon IC_{50} values as shown in table 10. There was a statistically significant difference between the values obtained from five varieties ($P<0.05$) as determined by one way ANOVA. It was observed that Jaffna variety was comparatively more effective in ABTS radical scavenging activity followed by PKM-1, PKM-2, ODC and Conventional variety. With both the leaves and seeds extract, a higher DPPH activity was observed as compared to ABTS (Fig. 6A-6D). The results with the antioxidant activities are in consonance with the earlier reports of Rajeshwari et al., 2013; Sravanthi and Roa, 2014).

DPPH and ABTS scavenging activity of leaf and seed extracts of different varieties of *Moringa oleifera* are shown in figure 6**.**

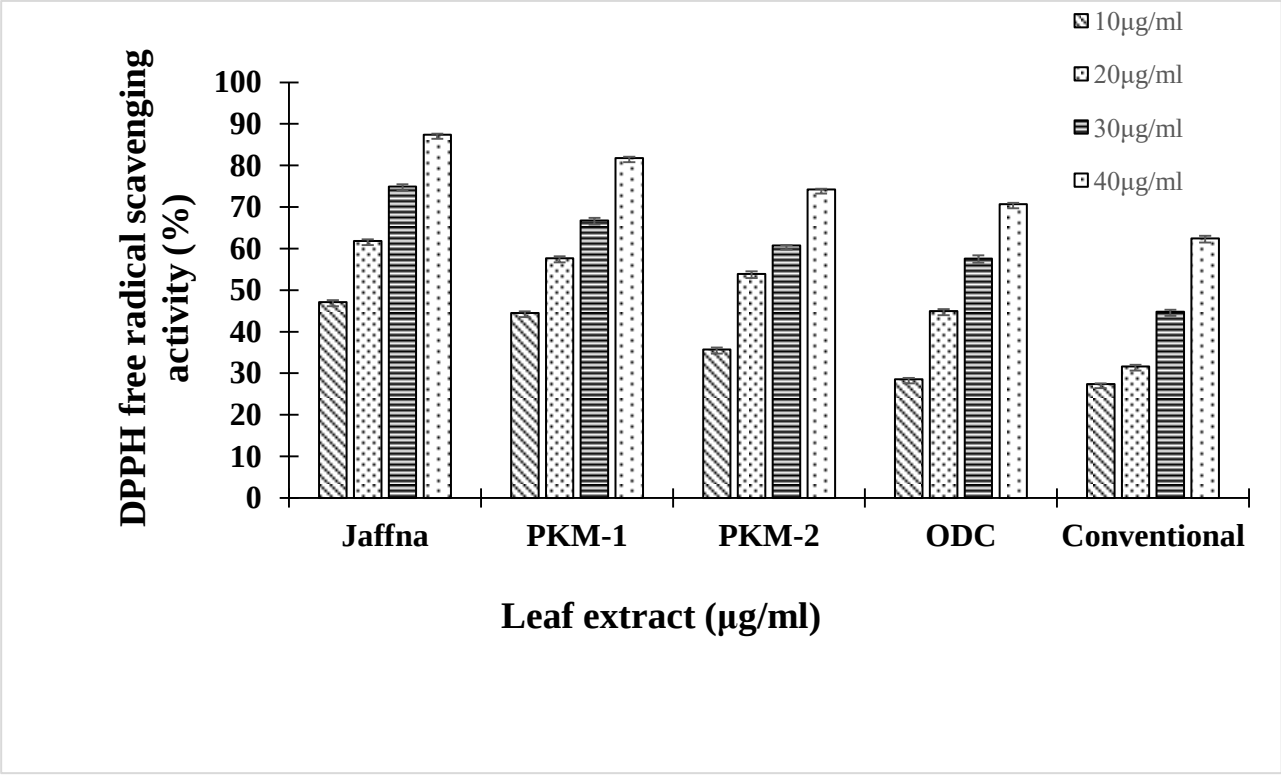


Figure 6 [A]: DPPH scavenging activity of leaf extracts of different varieties of *Moringa oleifera*

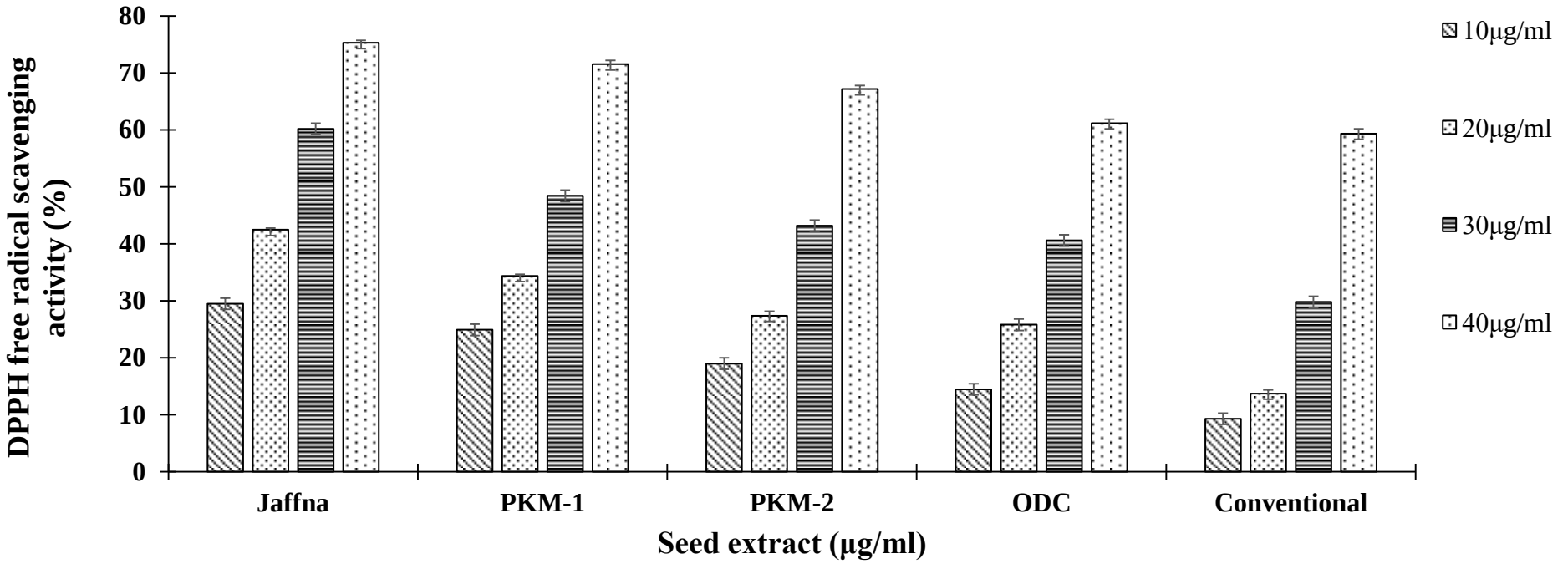


Figure 6[B]: DPPH scavenging activity of seed extracts of different varieties of *Moringa oleifera*

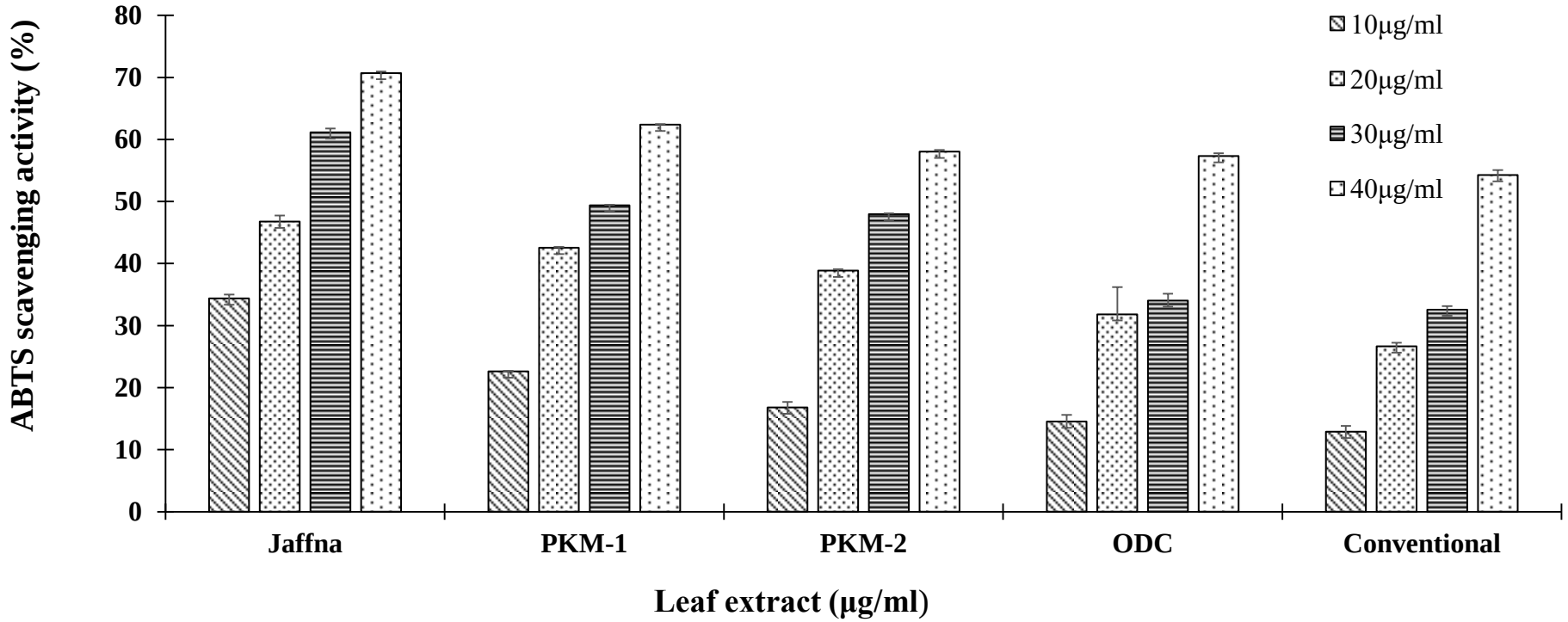


Figure 6[C]: ABTS scavenging activity of leaf extracts of different varieties of *Moringa oleifera*

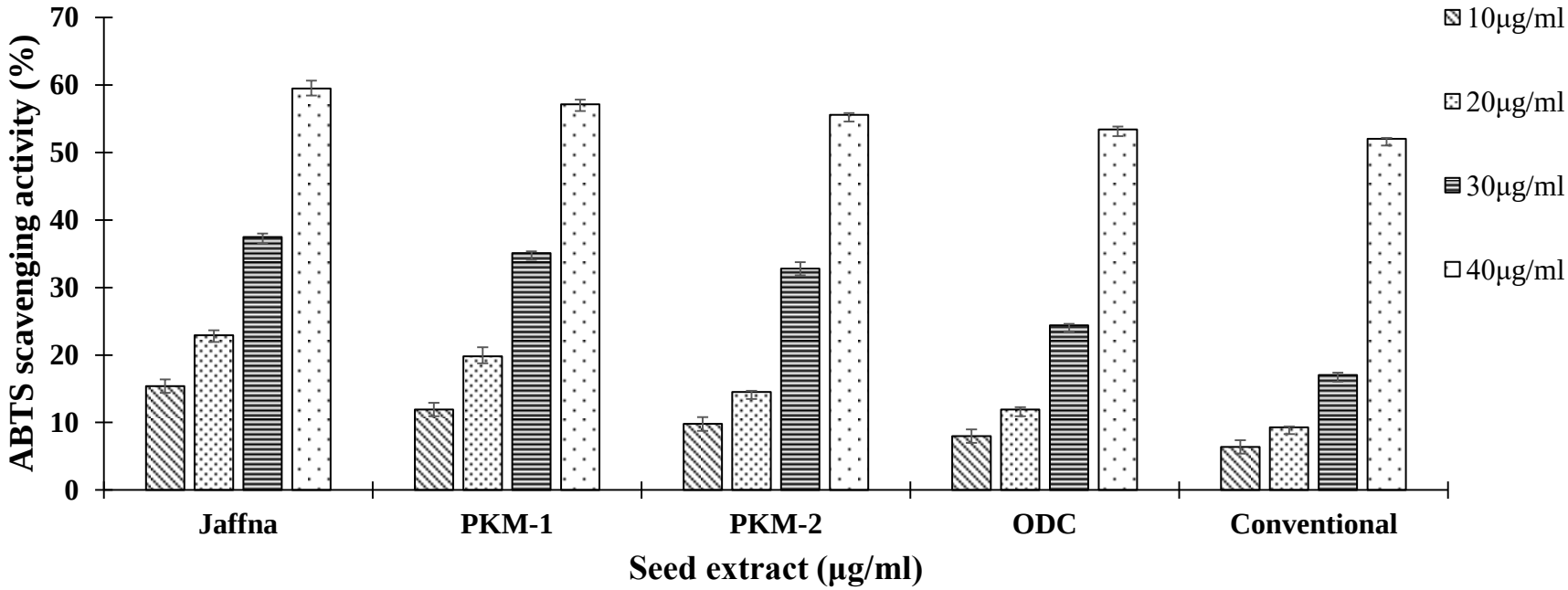


Figure 6[D]: ABTS scavenging activity of seed extracts of different varieties of *Moringa oleifera*

The trend of DPPH and ABTS scavenging activity of leaves and seeds of different varieties of *M.oleifera* based upon IC_{50} are shown in table 10.

Table 10: DPPH and ABTS scavenging activity of ethanolic leaves and seeds extracts of different varieties of *Moringa oleifera* varieties based on IC_{50} (µg/ml)

Varieties	**DPPH assay**		**ABTS assay**	
	Leaf	**Seed**	**Leaf**	**Seed**
Jaffna	11.46±0.24a*	23.36±2.81a	20.36±1.67a	29.72±0.31a
PKM-1	13.34±0.25b	29.52±0.57b	22.43±0.08b	32.14±0.56b
PKM-2	18.14±0.23c	33.59±0.63c	22.59±2.06c	32.50±0.71c
ODC	21.98±1.09d	35.31±0.55d	26.66±1.81d	33.35±0.99d
Conventional	26.61±0.45 e	37.50±1.18e	34.71±0.79e	35.83±0.78e

Each value is presented as mean of triplicate treatments.*LSD: least significantly different at $P \leq 0.05$ according to the posthoc test.

6.2.2. FRAP antioxidant activity

A large number of dreadful diseases are caused by the oxidative stresses. Phenols and the flavonoids are the natural antioxidants obtained from the plants, offers resistance against oxidative stresses by scavenge the free radicles, inhibit lipid peroxidation etc. (Masella et al., 2005; Dai and Mumper, 2010). The present study showed the antioxidant effect of five different varieties of *M. oleifera*, by using FRAP method (based on the transfer of electrons. (Ou et al., 2002; Prior et al., 2005). The present study revealed that the leaf extract of Jaffna variety showed best performance in the reducing power activity followed by other four varieties. The trend of the scavenging activity are represented as follow Jaffna (9.47 µg/ml, 18.48 µg/ml, 29.39µg/ml, and 35.37µg/ml) > PKM-1 (4.82 µg/ml, 7.63 µg/ml, 22.33 µg/ml, and 27.71µg/ml) > PKM-2 (2.10 µg/ml, 7.04 µg/ml, 13.18 µg/ml, and 21.78µg/ml) > ODC (0.17 µg/ml, 2.10 µg/ml, 4.41µg/ml and 13.94 µg/ml) > Conventional (0.05 µg/ml, 1.08 µg/ml, 2.86 µg/ml, and 5.40µg/ml). (Fig. 7).

All the samples showed reducing capacity in concentration dependent manner. In the study on the antioxidant potential of *juniperus oxycedrus* the leaf and the fruit extracts were examined by FRAP assay and showed potent antioxidant potential which is in accordance with the present study. The present study also exhibited similarities with other reports as well, where they found that flavonoids and phenols are directly responsible for antioxidant activity (Heim et al., 2002; Soong and Barlow, 2004; Balasundram et al., 2006; Loizzo et al., 2012).

Thus, the findings obtained by this study demonstrate Jaffna variety as therapeutic agent. Because Jaffna variety revealed the highest antioxidant values and constitutes a useful sources of antioxidant metabolites.

FRAP antioxidant activity are shown in figure 7.

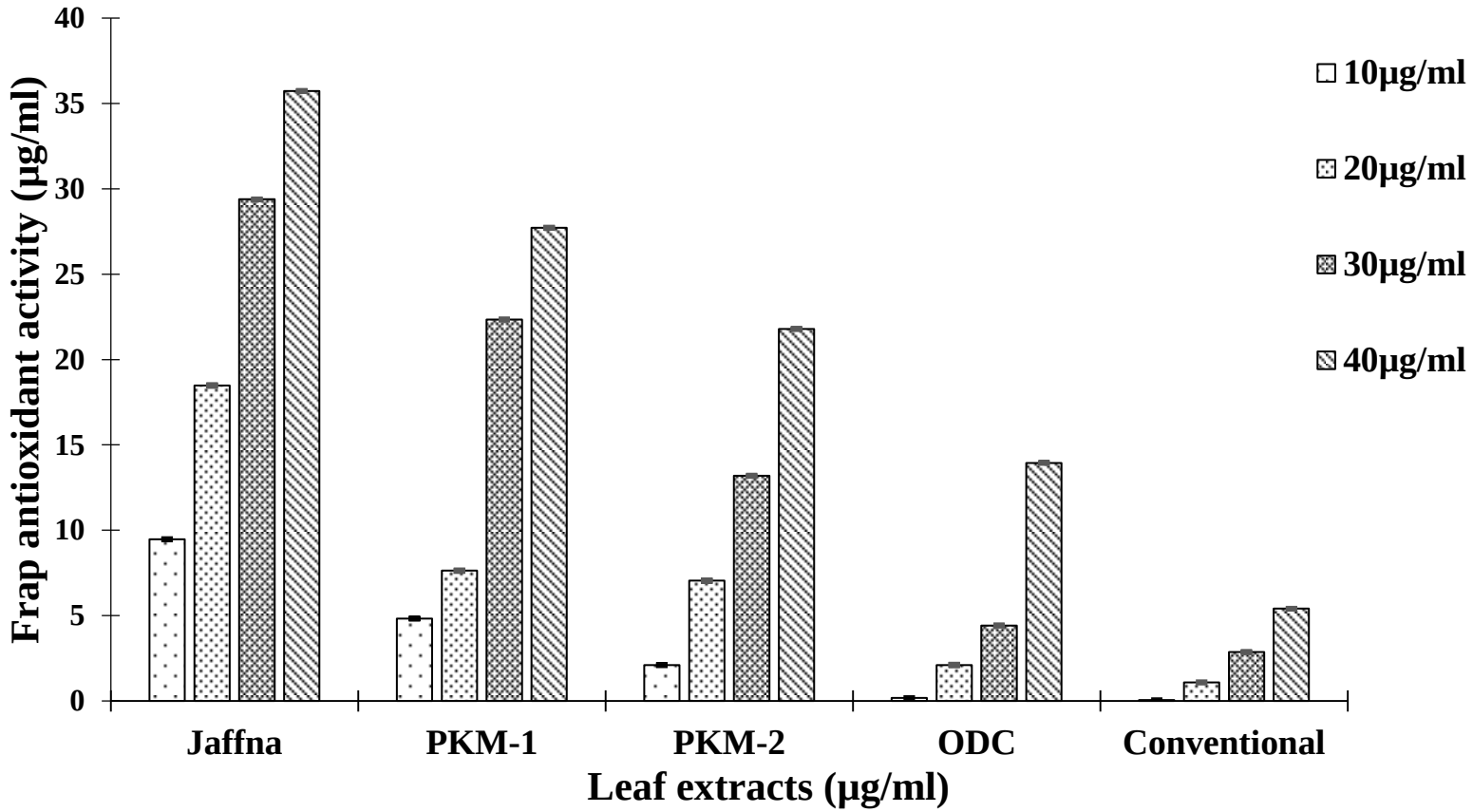


Figure 7: FRAP antioxidant activity of leaf extracts of different varieties of *Moringa oleifera*

6.2.3. Total phenolic (TPC) and total flavonoid content (TFC)

The leaves and seeds extract of *M. oleifera* are enriched with eloquent phenolic content. A similar trend of TPC was observed in leaves and seeds of the five varieties: Jaffna > PKM-1 > PKM-2 > ODC > Conventional. Among the five varieties, leaves and seeds of Jaffna exhibited the highest TPC. Moreover, the TPC values of the leaves of all the five varieties were more than that of the seeds. These results are in agreement with the findings of El Awady et al., (2016) and do Nascimento et al., (2017). The strong antioxidant activity of *M. oleifera* is due to the redox potential of phenolic contents present in it, which plays a key role in neutralizing free radicals (do Nascimento et al., 2017).

The trend of TFC values of leaves and seeds extract was Jaffna > PKM-1 > PKM-2 > ODC > Conventional. (Fig.7A-7D)Interestingly, the decreasing trend of TFC values of the five varieties was similar to that of the TPC values. The current study revealed the TPC and TFC values of all the five varieties were directly linked to the antioxidant activities, the same findings were observed by Siddhuraju and Becker (2003) and Pakade et al. (2013).

TPC and TFC values of all the five varieties of *M.oleifera* are shown in figure 8.

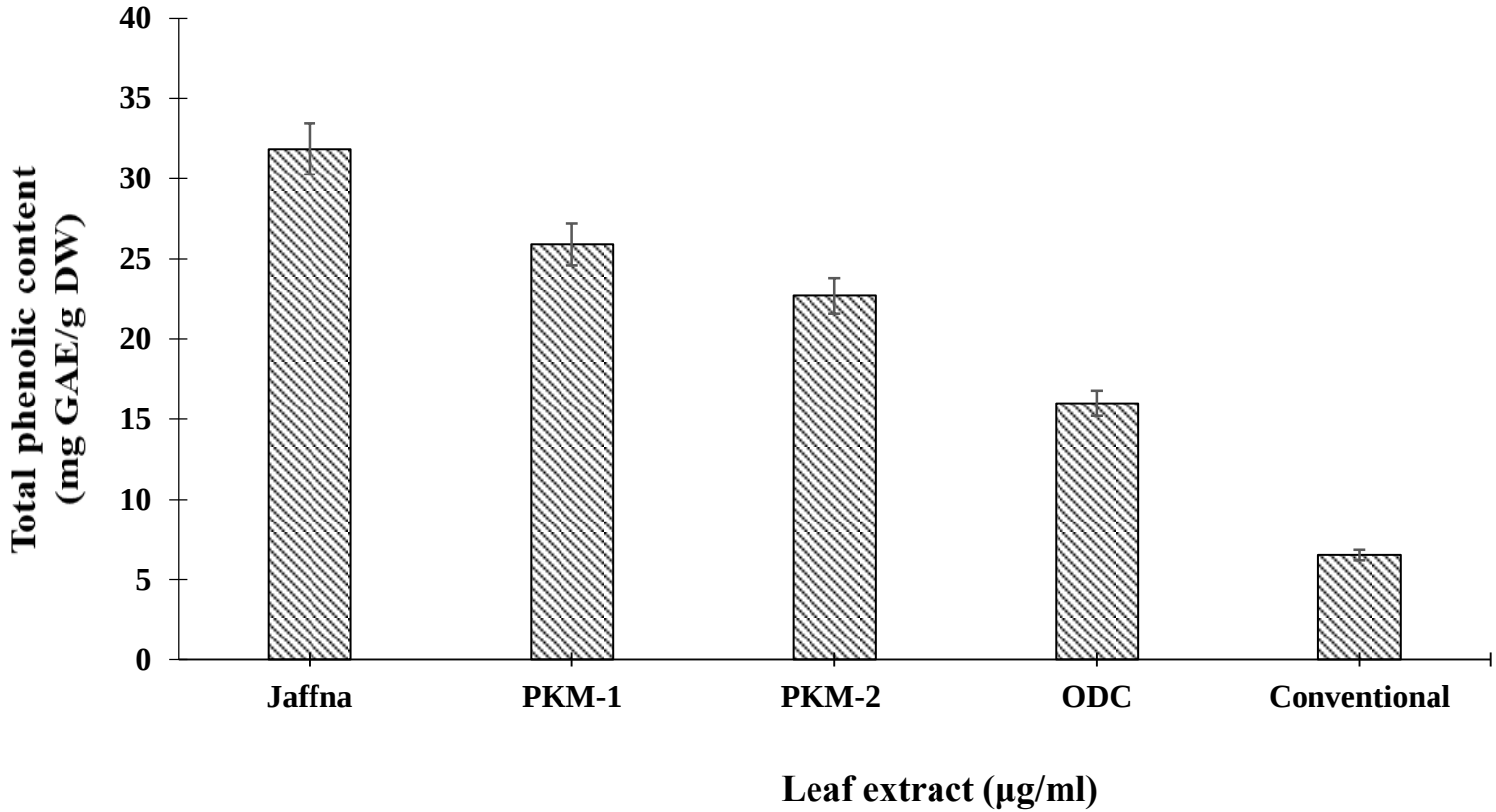


Figure 8[A]: TPC of leaf extracts of all the five varieties of *Moringa oleifera*

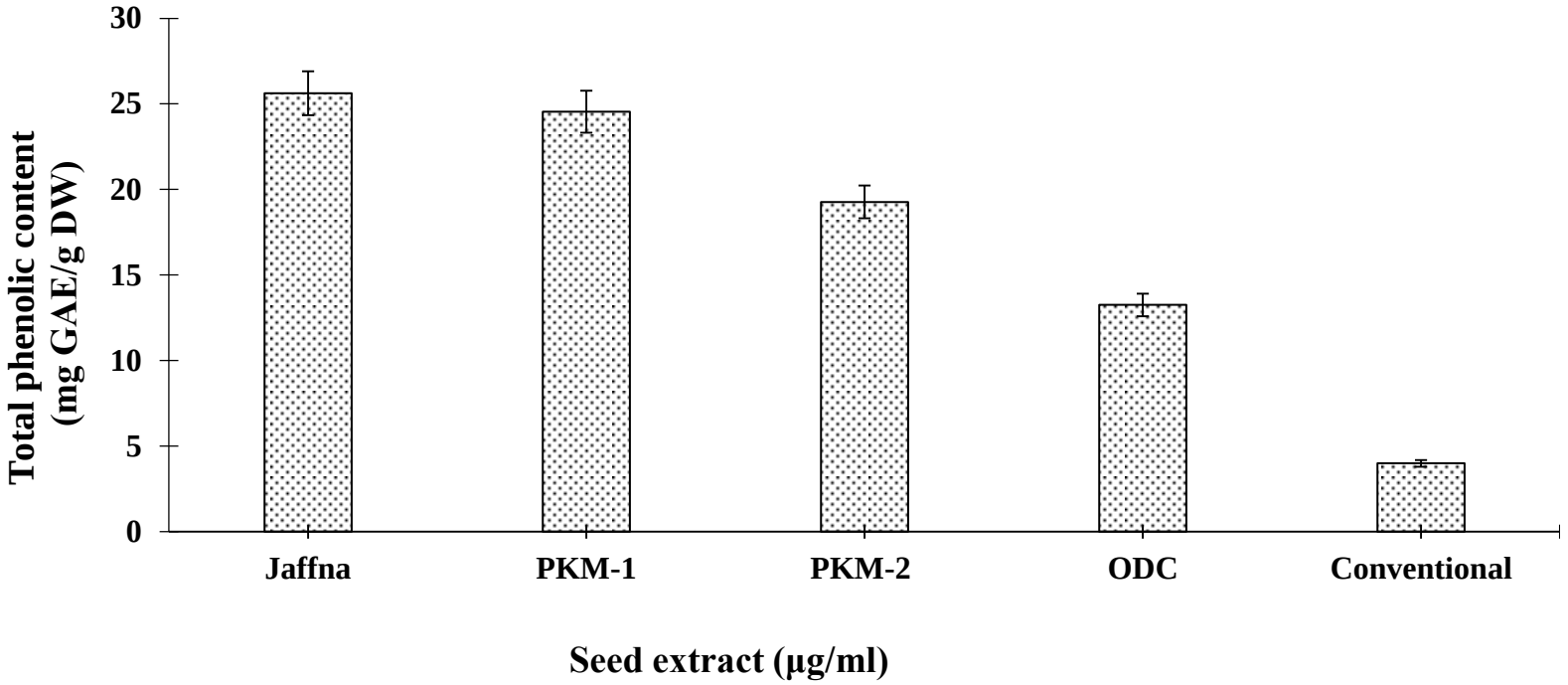


Figure 8[B]: TPC of seed extracts of all the five varieties of *Moringa oleifera*

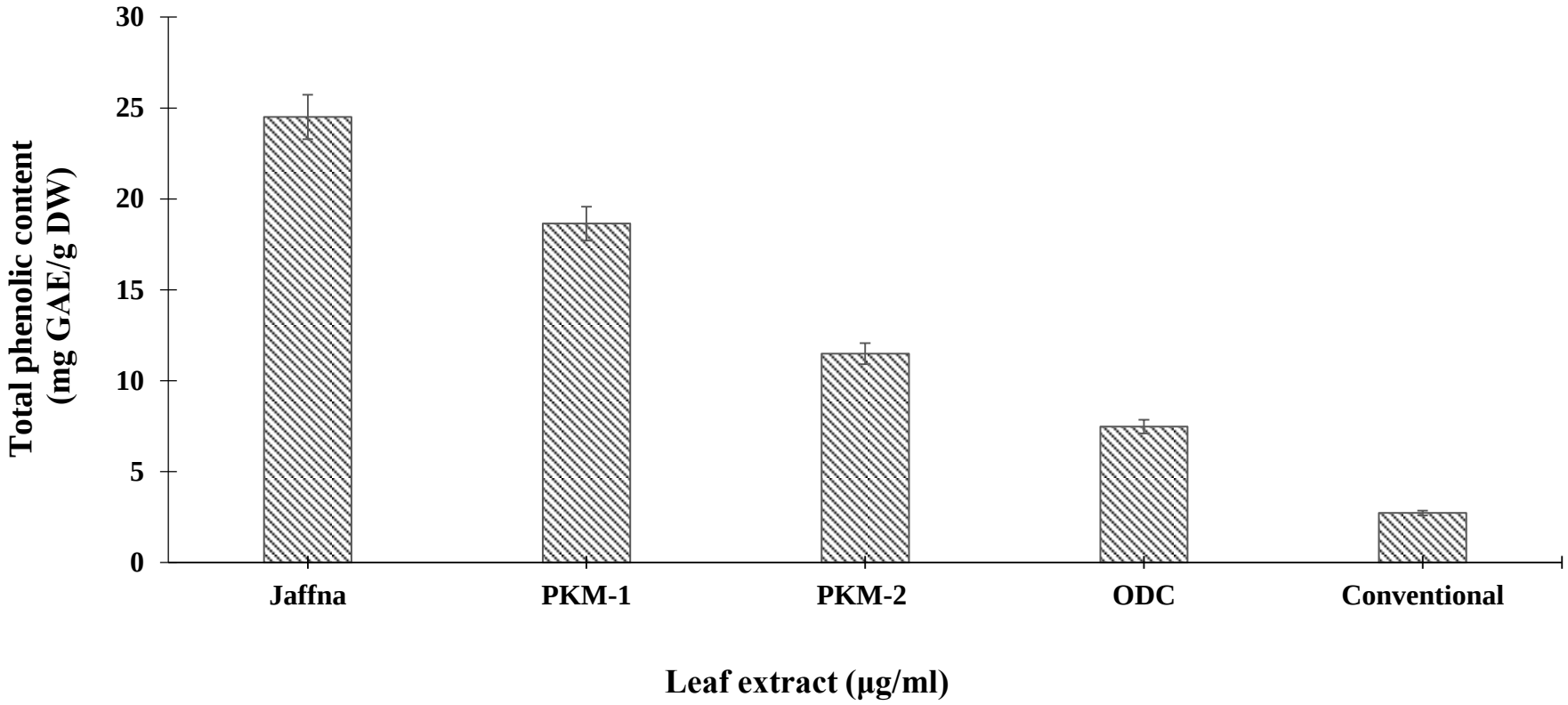


Figure 8[C]: TFC of leaf extracts of all the five varieties of *Moringa oleifera*

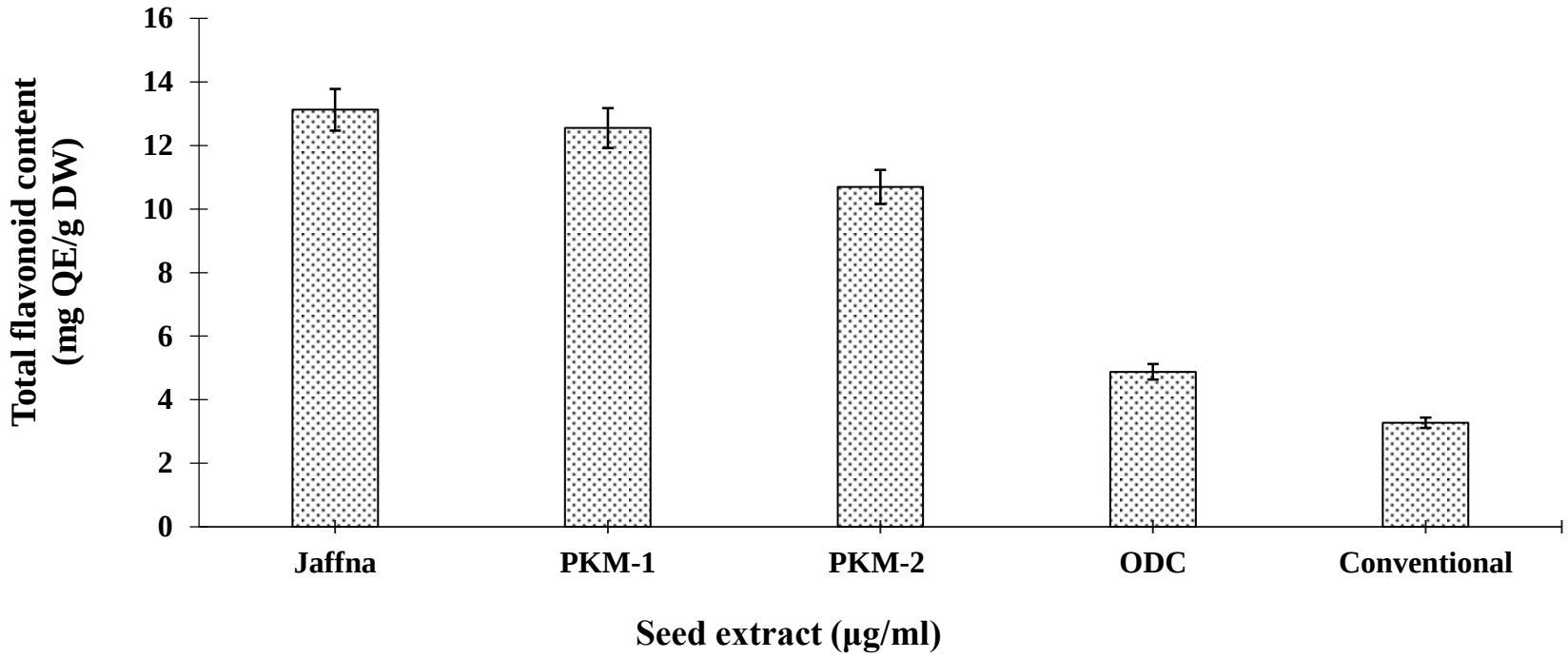


Figure 8[D]: TFC of seed extracts of all the five varieties of *Moringa oleifera*

6.3. Total sugar content

The total sugar content present in leaf extracts of five *M. oleifera* varieties was analysed at young and mature stages of growth. Maximum sugar content was recorded in Jaffna (0.39±0.04 and 0.51±0.01 µg/ml) followed by other varieties, PKM-1 (0.25±0.001 and 0.45 ± 0.006 µg/ml), PKM-2 (0.12±0.001 and 0.301±0.004 µg/ml), ODC (0.08±0.003 and 0.16±0.01 µg/ml) and Conventional (0.05±0.002 and 0.08 ± 0.01µg/ml) (Fig.8). Oxidation-reduction reactions are extremely important chemical reactions in maintaining proper health, by providing an energy source for the body. This process needs catalysts called as reducing agents, oxidising agents or reducing sugars (glucose, fructose, lactose, and maltose). Sugars (fructose, galactose, glucose, sucrose, lactose, maltose and raffinose) are the essential component of human diet (Fu et al., 2017). According to the Berkovich et al., 2013, *M. oleifera* contain reducing sugars and we also found sufficient amount of sugar content in different varieties of *M. oleifera* leading by the Jaffna variety. Thus, Jaffna variety can be consumed as the energy-booster and can be added in the diet plan of obese.

Total sugar content of leaf extracts of different varieties of *M. oleifera* are shown in figure 9.

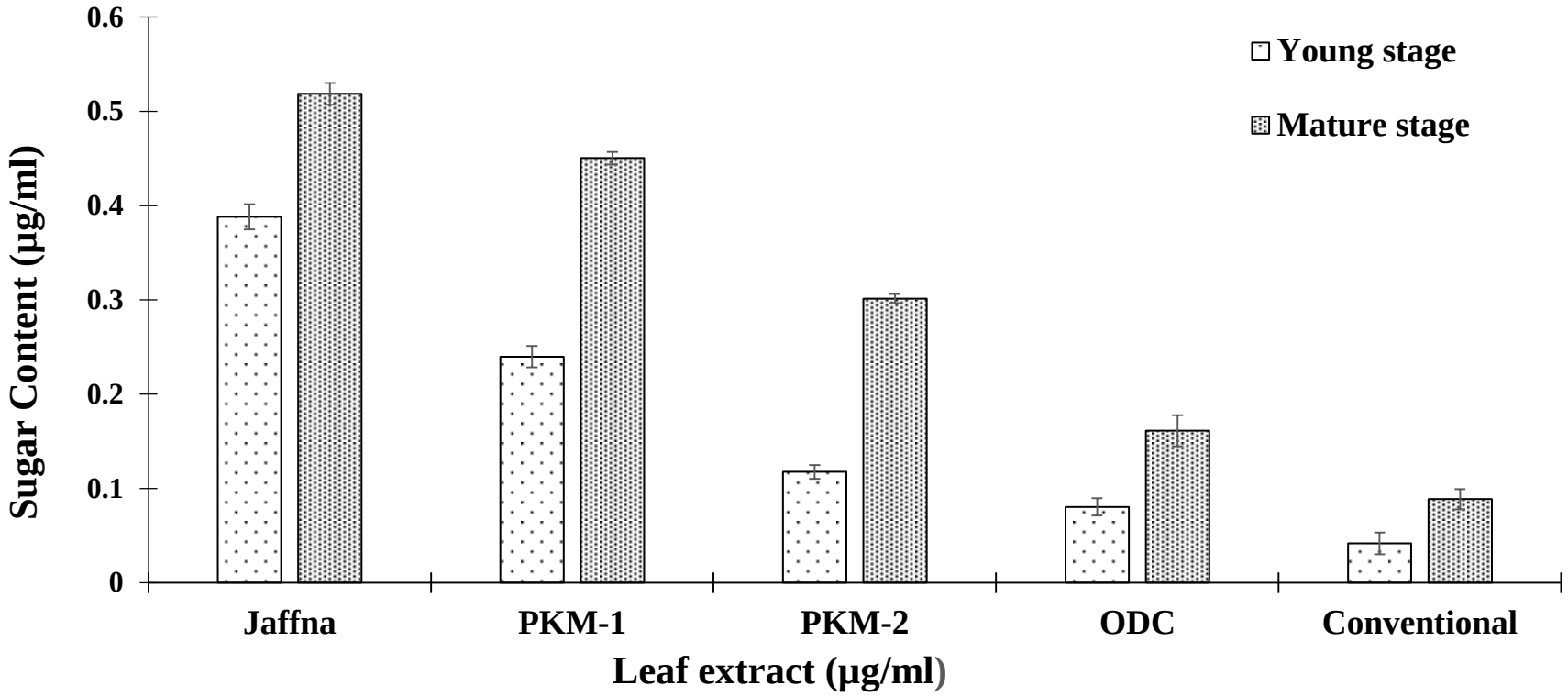


Figure 9: Total sugar content of leaf extracts of different varieties of *Moringa oleifera*

6.4. Proteins content

Plants are considered as the excellent source of proteins (building blocks and composed of essential amino acids) than animal products. Plant products like soya bean and quinoa are the complete source of protein. Our study is also concerned about the estimation of protein content among the commercially important varieties of *M. oleifera* at young and mature stages of growth. Our result revealed that among all the five varieties, Jaffna showed the highest values of protein content in comparison with the others. The trend observed was: Jaffna (0.695 ± 0.01 and 0.94 ± 0.01µg/ml) > PKM-1 (0.58±0.01and 0.88± 0.01 µg/ml) > PKM-2 (0.57 ±0.004 and 0.80 ±0.01 µg/ml) > ODC (0.44 ± 0.005 and 0.74 ± 0.01µg/ml) > Conventional (0.31 ± 0.02 and 0.64 ± 0.01 µg/ml) (Fig.9). A report by Sanchez-Machado et al. (2010), showed that the immature pods, mature pods, and flowers of *M.oleifera* contain 20.66%, 30%, and 31% of protein respectively. The aim of our study was to quantify the amount of protein present in leaves of five varieties of *M.oleifera*. Jaffna leaf extract showed higher amount of protein compared to others. Hence, Jaffna is a promising variety can used to treat the diseases (kwashiorkor, marasmus, edema, weak immune system, muscle shrinking, impaired mental health etc.) caused by the deficiency of proteins.

Protein content of leaf extracts of different varieties of *M. oleifera* are shown in figure 10.

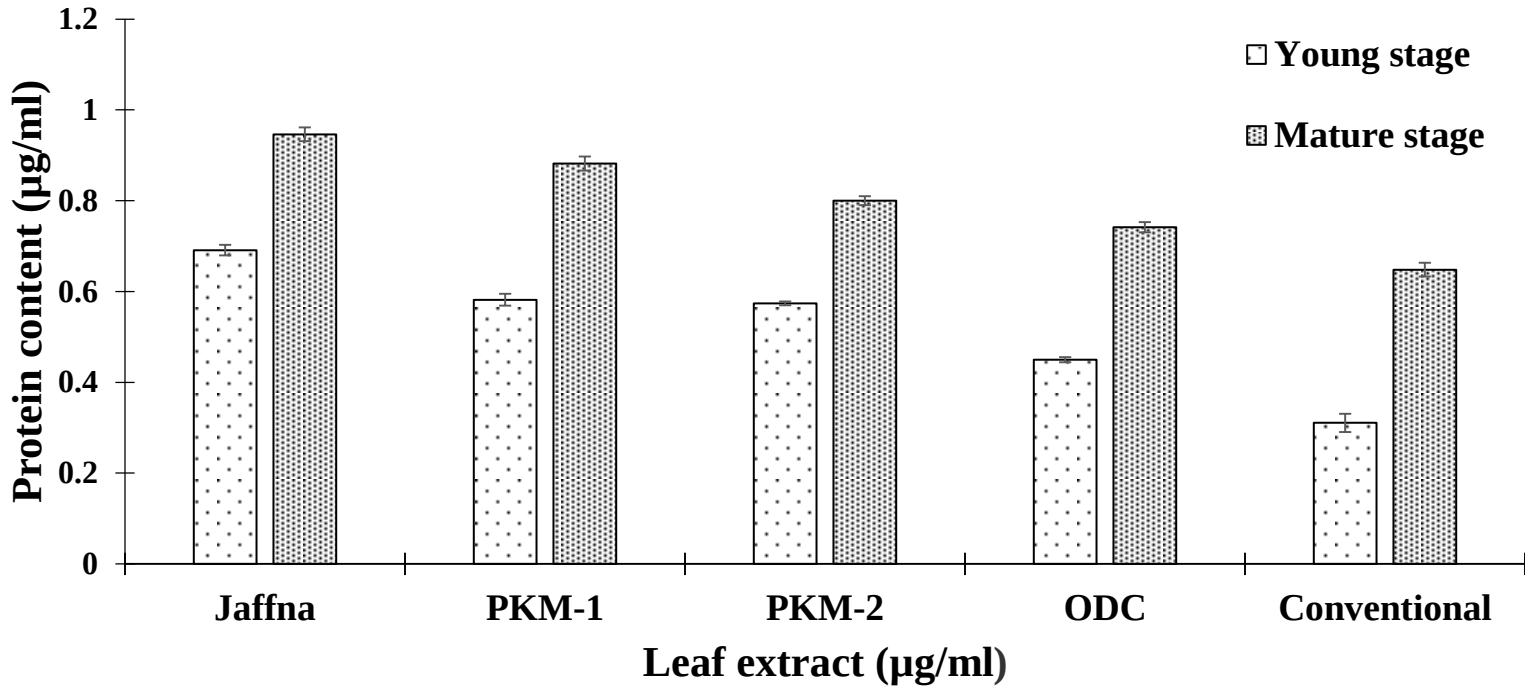


Figure 10: protein content of leaf extracts of different varieties of *Moringa oleifera*

6.5. Chlorophyll content

The chlorophyll molecules are the main pigments which absorbs the light in the photosystem reaction centres for photochemical reactions. They are having enormous number of therapeutic properties such as, anti-carcinogenic, anti-bacterial, anti-inflammatory, deodorizing, and wound healing activities) (Kang et al., 2018). In spite of all these health benefits, there are certain limitations in the production of chlorophylls at the commercial-scale as natural colorants because they are badly influenced by the environmental stresses (enzymes, light, acidic or alkaline pH, oxygen, and high temperature), result in discoloration and degradation of chlorophylls, (Schoefs, 2002).

In this study five different varieties of *M. oleifera* at three different stages (plantlet, vegetative and mature) were tested to estimate the amount of chlorophyll (chlorophyll a, chlorophyll b and total chlorophyll content). Jaffna variety showed the highest chlorophyll content at all the stages, as we compare it with other four varieties. The trend has been observed like Jaffna (1.31, 1.18 and 0.82 mg/g) > PKM-1 (1.14, 1.02 and 0.68 mg/g) > PKM-2 (0.96, 0.83 and 0.59) > ODC (0.86, 0.70 and 0.49mg/g) > Conventional (0.68, 0.58 and 0.30 mg/g) (Fig. 11).

A report by Khaleghi, (2012) revealed that the amount of chlorophyll a and total chlorophyll (Chl a+ Chl b) were reduced by the high water deficiency. Faisal and anis et al. (2006), compare the chlorophyll content of micro propagated plants with the seedling. They found more chlorophyll content in micro propagated plants [Chl b (0.61± 0.09 mg/g FW), (Chl a (0.91 ±0.19 mg/g FW)] as compare to the seedling [(chlorophyll a (0.83 ± 0.31mg/g FW) and chlorophyll b (0.53 ± 0.14 mg/g FW)].

In this study a comparison of chlorophyll content between five different varieties of *M. oleifera*, at three different stages of development was also done. In all the varieties a similar trend in chlorophyll content was observed as plantlet<vegetative<mature.

As a results found in this study demonstrated that Jaffna variety can be suggested as the carrier material because this variety provides maximum chlorophyll content and displayed better capacity for the defence of chlorophylls from the degradation.

Chlorophyll content of leaf extracts of different varieties of *M. oleifera* are shown in figure 11.

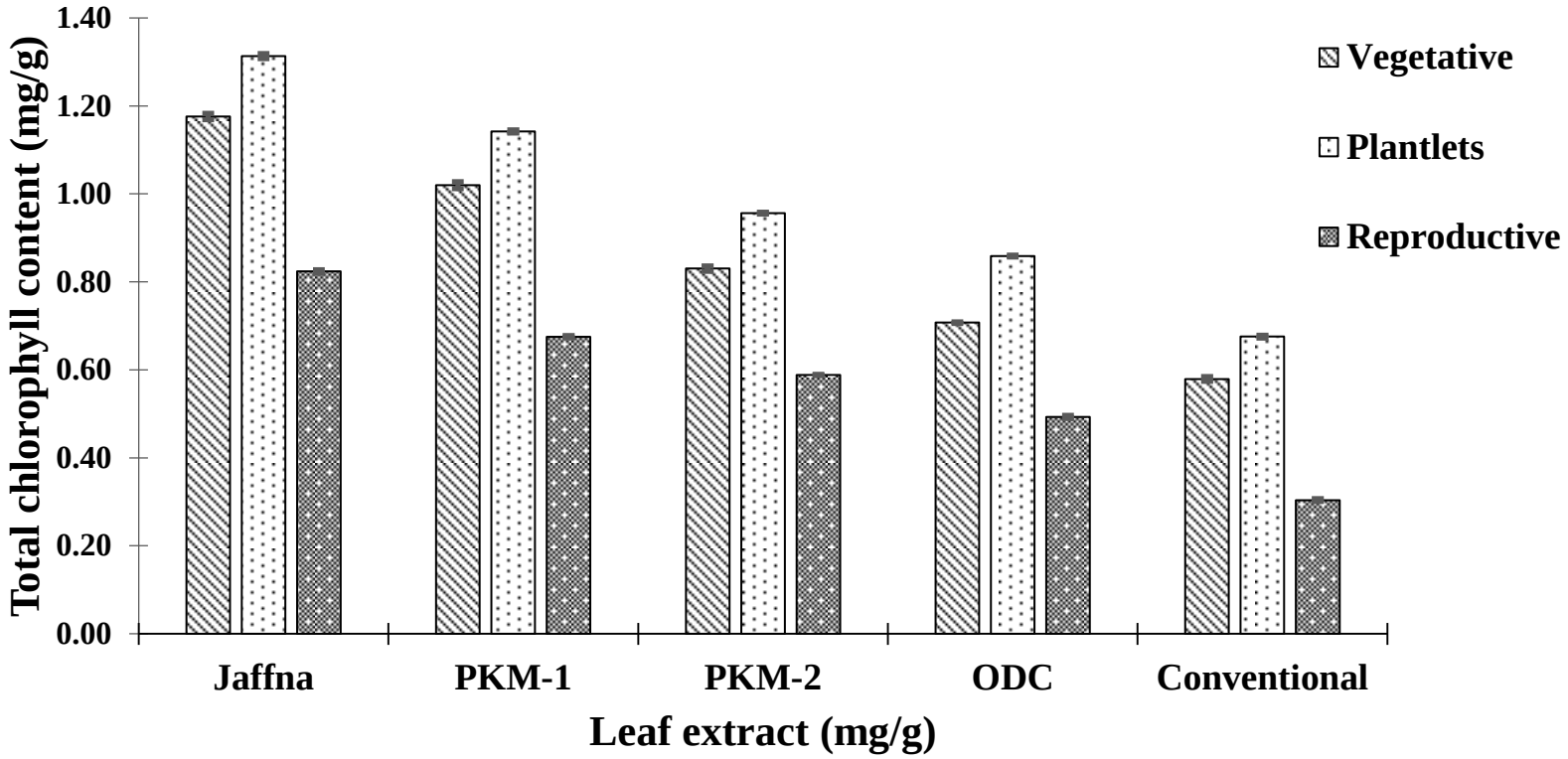


Figure 11: Chlorophyll content of *Moringa oleifera* leaf extracts

6.6. Mineral content

Minerals like calcium (Ca), potassium (K), sodium (Na), nitrogen (N), carbon (C), phosphorous (P), and Sulphur (S) are very important nutrients in plant and are essential in many processes needed to sustain plant growth and development. These minerals help plants (1) to tolerate in extreme conditions, (2), production of carbohydrates, (3) control root growth and (4) increase crop resistance to severe diseases. In humans, they also play a crucial role in maintaining good health.

Mineral contents in the five different varieties of *M. oleifera* were measured by using flame photometer (Na and K), spectrophotometer (P, and S), Kjeldahl method (N), and titration (Ca and C). The mineral analysis of five different varieties of *M. oleifera* confirming the presence of 7 elements (calcium (Ca), potassium (K), sodium (Na), nitrogen (N), carbon (C), phosphorous (P), and Sulphur (S) are shown in the Table 11.

Jaffna exhibited the highest mineral content (Na: 10.98%, K: 15.14%, P: 1.11%, S: 1.13%, Ca: 5.9%, N: 2.32%, and C: 5.7% followed by PKM-1, PKM-2, ODC and Conventional. This result indicates that Jaffna variety of *M. oleifera* leaves are a promising source of minerals. Our results are in agreement with that of Sodamade et al. (2013), Oluwole et al. (2013) and El Sohaimy et al. (2015). Jaffna leaves contained a high level of K and Na (15.14±0.1 and 10.98±0.1%) respectively. K works with Na (important electrolytes) and helps in maintaining the water balance and the blood pressure. It also prevents heart diseases, osteoporosis, and kidney stones of the body (Elson and Haas, 2007).

In case of plants, K also plays a key role in regulating enzymatic activities; provide strength against drought and stress and in translocation of photosynthetic products between plant tissues (Lei, 2011). Calcium level in Jaffna leaves was 5.9 ± 0.2% that is extremely important for human body as it helps in oocyte activation, muscle contraction , maintaining strong teeth and bones, nerves impulse, blood clotting, regulation of heart beats and fluid balance in the cells (Pravina et al., 2013).

These minerals are important for maintaining various physiological processes. Sulphur plays important role in the production of insulin (Hewlings and Kalman, 2019). Nitrogen helps in protein synthesis, influence growth and provide strong immune system (Leghari et al., 2016). Phosphorous play a vital role in the formation of proteins, bones and teeth, in repairment and maintenance of cells. It also helps in the utilization of carbohydrates and fats inside the body (Takeda et al., 2004).

Carbon is considered as the basic building block of cells inside the body, as it plays a key role in cellular respiration (Brown and Schwartz, 2009). The obtained results are in consonance with the previous reports (Ijarotimi et al., 2013; McCall et al., 2014; Gupta et al., 2014). Now, it can be concluded that the Jaffna variety of *M. oleifera* is the most nutrient-rich variety, among the five. It contains macro-essential minerals like Na, K, Ca, N, S, C, and P, which make it a potential source of food and is suitable to combat malnutrition.

The mineral analysis of five different varieties of *M. oleifera* confirming the presence of 7 elements (calcium (Ca), potassium (K), sodium (Na), nitrogen (N), carbon (C), phosphorous (P), and Sulphur (S) are shown in the Table 11.

Table 11: Percentage of mineral content of leaf extracts of different varieties of *Moringa oleifera*

Mineral	Jaffna	PKM-1	PKM-2	ODC	Conventional
K	15.14±1.1	11.07±1	8.50±1	6.77±1	4.94±0.5
Na	10.98±1	9.77±1	8.90±1	7.70±1	6.61±1
Ca	5.90±0.2	4.96±0.2	3.96±0.3	3.02±0.1	2.32±0.1
C	5.70±0.1	4.99±0.1	4.17±0.1	3.79±0.1	2.89±0.1
N	2.32±0.1	2.08±0.1	1.68±0.1	1.34±0.1	0.99±0.1
P	1.11±0.08	0.86±0.02	0.78±0.05	0.63±0.04	0.51±0.02
S	1.13±0.09	0.84±0.03	0.76±0.05	0.58±0.06	0.43±0.06

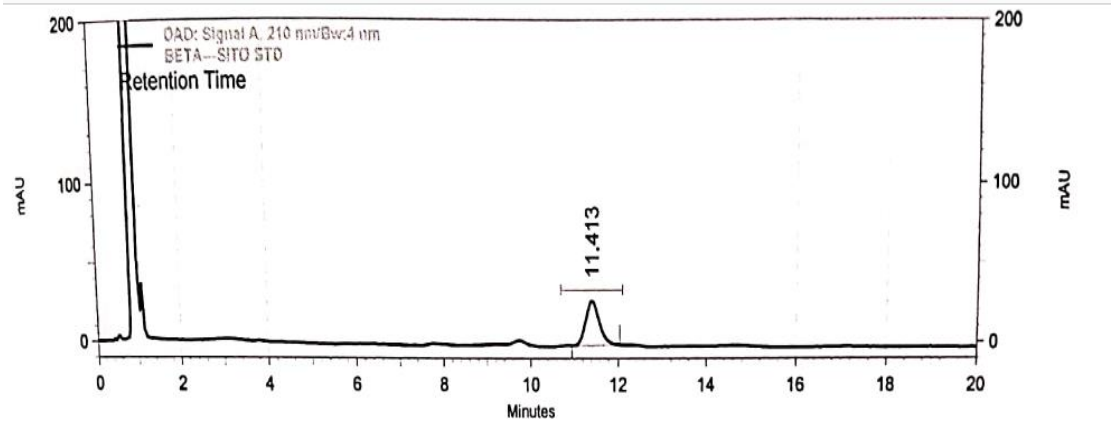
6.7. HPLC analysis

Medicinal plants have been well accepted as major source of immensely effective traditional drugs for the treatment of several dreadful diseases like cancer. These plants have been used clinically for the development of anticancer compounds to the anticancer agents (Kurokawa et al., 2016). A plentiful amount of bioactive compounds present in *M. oleifera*, have anti-cancer properties against kidney, lungs, liver, breast, esophagus, pancreatic and skin cancer (Muhammad et al., 2016).

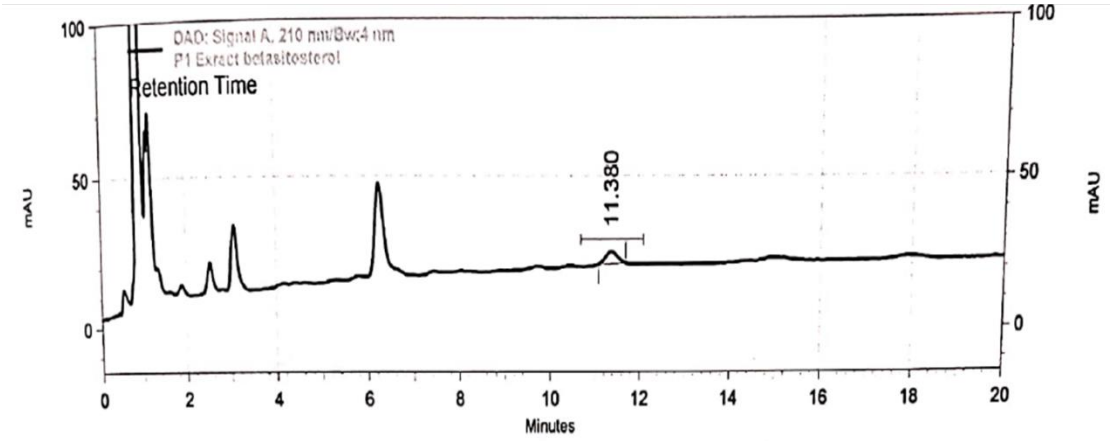
To validate these findings, the leaf extracts of all five varieties of *M. oleifera* were subjected to HPLC for the quantification of β-sitosterol, quercetin, khaempherol and moringine content. Estimation was determined by the developed analytical HPLC method and content of compounds in the sample were identified and quantified by comparing the peak area with that of standards of the compounds. It has been found that the Jaffna variety showed the highest β-sitosterol (0.244%), quercetin (0.216%), khaempherol (0.013%), and moringin (0.063%) content, followed by PKM-1 β-sitosterol (0.236%), quercetin (0.154%), khaempherol (0.012%), and moringin (0.057%), PKM-2 β-sitosterol (0.204%), quercetin (0.127%), khaempherol (0.004%), and moringin (0.46%), ODC β-sitosterol (0.110%), quercetin (0.073%), khaempherol (0.002%), and moringin (0.43%), Conventional varieties β-sitosterol (0.056%), quercetin (0.59%), khaempherol (0.001%), and moringin (0.035%). (Table 12).

The present study showed that the different varieties of *M. oleifera* contain sufficient amount of anticancer compounds such as β-sitosterol, quercetin, khaempherol, and moringin. The obtained results were in accordance with the findings of Fahey (2005), who reported the presence of phytochemicals (zeatin, caffeoylquinic acid, kaempferitrin, Isoquercitrin, rhamnetin, rhamnose, glucosinolates and isothiocyanates) in the same plant. Likewise, HPLC and MS analysis performed by (Singh et al., 2009), showed the presence of chlorogenic acid, vanillin, gallic acid, ferulic acid, and ellagic acid, from seeds, fruits, and leaves of *M. oleifera*. Muhammad et al., (2016) also indicates that the

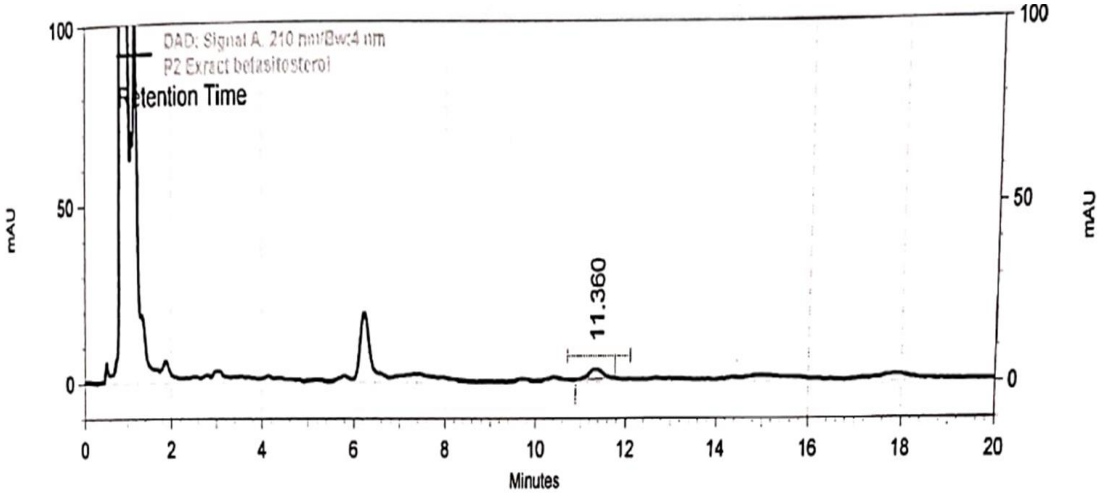
leaf extracts of *M. oleifera* contain ample number of unique compounds such as, o-coumaric acid, epicatechin, carotenoids, niazirinin, niaziminin A, niazirin, 4-[(4'-O-acetyl-L rhamnosyloxy) benzyl] isothiocyanate, 3-caffeoylquinic, and 5-caffeoylquinic acid. The comparison between the five varieties of *M.oleifera* indicates that the Jaffna variety is endowed with high anti-cancer compounds than other four varieties. : On the basis of the aforementioned results we suggest to cultivate 'Jaffna variety' in cancer-affected zones of Punjab. The leaf extracts of all five varieties of *M. oleifera* were subjected to HPLC for the quantification of β-sitosterol, quercetin, khaempherol and moringine content are shown in figure 12.


DAD: Signal A, 210 nm/Bw:4 nm
BETA---SITO STD
Retention Time
11.413
mAU
Minutes

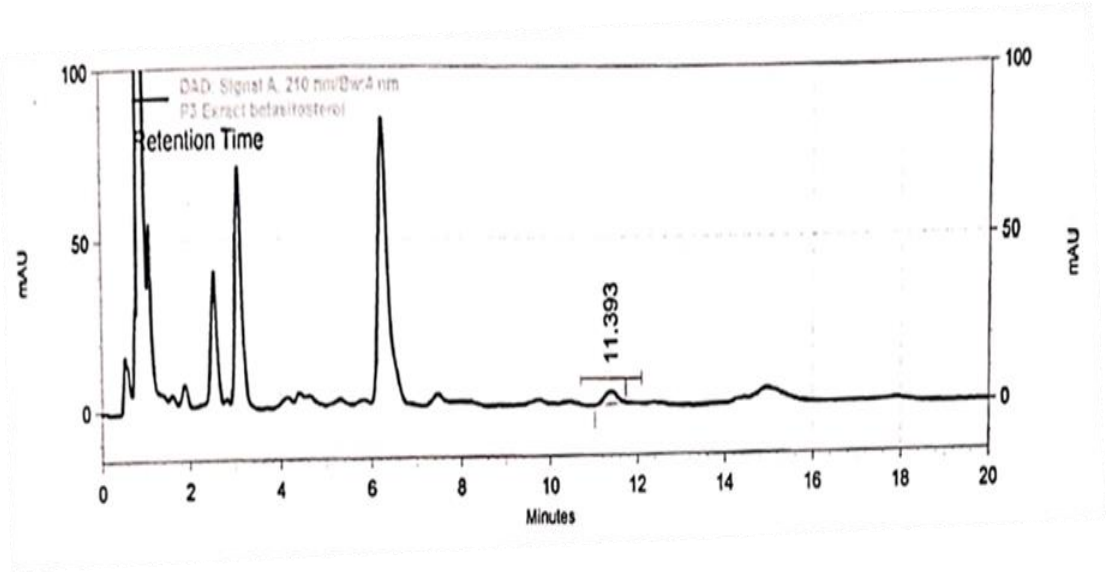
[A]


DAD: Signal A, 210 nm/Bw:4 nm
P1 Exract betasitosterol
Retention Time
11.380
mAU
Minutes

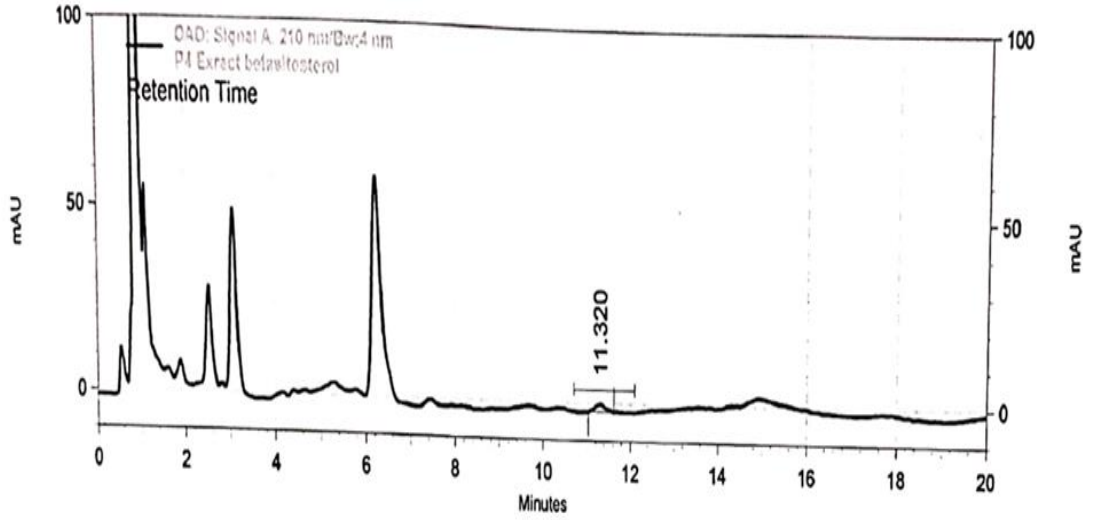
[B]


DAD: Signal A, 210 nm/Bw:4 nm
P2 Exract betasitosterol
Retention Time
11.360
mAU
Minutes

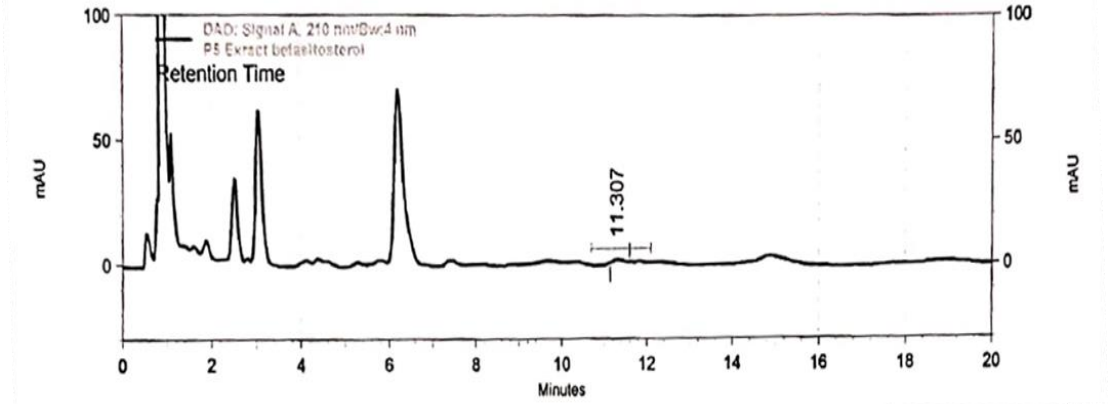
[C]


DAD: Signal A, 210 nm/Bw:4 nm
P3 Exract betasitosterol
Retention Time
11.393
mAU
Minutes

[D]

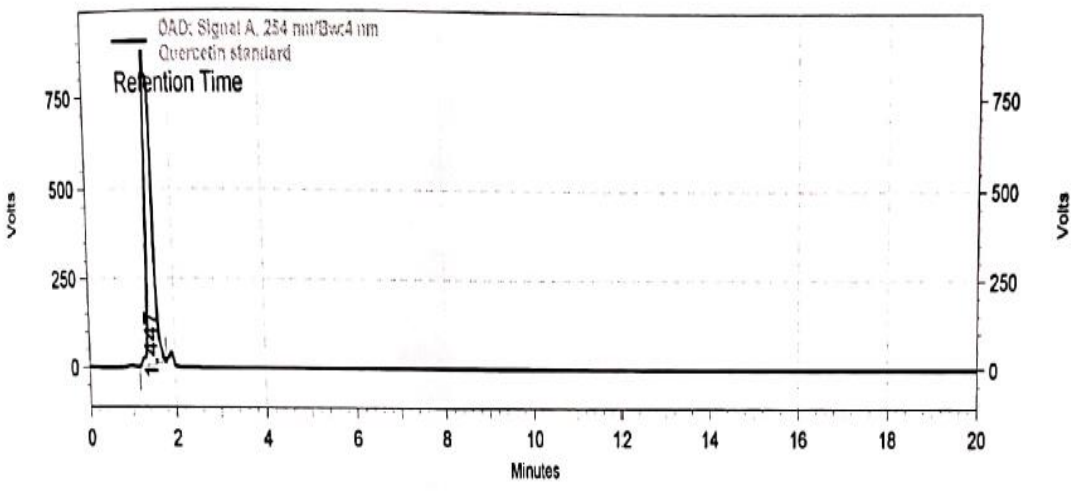


[E]

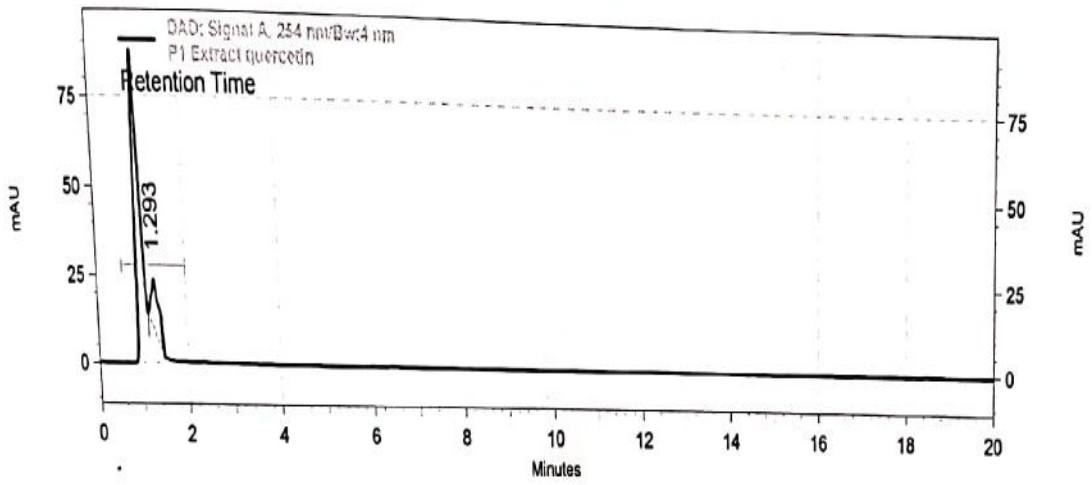


[F]

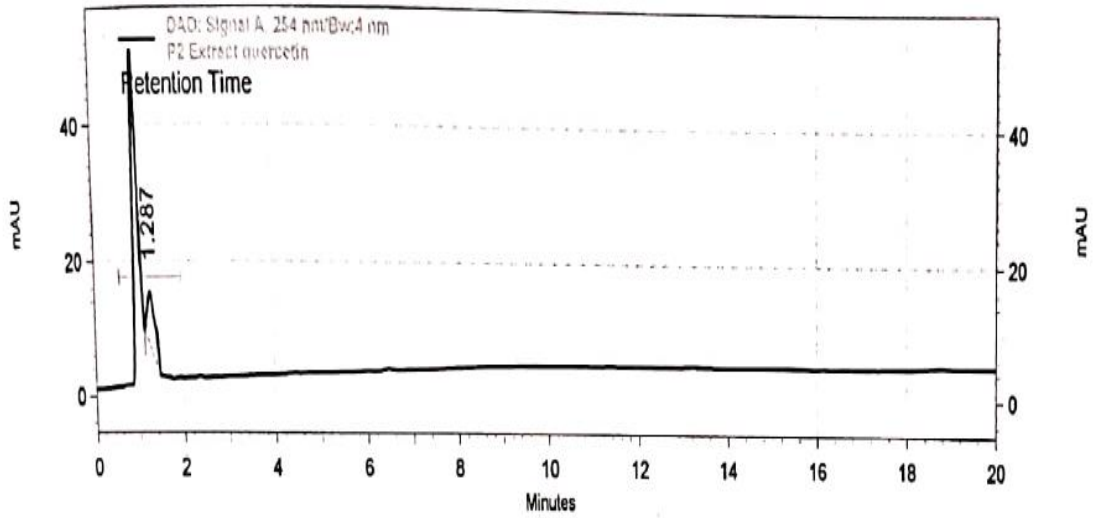
Figure 12[A]: HPLC chromatogram for estimation of β-sitosterol content in *Moringa oleifera* varieties leaf extracts. [A] Standard compound [B] Jaffna [C] PKM-1 [D] PKM-2 [E] ODC and [F] Conventional.


DAD: Signal A, 254 nm/Bw:4 nm
Quercetin standard
Retention Time
1.247
Volts
Minutes

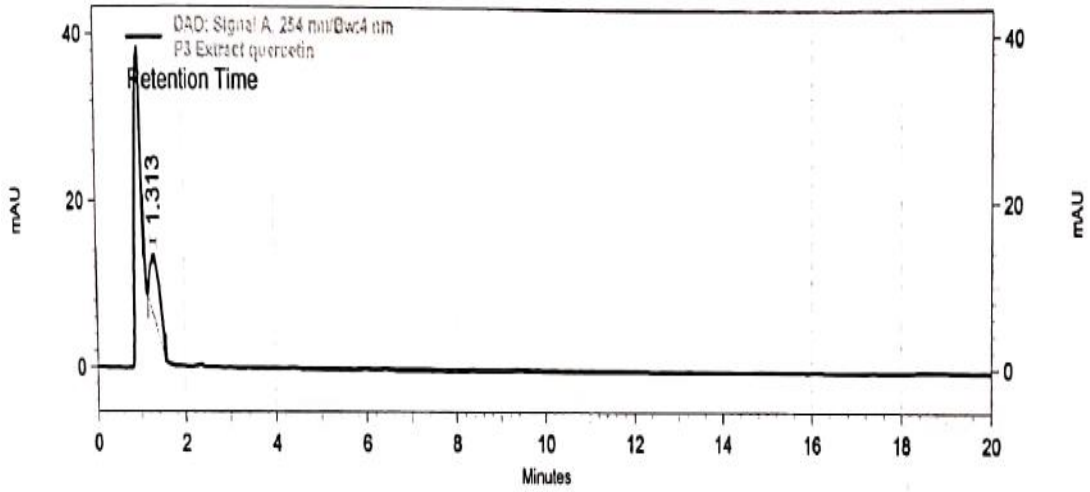
[A]


DAD: Signal A, 254 nm/Bw:4 nm
P1 Extract quercetin
Retention Time
1.293
mAU
Minutes

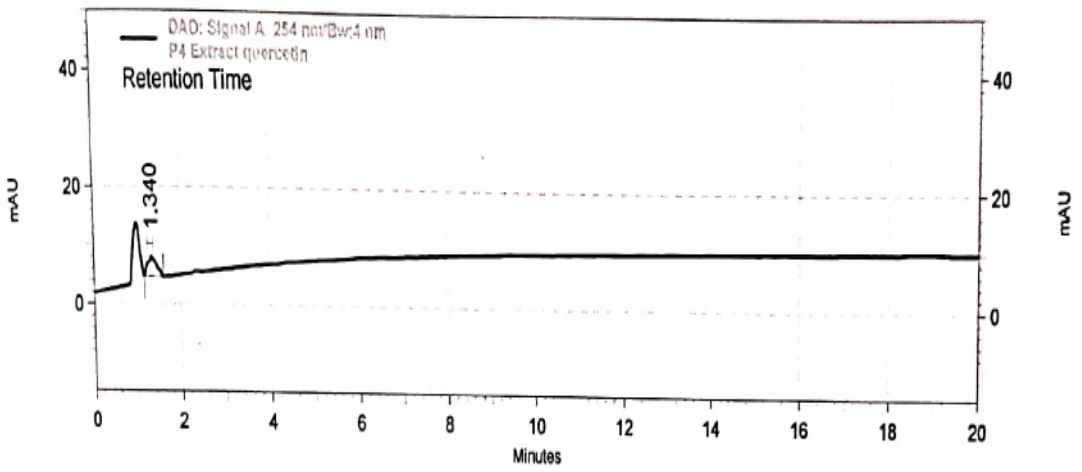
[B]


DAD: Signal A, 254 nm/Bw:4 nm
P2 Extract quercetin
Retention Time
1.287
mAU
Minutes

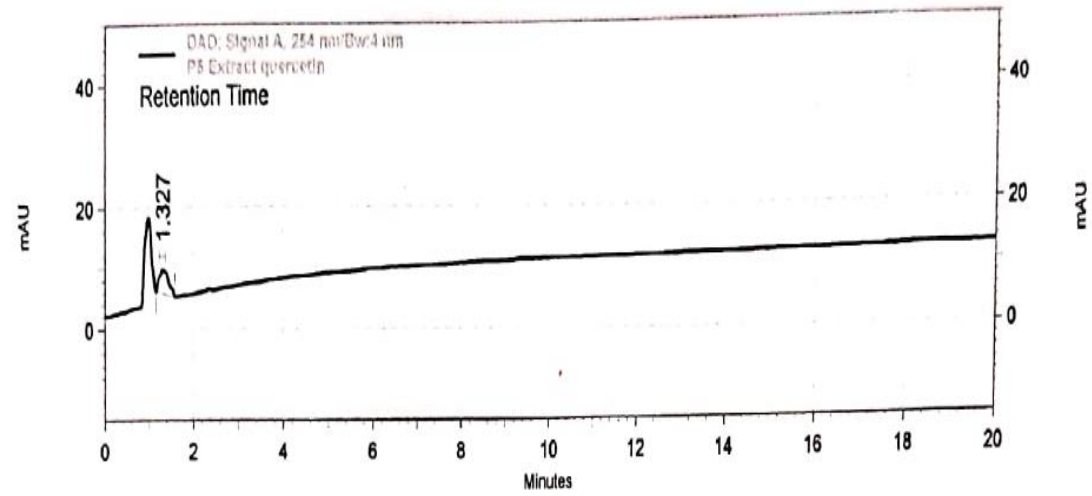
[C]


DAD: Signal A, 254 nm/Bw:4 nm
P3 Extract quercetin
Retention Time
1.313
mAU
Minutes

[D]

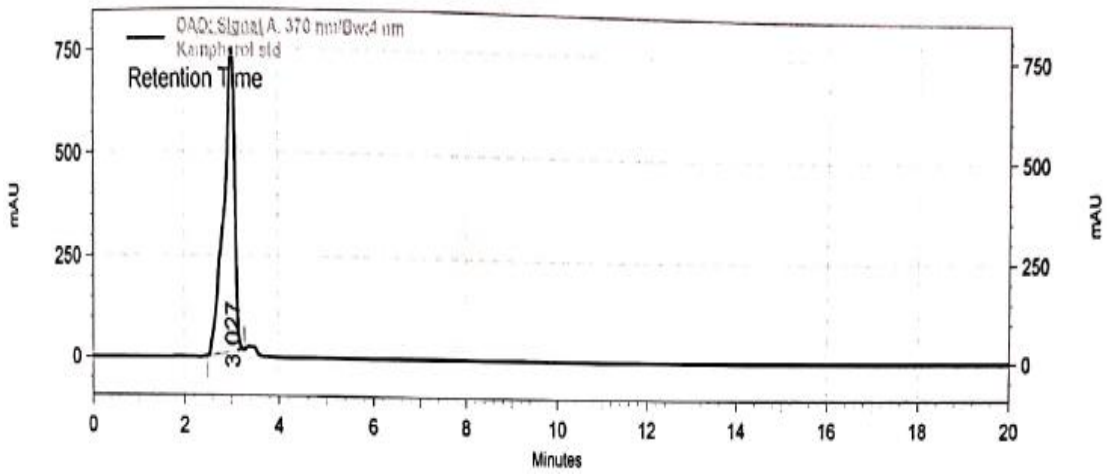


[E]

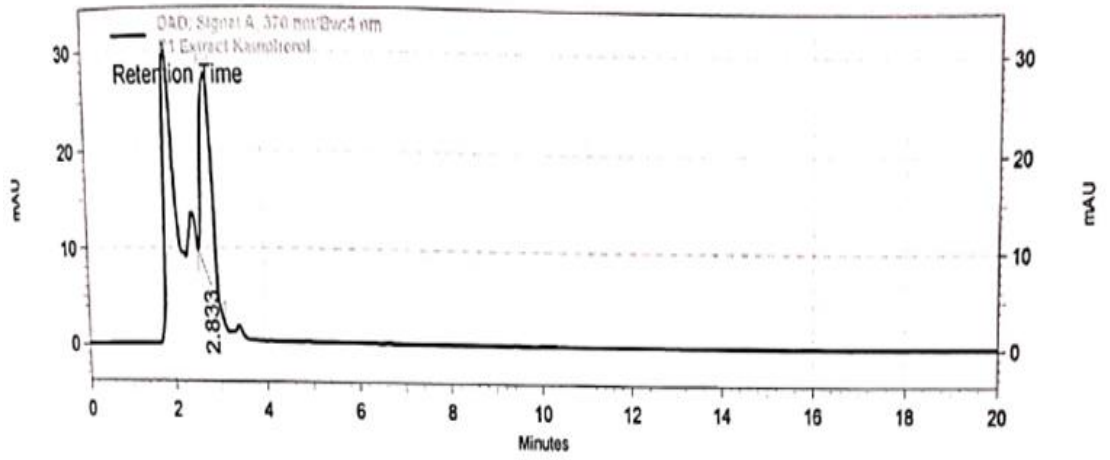


[F]

Figure 12[B]: HPLC chromatogram for estimation of Quercitin content in *Moringa oleifera* varieties leaf extracts. [A] Standard compound [B] Jaffna, [C] PKM-1 [D] PKM-2 [F] ODC and [F] Conventional


DAD1 Signal A, 370 nm/Bw:4 nm
Kamphenol std
Retention Time
3.027
mAU
Minutes

[A]


DAD1 Signal A, 370 nm/Bw:4 nm
Retention Time
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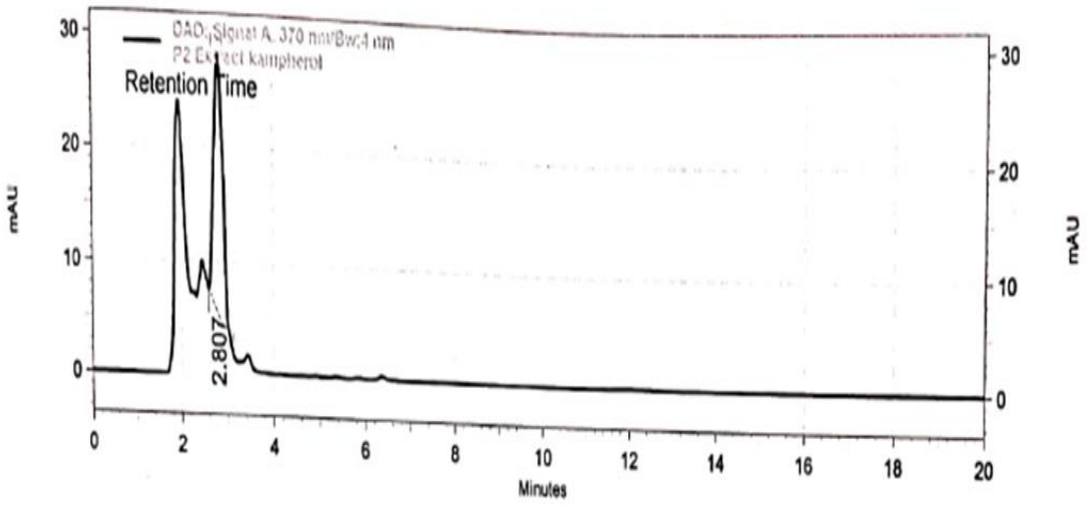
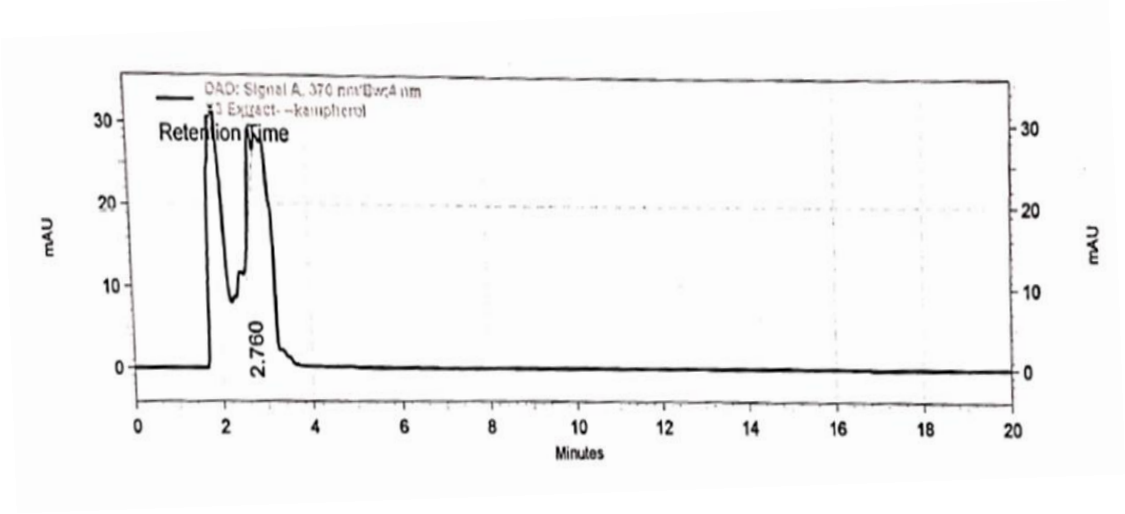
mAU
Minutes

[B]


Retention Time
2.807
mAU
Minutes

[C]


Retention Time
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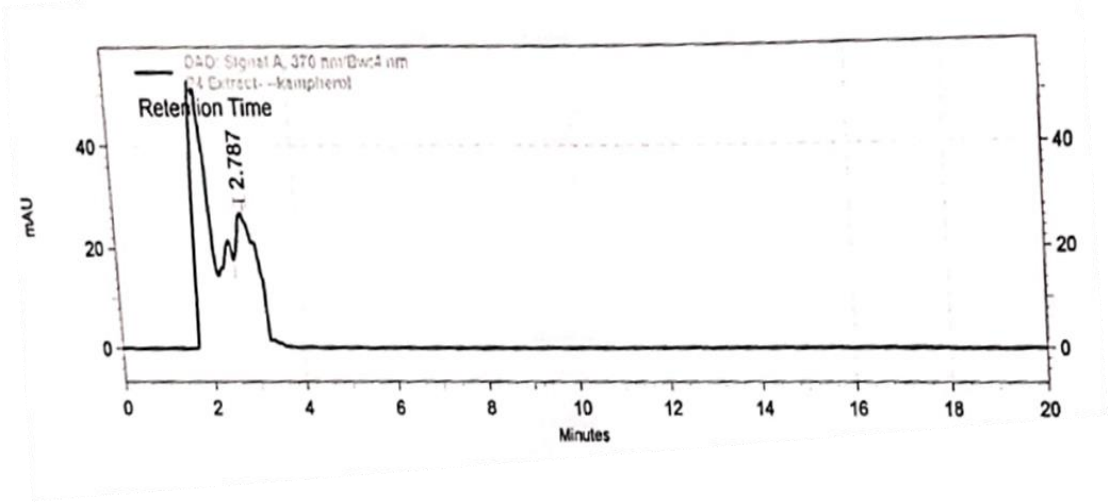
mAU
Minutes

[D]



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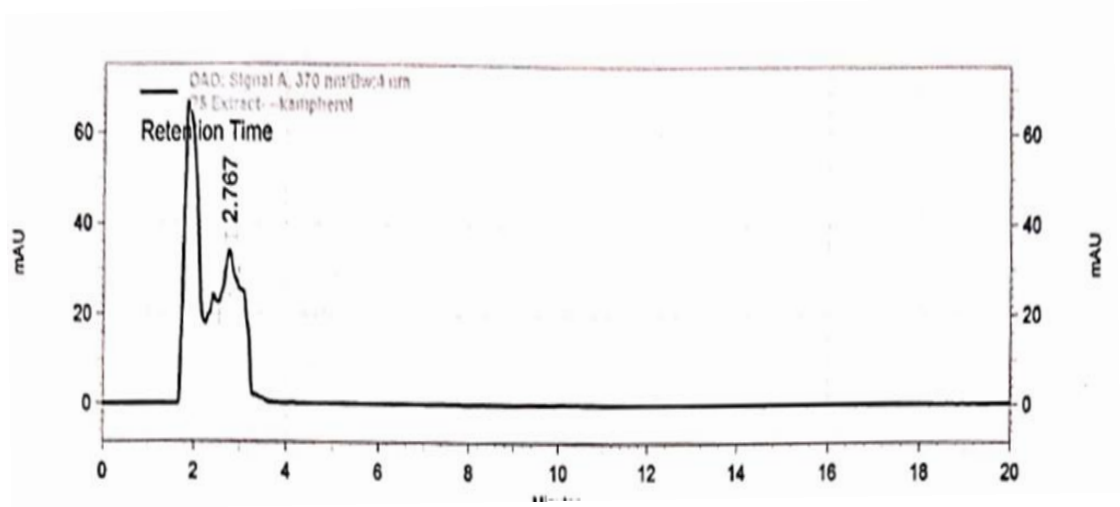
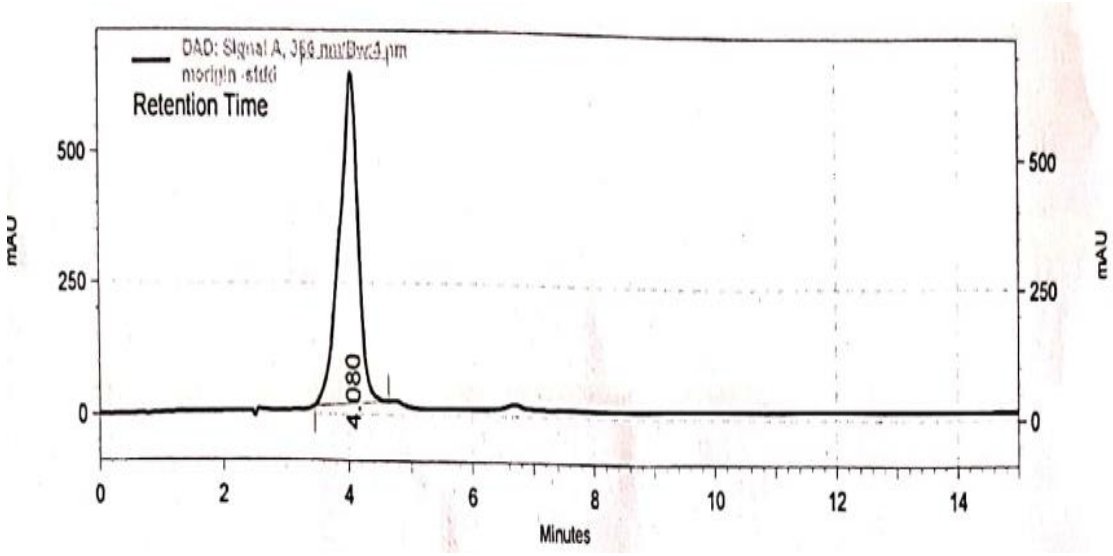


[F]

Figure 12[C]: HPLC chromatogram for estimation of Kaempferol content in *Moringa oleifera* varieties leaf extracts. [A] Standard compound [B] Jaffna [C] PKM-1, [D] PKM-2 [F] ODC and [F] Conventional


DAD: Signal A, 366 nm/Bw:4 nm
morigin -std6
Retention Time
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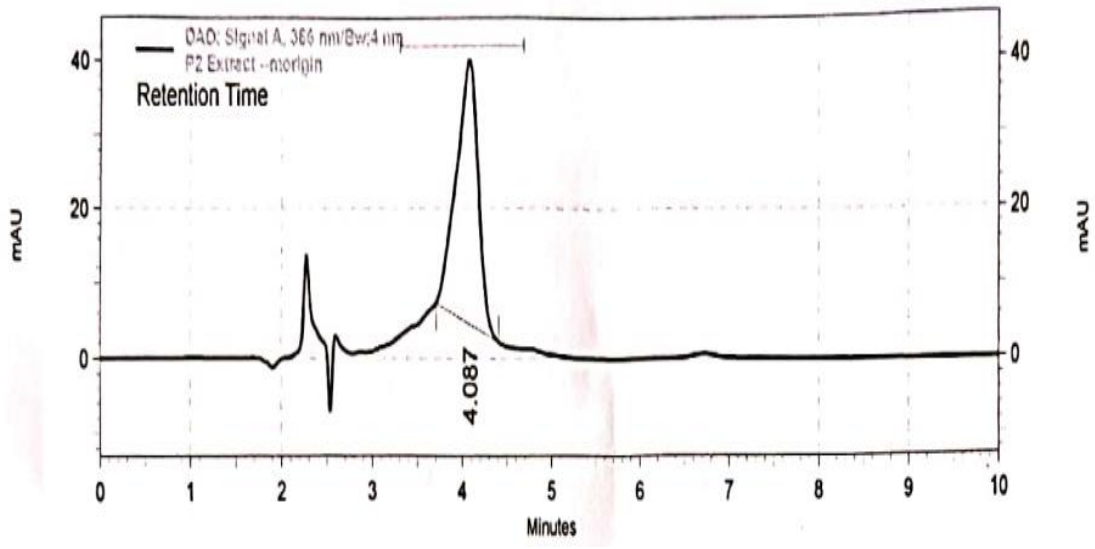
250
0
mAU
4.080
0
2
4
6
8
10
12
14
Minutes

[A]


DAD: Signal A, 366 nm/Bw:4 nm
P2 Extract --morigin
Retention Time
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20
0
mAU
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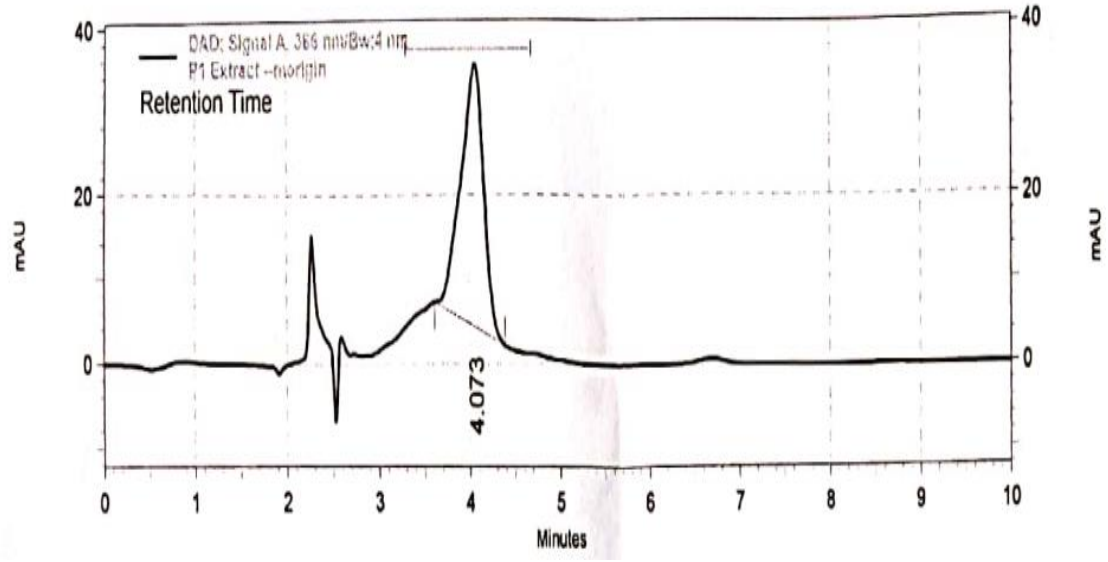
0
1
2
3
4
5
6
7
8
9
10
Minutes

[B]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P1 Extract --morigin
Retention Time
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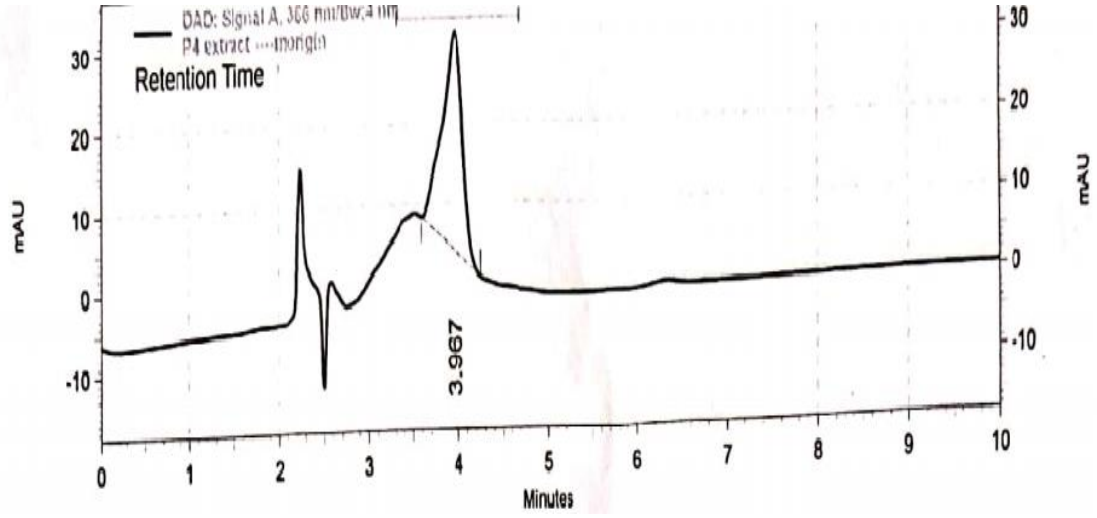
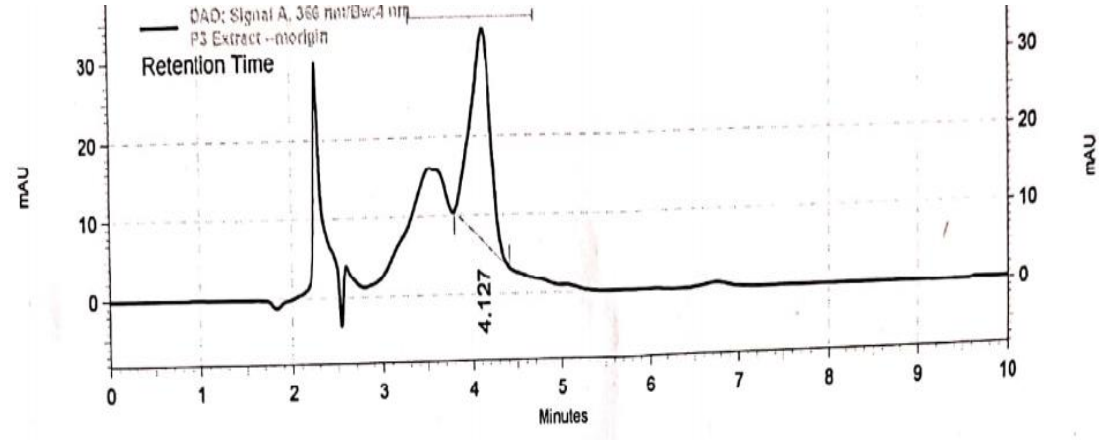
mAU
Minutes


DAD: Signal A, 366 nm/Bw:4 nm
P4 extract ----morigin
Retention Time
3.967
mAU
Minutes

[C]

[D]



[E]

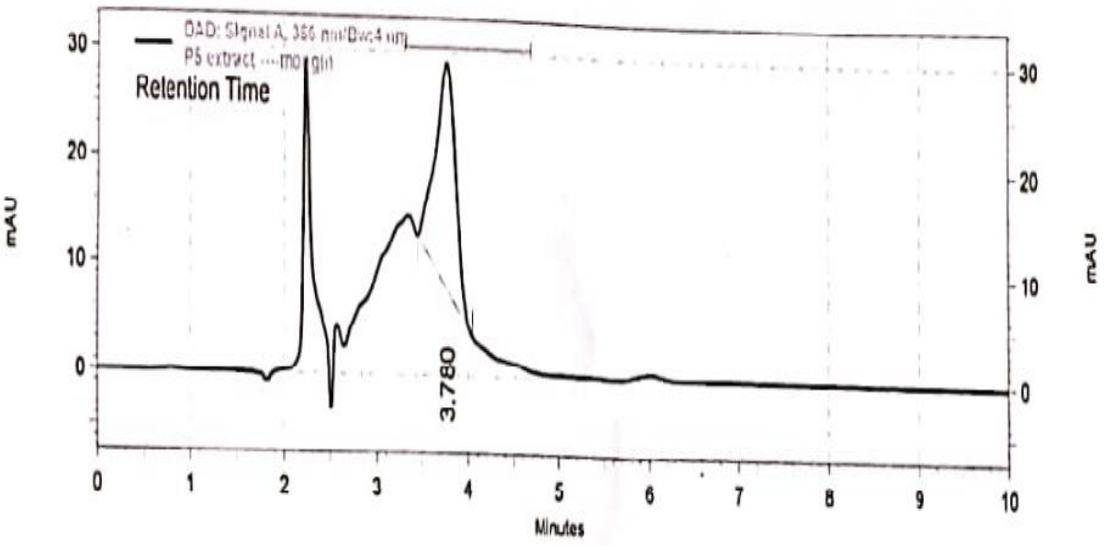


Figure 12[D]: HPLC chromatogram for estimation of Moringin content in **[F]** *Moringa oleifera* varieties leaf extracts. [A] Standard compound [B] Jaffna [C] PKM-1 [D] PKM-2 [F] ODC and [F] Conventional**.**

Table 12: HPLC analysis for estimation of bioactive compounds in five different varieties of *M.oleifera*.

Variety	Bioactive compound							
	β-sitosterol		Quercitin		Kaempferol		Moringin	
	Area	% of content	Area	% of content	Area	% of content	Area	% of content
Jaffna	154084	0.244	355765	0.216	663751	0.013	1254800	0.063
PKM-1	149743	0.236	234489	0.155	652556	0.012	1133682	0.057
PKM-2	129271	0.204	204488	0.127	239864	0.004	898404	0.046
ODC	72338	0.110	118729	0.073	111264	0.002	864931	0.043
Conventional	35945	0.056	94796	0.030	71290	0.001	671978	0.03

6.8. Antibacterial activity of different varieties of *Moringa oleifera*

Antibacterial activity of aqueous and ethanolic leaves and seeds extract was analysed separately by using disc-diffusion method. Among the two solvents, the ethanolic leaf extract of Jaffna variety exhibited the highest antibacterial activity as indicated by the maximum zone of inhibition (average values): 27.80, 26.00, 23.60 and 25.00 mm against *P. aeruginosa*, *B.subtilis*, *E. coli*, and *S. aureus* respectively (Table 13) followed by PKM-1, PKM-2, ODC, and Conventional. Similarly, using the ethanolic seeds extract a decreasing trend of bacterial zone of inhibition: Jaffna (25.86, 23.80, 22.56; and 20.66 mm $p < 0.05$) > PKM-1 (24.63, 20.2, 20.06 and 19.83 mm; $p < 0.05$), > PKM-2 (21.73, 20.13, 17.70 and 18.26 mm; $p < 0.05$) > ODC (18.60, 13.06, 12.70and 9.70 mm; $p < 0.05$) > Conventional (16.70, 14.70, 9.50 and 8.70 mm; $p < 0.05$) was observed.

It was interesting to observe that in all the four experiments (ethanol: leaf extract; aqueous: leaf extract; ethanol: seed extract; and aqueous: seed extracts) Jaffna variety exhibited the maximum zone of inhibition. The antibacterial activity increased significantly with the increasing concentration of the extract (100 µg being the maximum

concentration). A similar trend (Jaffna > PKM-1 > PKM-2 > ODC > Conventional) of decreasing order of bacterial zone of inhibition was observed in each experiment (Fig. 13).

Aqueous extract of all the varieties exhibited the least antibacterial activity as compared to the ethanolic extracts. Our results are in agreement with the findings of Nepolean et al., 2009 and Kalpana et al., 2013 as they also reported that the ethanolic extract of Moringa leaf exhibit better antibacterial activity than the aqueous extract. In addition to it, the ethanolic leaves extracts showed better zone of inhibition than the ethanolic seeds extracts. The antibacterial activty of leaves and seeds of *M. oleifera* is due to the presence of pterygospermin, moringine and benzyl isothiocyanate (Jahn et al., 1986; Gopalakrishnan et al., 2016).

Anti-bacterial activity of ethanolic and aqueous leaf and seed extracts of Jaffna, PKM-1, PKM-2, ODC, and Conventional variety on bacterial strains **[A]** *P. aeruginosa*, **[B]** *B. subtilis* **[C]** *E. coli* and **[D]** *S. aureus* are shown in figure 13A-D.

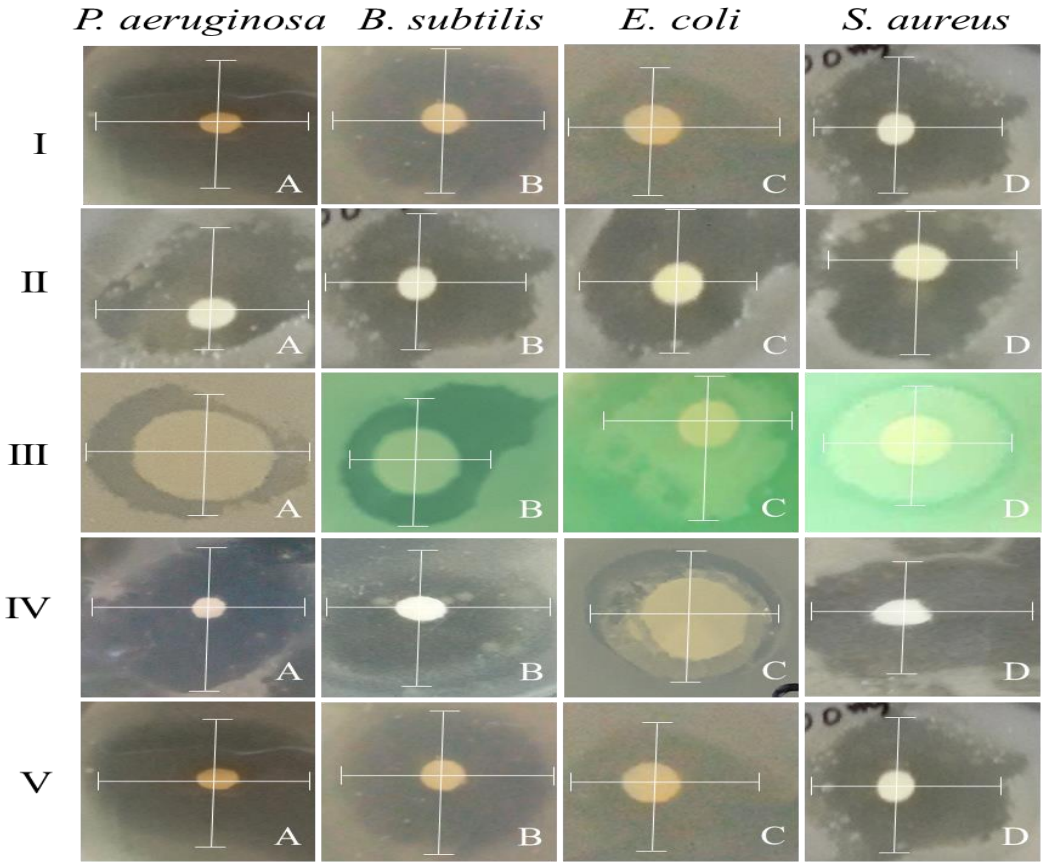


Figure 13[A]: Anti-bacterial activity of aqueous leaf extract of Jaffna (*panel I*), PKM-1(*panel II*), PKM-2 (*panel III*), ODC (*panel IV*), and Conventional variety (*panel V*), on bacterial strains **[A]** *P. aeruginosa*, **[B]** *B. subtilis* **[C]** *E. coli* and **[D]** *S. aureus*.

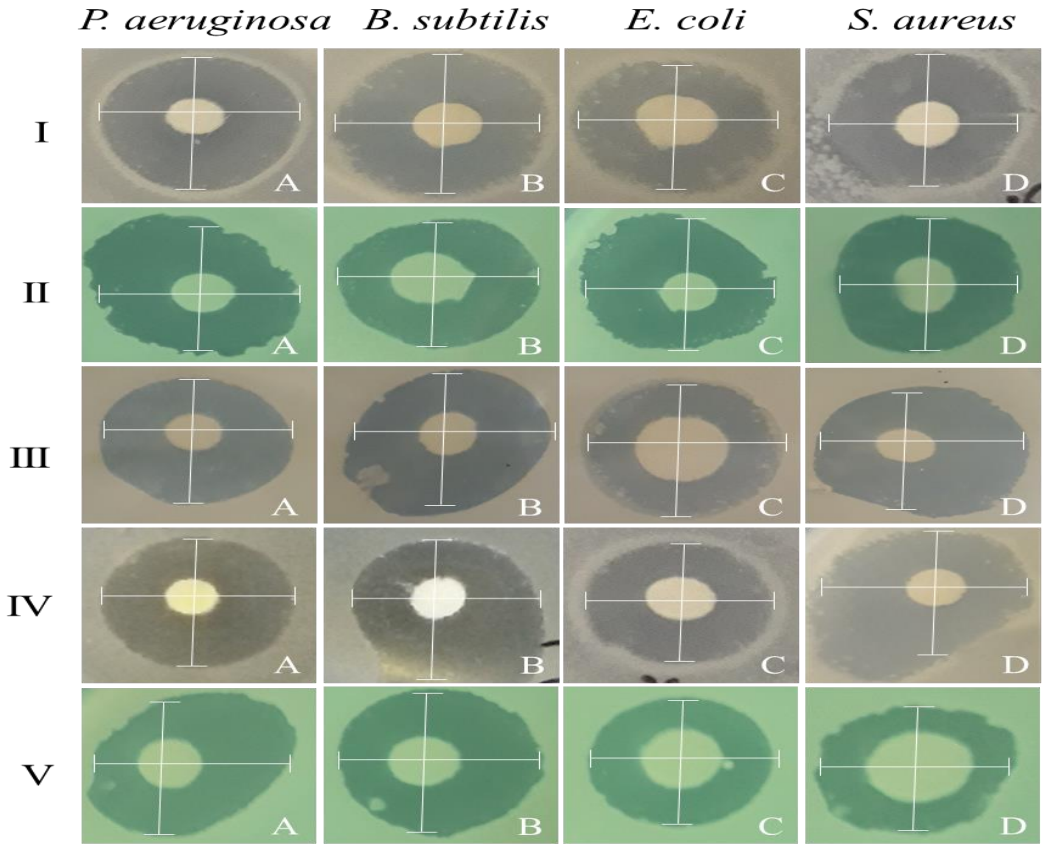


Figure 13[B]: Anti-bacterial activity of ethanol leaf extract of Jaffna (*panel I*), PKM-1(*panel II*), PKM-2 (*panel III*), ODC (*panel IV*), and Conventional variety (*panel V*), on bacterial strains **[A]** *P. aeruginosa*, **[B]** *B. subtilis* **[C]** *E. coli* and **[D]** *S. aureus*.

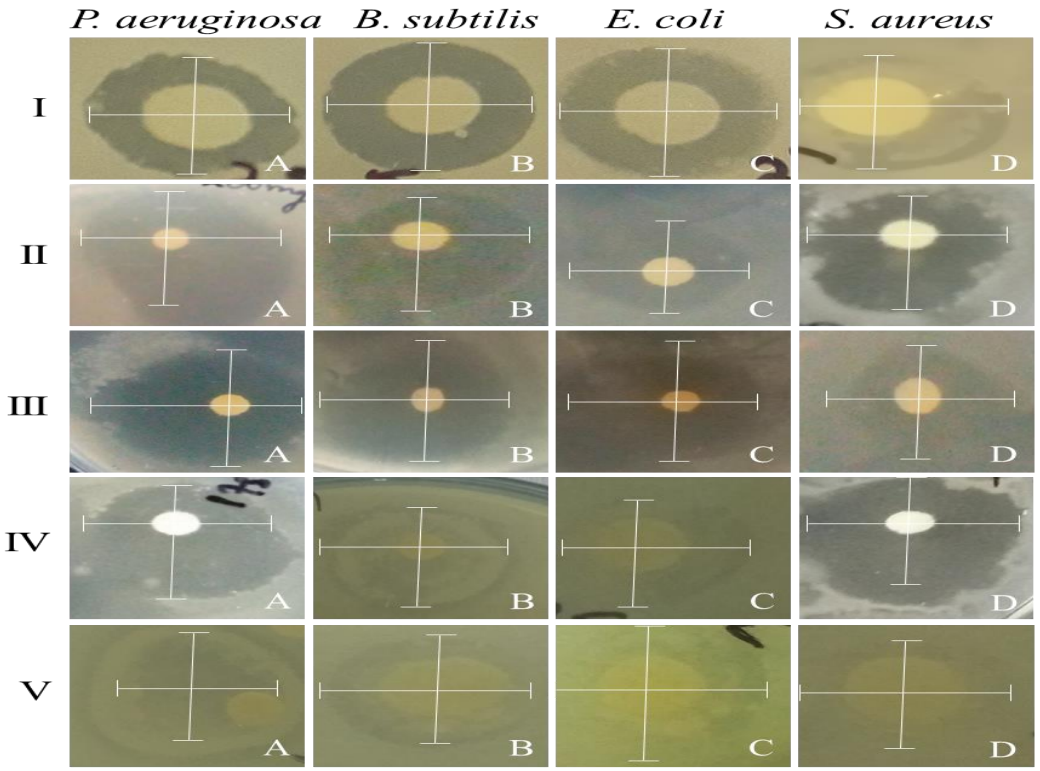


Figure 13[C]: Anti-bacterial activity of aqueous seed extract of Jaffna (*panel I*), PKM-1(*panel II*), PKM-2 (*panel III*), ODC (*panel IV*), and Conventional variety (*panel V*), on bacterial strains **[A]** *P. aeruginosa*, **[B]** *B. subtilis* **[C]** *E. coli* and **[D]** *S. aureus*.

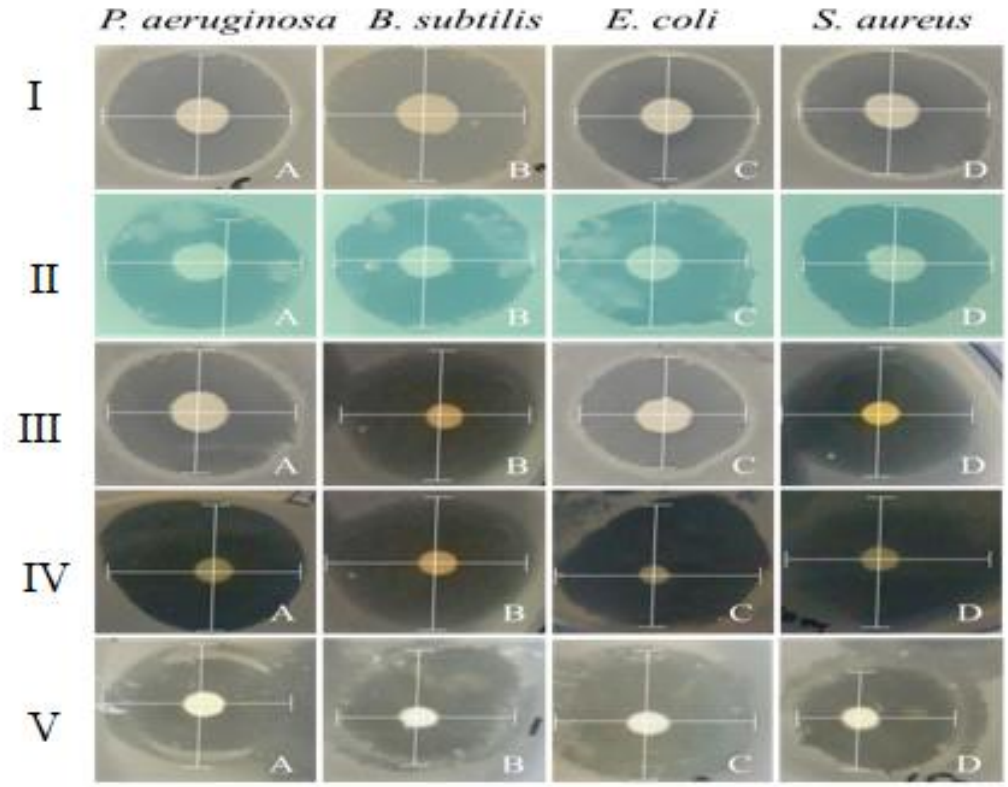


Figure 13[D]: Anti-bacterial activity of ethanol seed extract of Jaffna (*panel I*), PKM-1(*panel II*), PKM-2 (*panel III*), ODC (*panel IV*), and Conventional variety (*panel V*), on bacterial strains **[A]** *P. aeruginosa*, **[B]** *B. subtilis* **[C]** *E. coli* and **[D]** *S. aureus*.

Bacterial zone of inhibition of ethanolic leaf and seed extracts of different varieties of *M. oleifera* are listed in table 13.

Table 13: Bacterial zone of inhibition values using leaf and seed extracts of *Moringa oleifera* varieties

Ethanolic leaf extract					**Ethanolic seed extract**			
Varieties	***P. aeruginosa***	***B. subtilis***	***E. coli***	***S. aureus***	***P. aeruginosa***	***B. subtilis***	***E. coli***	***S. aureus***
Jaffna	27.8±0.43	26.0±0.80	23.6±0.36	25.0±0.40	25.86±0.15	23.8±0.20	22.56±0.45	20.66±0.30
PKM-1	23.9±0.26	24.90±0.45	22.4±0.50	23.0±0.10	24.63±0.35	20.20±0.20	20.06±0.81	19.83±0.15
PKM-2	23.23±0.20	20.33±0.61	19.83±0.85	19.83±0.15	21.73±0.25	20.13±0.51	17.70±0.36	18.26±0.30
ODC	20.26±0.30	20.83±0.20	18.0±0.40	16.73±0.25	18.60±0.36	13.66±0.41	12.70±0.20	9.70±0.30
Conventional	18.43±0.40	18.46±0.61	13.66±0.41	14.63±0.40	16.70±0.30	14.70±0.43	9.50±0.40	8.70±0.25
Aqueous leaf extract					**Aqueous seed extract**			
Jaffna	16.83±0.20	14.66±0.41	13.63±0.65	11.83±0.15	16.66±0.35	14.86±0.15	12.53±0.45	10.63±0.30
PKM-1	15.83±0.20	12.66±0.41	10.13±0.61	10.7±0.26	15.73±0.30	12.7±0.30	10.66±0.35	9.66±0.30
PKM-2	13.80±0.20	11.20±0.20	9.76±0.25	13.36±3.78	13.63±0.47	10.56±0.40	8.63±0.40	7.70±0.30
ODC	13.63±0.35	13.83±0.41	8.60±0.45	8.86±0.85	11.80±0.35	9.33±0.20	8.633±0.47	10.60±0.36
Conventional	9.96±0.90	10.03±0.90	6.76±0.32	5.73±0.30	9.70±0.26	8.56±0.450	6.93±0.50	3.53±0.26

6.9. Plant growth promoting activity

A significant increase in growth characteristics was observed in plants sprayed with leaf extracts of different varieties of *M. oleifera* as compared to the untreated control. The shoot length, root length, number of leaves, plant dry weight, chlorophyll a and b, carotenoids, total soluble sugar, total protein content, total phenolic and flavonoid contents of *S. rebaudiana* were maximally increased by the foliar spray of Jaffna (35.43 ± 0.2 cm, 30.7 ± 0.5cm, 138 ± 3.5, 4.9 ± 0.43 g, 0.85 ± 0.03mg/g, 0.87 ± 0.01 mg/g, 0.45 ± 0.01 mg/g, 25.69 ± 0.63 mg/g, 8.43 ± 0.84 mg/g, 420.45 ± 0.503 mg/g and 194.43 ± 0.25 mg/g respectively), followed by the PKM-1, PKM-2, ODC and Conventional (Table 14). The reason for the maximal enhancement of the plant growth characteristics of *S. rebaudiana* plants treated with Jaffna leaf extracts may be that it contains a high content of essential macro and micronutrients such as calcium, potassium, zinc and phytohormones such as auxin, gibberellins and zeatin that favoured a rapid cell division, cell enlargement and multiplication (Merwad and Abdel-Fattah, 2016; Waqas et al., 2017; Rehman et al., 2017; Soliman and Shanan, 2017).

6.9.1. Chlorophyll content of *Stevia rebaudiana*

The maximum photosynthetic pigment content was observed in plants treated with of Jaffna leaf extract. The increased chlorophyll content in stevia may be due to the internal hormonal activation or due to the presence of a high content of iron and magnesium. These elements regulate biosynthesis of chlorophyll by catalyzing the conversion of proporphyrine to chlorophyllide. The cytokinin present in *M. oleifera* leaf extract prevented the premature leaf senescence, enhanced the biosynthesis of chlorophyll and also prevented its degradation in *Stevia* plants (Yasmeen et al., 2014; Basra, 2016).

6.9.2. Total soluble sugar content (TSS) of *Stevia rebaudiana*

As already mentioned the highest total soluble sugar content was observed in plants treated with Jaffna leaf extract. This may be due to the enhanced chlorophyll pigments and the photosynthesis which lead to the accumulation of a high sugar content in Jaffna extract treated *Stevia* plants. *M. oleifera* is a potent source of cytokinin which plays a vital role in sustaining the metabolic state and normal photosynthate exporting phase of mature leaves. Cytokinins from Jaffna variety of *M.oleifera* may have improved the sink and source capacity of *S. rebaudiana* and it might be the cause of increment in its sugar content (Crouch and Staden, 1991).

6.9.3. Total soluble protein (TSP) content of *Stevia rebaudiana*

The maximum total soluble protein (TSP) content was reported in plants treated with foliar spray of Jaffna. A higher amount of TSP can be attributed to a high endogenous profile of *M. oleifera* leaf extract that comprises hormones such as cytokinins, auxins etc., vitamins, and minerals and other bioactive compounds. Such attributes directly or indirectly promotes enzymatic actions, which in turn increases the cell division and proten production (El-Tohamy et al., 2007).

The growth characteristics (The shoot length, root length, number of leaves, plant dry weight, chlorophyll a and b, carotenoids, total soluble sugar, total protein content, total phenolic and flavonoid contents) of *S. rebaudiana* was observed after the foliar spray of leaf extracts of different varieties of *M. oleifera* and are listed in table 13.

Table 14: Enhancement in growth attributes of *S. rebaudiana* by *M. oleifera* foliar spray

Foliar spray	Shoot length (cm)	Root length (cm)	No. of leaves	Plant dry weight (gm)	Chlorophyll a content (mg/g FW)	Chlorophyll b content (mg/g FW)	Carotenoids content (mg/g FW)	Total soluble sugars content (mg/g FW)	Total protein content (mg/g FW)	Total Phenolic content (µg/g FW)	Total flavonoid content (µg/g FW)
JSS	35.43 ± 0.2	30.7 ± 0.5	138 ± 3.5	4.9 ± 0.43	0.85 ± 0.03	0.87 ± 0.01	0.45 ± 0.01	25.69 ± 0.63	8.43 ± 0.84	420.46 ± 0.503	194.43 ± 0.25
P1SS	33.26 ± 0.3	22.4 ± 0.3	122 ± 3.6	4.46 ± 0.34	0.83 ± 0.01	0.86 ± 0.01	0.41 ± 0.01	22.8 ± 0.82	7.75 ± 0.49	387.2 ± 0.2	185.5± 0.36
P2SS	28.4 ± 0.3	25.5 ± 0.2	103 ± 4.3	4.01 ± 0.20	0.81 ± 0.01	0.83 ± 0.01	0.35 ± 0.01	17.84 ± 0.84	7.28 ± 0.78	356.36 ± 0.32	162.13 ± 0.9
OSS	27.1 ± 0.26	15.7 ± 0.3	88 ± 3.5	3.13 ± 0.48	0.79 ± 0.01	0.82 ± 0.01	0.30 ± 0.02	14.83 ± 0.79	6.69 ± 0.53	308.36 ± 0.1527	135.3 ± 0.26
CSS	20.63 ± 0.2	12.5 ± 0.9	64 ± 4.04	2.82 ± 0.04	0.78 ± 0.01	0.80 ± 0.01	0.27 ± 0.02	12.05 ± 0.81	5.66 ± 0.55	280.4 ± 0.4	120.86 ± 0.9
WSS	12.46 ± 0.4	6.5 ± 0.30	48 ± 3.05	1.32 ± 0.01	0.75 ± 0.03	0.78 ± 0.02	0.25 ± 0.02	9.31± 0.78	4.40 ± 0.62	230.41 ± 0.25	112.21 ± 0.66

6.9.4. DPPH radical scavenging activity of *Stevia rebaudiana*

The highest DPPH radical scavenging activity was recorded in leaf extracts of the *Stevia* plants treated with foliar spray of Jaffna. The untreated stevia plants showed a lower value of DPPH radical scavenging activity than the treated ones (Fig. 10). This is also confirmed by the phenolic and flavonoid content values which are highest in plants treated with Jaffna leaf extract. This might be linked with the presence of high amount of vitamin C, ascorbate, carotenoids, phenols and flavonoids in *Moringa* leaves (Abdel-Kader et al., 2016). A high TPC and TFC values of *M. oleifera* leaf extract treated plants can also be attributed to the presence of several phytochemicals such as kaempferol, quercetin, rhamnetin, isoquercetin, kaempferitrin present in *Moringa* leaves, which also the enhanced antioxidant potential of *Stevia*. Also, *Moringa* is a potent source of cytokinins which are known to perceive signals and generate higher phenolics and other plant secondary metabolites (Yasmeen et al., 2014; Basra, 2016). In addition, *Moringa* leaves are a potent source of glycoside compounds, glucosinolates and isothiocyanates, which may lead to the formation of phytochemical compounds, which in turn can enhance the antioxidant activity. The increase in phenolic contents may also be linked with the high vitamin C content of *Moringa* leaf extract. Total phenolic content and antioxidant activity have a strong relationship. Phenolic compounds are a major contributor of antioxidant activity as phenols have high free radicals scavenging ability due to their hydroxyl groups (Wu Lin, 2000).

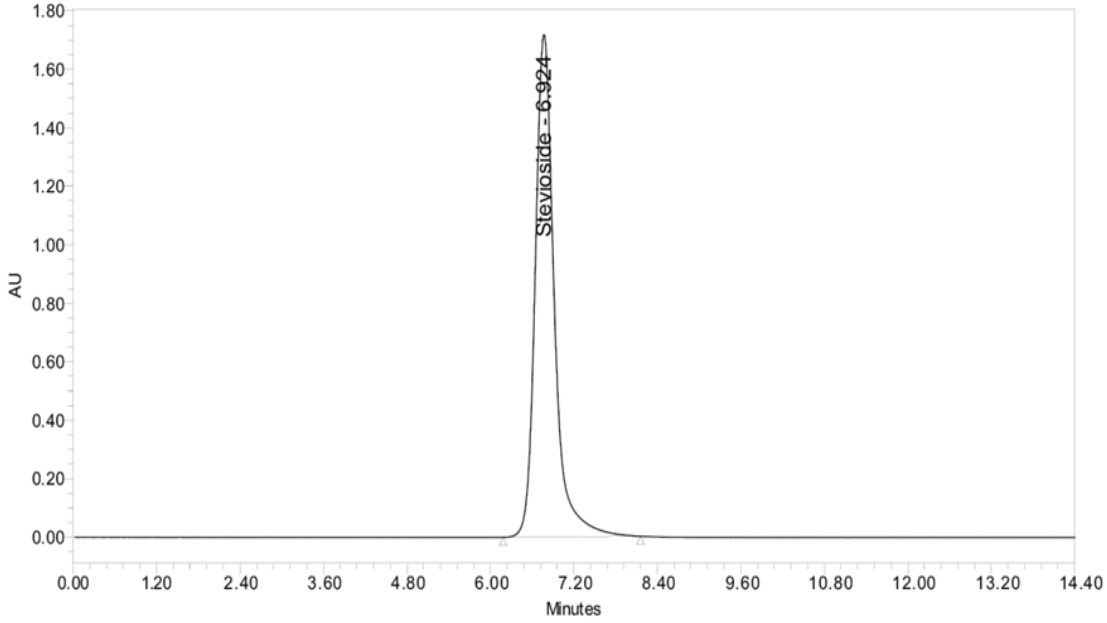
6.9.5. Stevioside and Zeatin contents in treated *Stevia rebaudiana*

The decreasing order of stevioside and zeatin contents in treated *Stevia*, as determined by HPLC were: JSS (7.73%; 0.0063%) > P1SS sprayed *Stevia* (6.93%; 0.0056%) > P2SS sprayed *Stevia* (6.45%; 0.0051%) > OSS sprayed *Stevia* (4.14%; 0.0048%) > CSS (3.86%; 0.0042%) > WSS (2.94%; 0.00088%) respectively. The highest stevioside and zeatin content was recorded in *Stevia* plants treated with foliar spray of Jaffna, than the other varieties (Table 15 and Fig. 14A-G and 15 A-G) shows the chromatograms of each samples as analysed by HPLC.

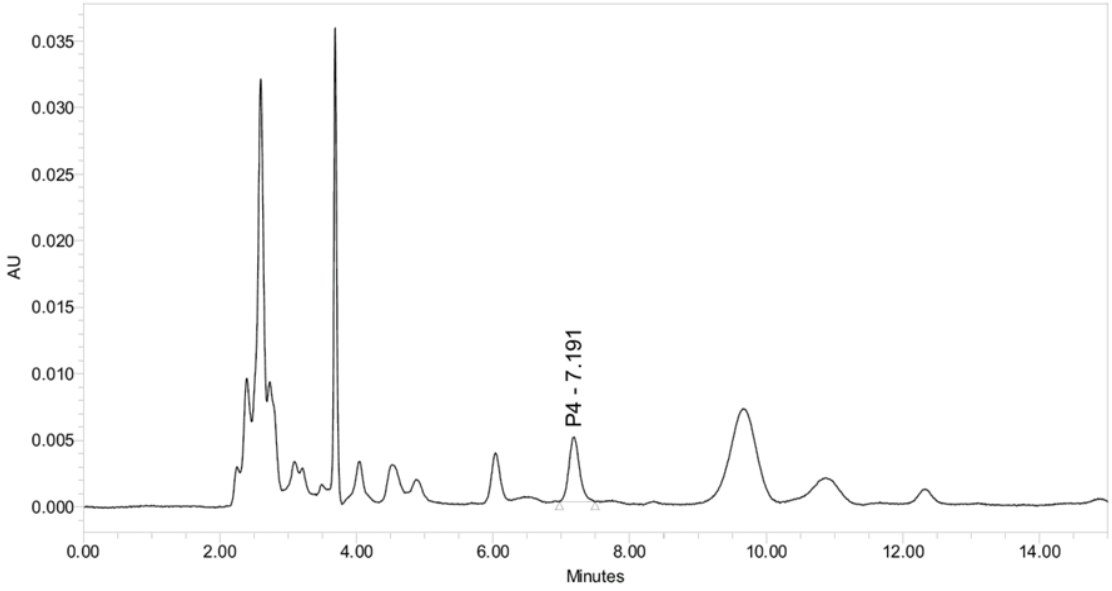
Table 15: Effect of Moringa leaf extract on stevioside and zeatin content of *S. rebaudiana*

Treatment	Stevioside content (%)	Zeatin content (%)
JSS	7.73a ± 0.03*	0.0063a ± 0.0001528*
P1SS	6.93b ± 0.02*	0.0056b ± 0.0001528*
P2SS	6.45c ± 0.02*	0.0051c ± 0.0001000*
OSS	4.14d ± 0.03*	0.0048d ± 0.0001000*
CSS	3.85e ± 0.03*	0.0042e ± 0.0001528*
WSS	2.94f ± 0.03*	0.00058f ± 0.0000306*

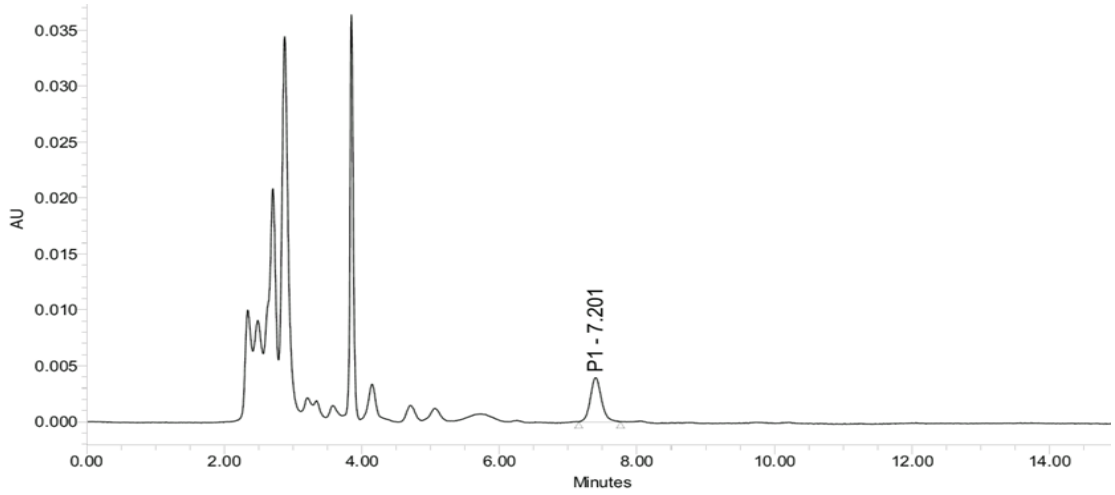
The leaf extracts of *Stevia rebaudiana* sprayed by different varieties of *M.oleifera* are were subjected to HPLC for the quantification of stevioside and Zeatin content are shown in figure 14 and 15.



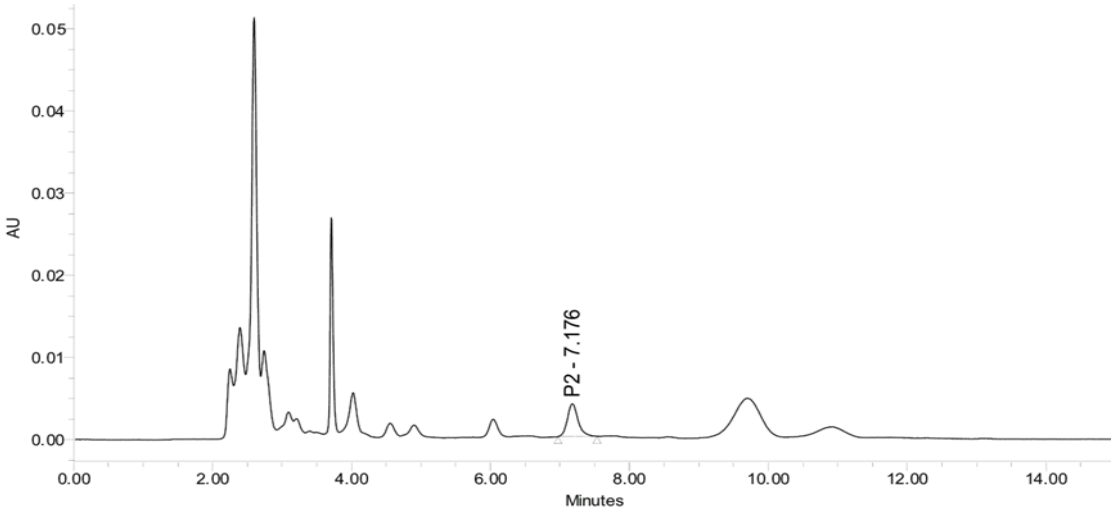
[A]



[B]


AU
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
P1 - 7.201
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
Minutes

[C]


AU
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0.04
0.03
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0.01
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Minutes

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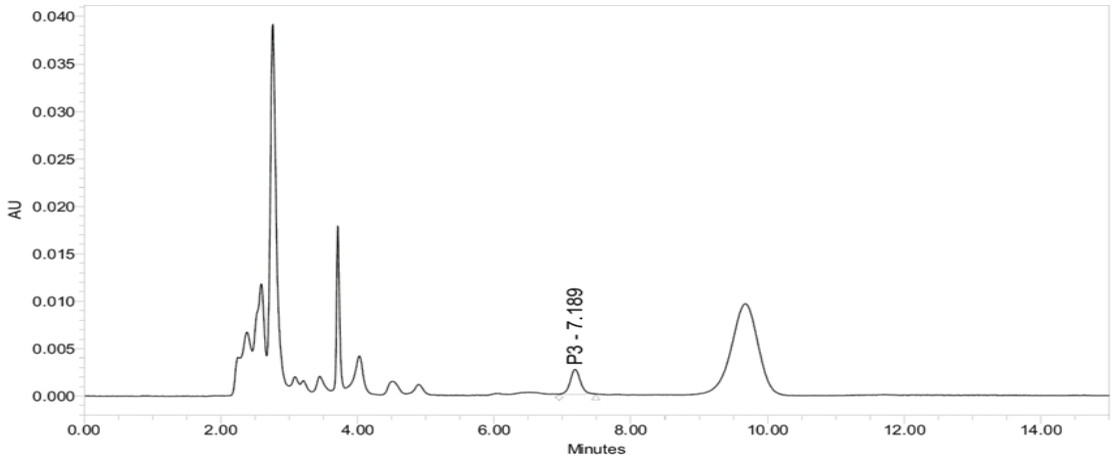
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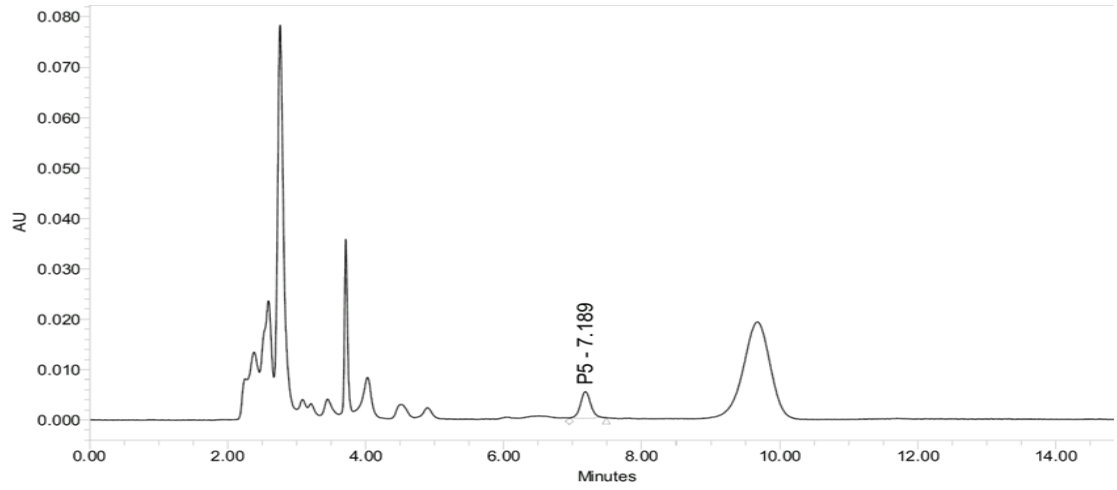
[E]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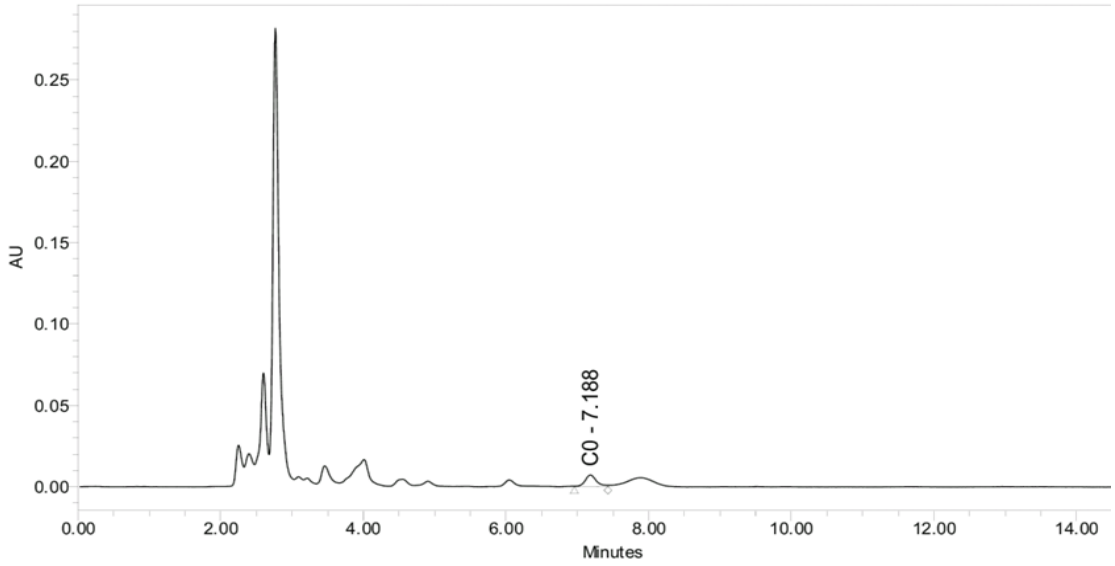
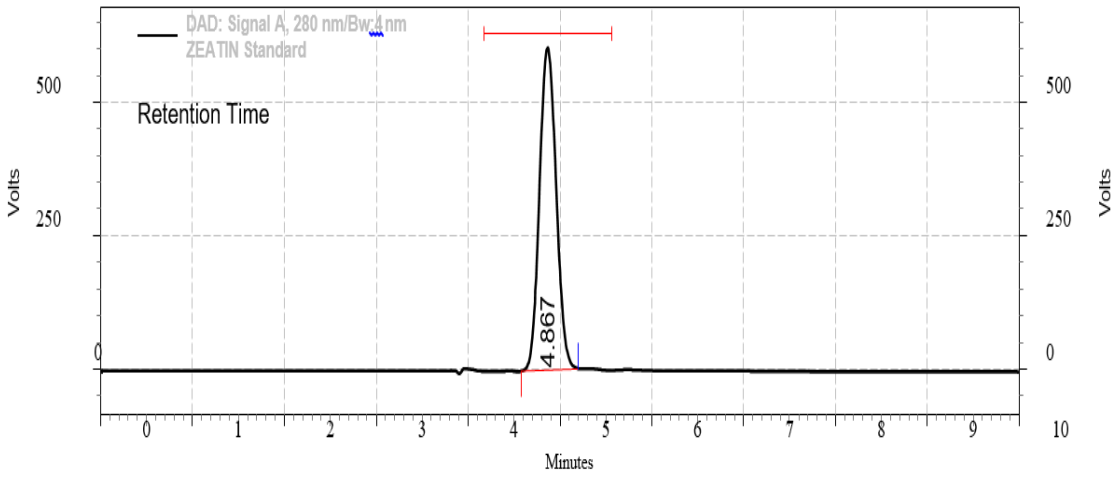
[F]



[G]

Figure 14: Chromatograms for stevioside content determination **[A]** stevioside standard; **[B]** Jaffna sprayed *Stevia*; **[C]** PKM-1 sprayed *Stevia*; **[D]** PKM-2 sprayed *Stevia*; **[E]** ODC sprayed *Stevia*; **[F]** Conventional sprayed *Stevia*; **[G]** Control (untreated *Stevia*)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ZEATIN Standard
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Volts
Minutes

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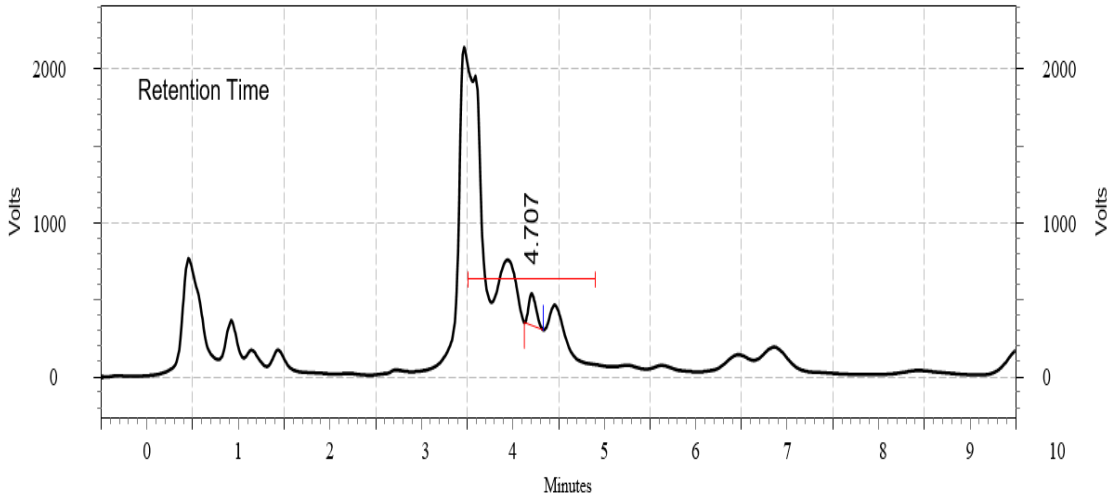
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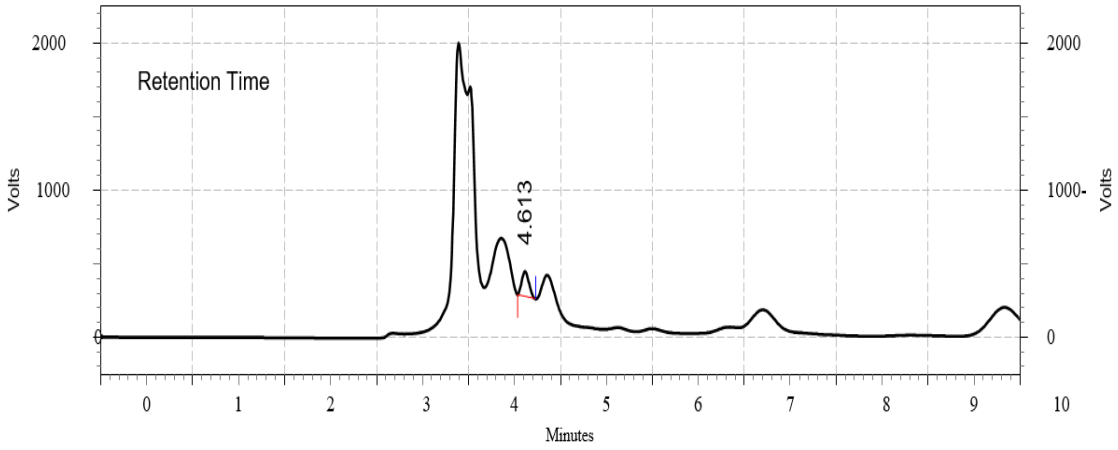
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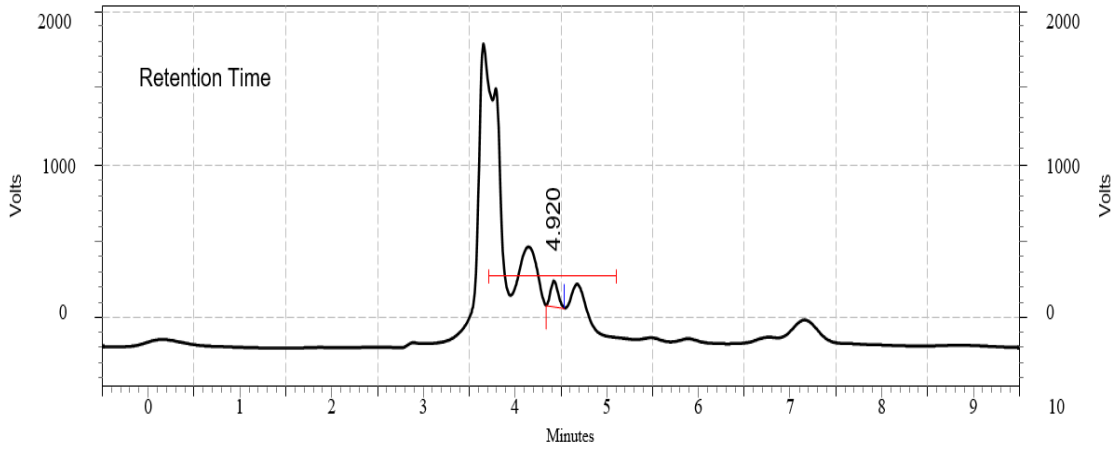
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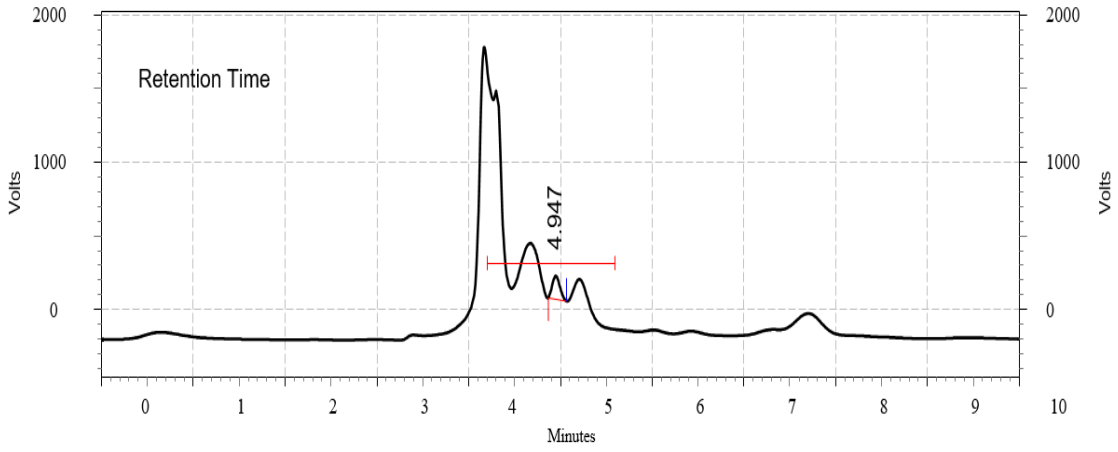
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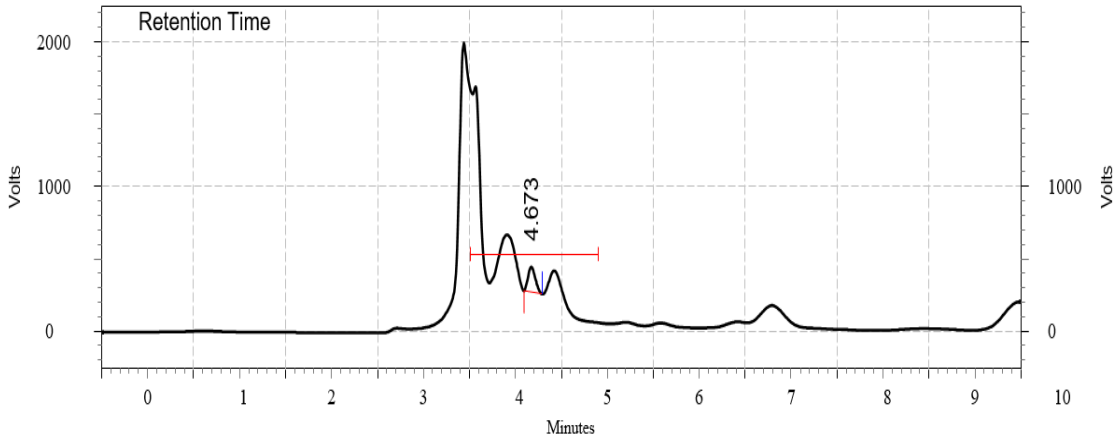
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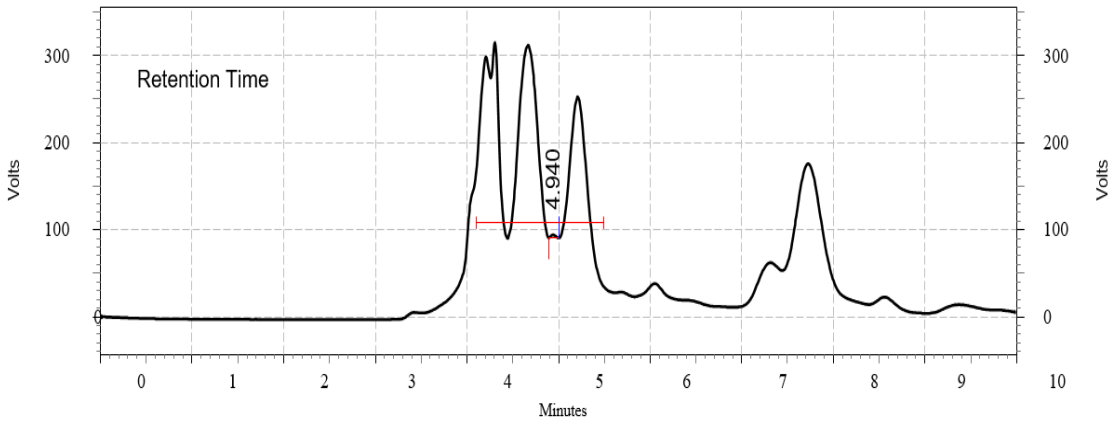

[G]

Figure 15: Chromatograms for zeatin content determination **[A]** Zeatin standard; **[B]** Jaffna sprayed *Stevia*; **[C]** PKM-1 sprayed *Stevia*; **[D]** PKM-2 sprayed *Stevia*; **[E]** ODC sprayed *Stevia*; **[F]** Conventional sprayed *Stevia*; **[G]** Control (untreated *Stevia*)

6.9.6. Mineral content of treated *Stevia rebaudiana*

Minerals like calcium (Ca), potassium (K), sodium (Na), and lithium (Li) are very important nutrients in plant and are essential in many processes needed to sustain plant growth and development. These minerals help plants to tolerate in extreme conditions, production of carbohydrates, control root growth and also increase crop resistance to severe diseases. In humans, they also play a crucial role in maintaining good health. The mineral contents in the treated *S. rebaudiana* were measured by using flame photometer (Zia et al., 2007). Jaffna sprayed *Stevia* exhibited the highest mineral content (potassium: 0.44%, sodium: 0.95%: lithium: 0.62% and calcium: 0.89%), followed by PKM-1, PKM-2, ODC and Conventional (Fig.16 and Table 17).

The extracts of some plants, trees, and crop residues have been reported to influence the growth and yield of other plants (Ahmed and Nimer, 2002; Farooq et al., 2008; Mvumi et al., 2018). The MLE is one of the examples of the aforesaid statement. It has been found that the foliar application of MLE has accelerated the growth of peanut, tomato, wheat,

and corn at early vegetative growth stage, improved resistance to diseases and has generally increased the yield by 20 to 35% (Fuglie, 2000; Imran et al., 2018). In the present study, the exogenously applied MLEs also effectively improved the stevioside and zeatin content in *Stevia* plant as compared to untreated control. This is because of the fact that the control plants did not receive any exogenous supply of the plant growth enhancer. To validate these findings, the leaf extracts of all five varieties of *M. oleifera* were subjected to HPLC for the quantification of zeatin content.

It has been found that the Jaffna variety showed the highest zeatin content and this is the reason why the treated *Stevia* plants exhibited the highest zeatin content. This alteration in the endogenous zeatin level in *Stevia* can be linked with the increment in isopentyl amino purine (precursor of zeatin) content due to *Moringa* foliar spray, which enhanced the zeatin content.

Additionally, there was a decline in Abscisic acid (ABA) content in *Stevia* after the treatment. A reduction in ABA content is followed by the accumulation of the common precursor isopentyl pyrophosphate, which leads to the biosynthesis of zeatin. In a similar report, foliar spray of salicylic acid in sunflower and *Phaseolus vulgaris* increased the level of other phytohormones and decreased the ABA content (Dawood et al., 2012; Elzaawely et al., 2018). MLE act as biostimulant because they are rich in phytohormones such as zeatin, gibberellic acid, and IAA (Fuglie, 1999). Stevioside and Gibberellic acid (GA) share a common metabolic pathway. Due to the application of GA in *Stevia* through *Moringa* foliar spray, the amount of GA increases within the cell and it retards the process of gibberellin biosynthesis and the pathway is diverted to stevioside accumulation which results in increasing the production of stevioside in *Stevia* upon treatment (Brandle and Telmer, 2007).

Similar findings were reported by Modi (2011), in which GA foliar spray led to an increment in stevioside content in *S. rebaudiana*. This point is also strengthened by the research carried out by Karimi (2014), in which anti-gibberelins such as CCC

(Chlorocholine chloride) and PBZ (Paclobutrazol) were used for increment in steviol glycoside production and antioxidant capacity of *Stevia*. The present research also focuses on the effect of MLEs on plant growth promoting characteristics of *Stevia rebaudiana.*

The growth promoting effect of *Moringa* foliar spray is due to the presence of nutrients like calcium, potassium, iron, sodium, lithium, amino acids and multivitamins. It enhances the stevioside, zeatin, and mineral content in *Stevia.* Similar results were observed when a foliar spray of free amino acids, N, P and K, was used for increment of stevioside and mineral content in *Stevia* (Acuna et al., 1997).

In the like manner, the results of the certain reports brings into the limelight that the foliar spray of *Moringa* leaf and seed extracts accelerate the growth of several plants such as sunflower (Iqbal, 2014), maize (Maswada et al., 2018), tomato (Culver and Fanuel, 2012; Mvumi et al., 2018) and legumes (Abohassan et al., 2018) etc. by alleviating plant macro and micro-nutrient deficiencies and also enriching the soil. Thus, it is a promising alternative to expensive inorganic fertilizers which benefit the farmers. In the light of all these reports, it is found that MLEs which have a number of plant growth promoters, minerals, nutrients, and vitamins in a naturally balanced composition, can be beneficial for the growth and development of other plants.

The phytohormone zeatin (cytokinin) promotes cell division and enhances growth of lateral buds (Hare and Staden, 1997; Schäfer et al., 2015). The extracts of some plants, trees, and crop residues have been reported to influence the growth, yield and physiochemical properties of other plants (Ahmed and Nimer, 2002; Farooq et al., 2008; Mvumi et al., 2018). The MLE is one of the examples of the aforesaid statement. It has been found that the foliar application of MLE has accelerated the growth of peanut, tomato, wheat, and corn at early vegetative growth stage, improved resistance to diseases and has generally increased the yield by 20 to 35% (Fuglie, 2000; Imran et al., 2018).

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because of the fact that the control plants did not receive any exogenous supply of the plant growth enhancer. To validate these findings, the leaf extracts of all five varieties of *M. oleifera* were subjected to HPLC for the quantification of zeatin content. It has been found that the Jaffna variety showed the highest zeatin content and this is the reason why the treated *Stevia* plants exhibited the highest zeatin content. This alteration in the endogenous zeatin level in *Stevia* can be linked with the increment in isopentyl amino purine (precursor of zeatin) content due to *Moringa* foliar spray, which enhanced the zeatin content.

Additionally, there was a decline in Abscisic acid (ABA) content in *Stevia* after the treatment. A reduction in ABA content is followed by the accumulation of the common precursor isopentyl pyrophosphate, which leads to the biosynthesis of zeatin. In a similar report, foliar spray of salicylic acid in sunflower and *Phaseolus vulgaris* increased the level of other phytohormones and decreased the ABA content (Dawood et al., 2012; Elzaawely et al., 2018). MLE act as biostimulant because they are rich in phytohormones such as zeatin, gibberellic acid, IAA (Fuglie, 1999). Stevioside and Gibberellic acid (GA) share a common metabolic pathway.

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The present research also focuses on the effect of MLEs on plant growth promoting characteristics of *Stevia rebaudiana.* The growth promoting effect of *Moringa* foliar spray is due to the presence of nutrients like calcium, potassium, iron, sodium, lithium,

amino acids and multivitamins. It enhances the stevioside, zeatin, and mineral content in *Stevia.* Similar results were observed when a foliar spray of free amino acids, N, P and K, was used for increment of stevioside and mineral content in *Stevia* (Acuna et al., 1997).

In the like manner, the results of the certain reports brings into the limelight that the foliar spray of *Moringa* leaf and seed extracts accelerate the growth of several plants such as sunflower (Iqbal, 2014), maize (Maswada et al., 2018), tomato (Culver and Fanuel, 2012; Mvumi et al., 2018) and legumes (Abohassan et al., 2018) etc. by alleviating plant macro and micro-nutrient deficiencies and also enriching the soil.

Thus, it is a promising alternative to expensive inorganic fertilizers which benefit the farmers. In the light of all these reports, it is found that MLEs which have a number of plant growth promoters, minerals, nutrients, and vitamins in a naturally balanced composition can be beneficial for the growth and development of other plants.

The mineral analysis of five different varieties of *M. oleifera* confirming the presence of 4 elements calcium (Ca), potassium (K), sodium (Na), and lithium (Li) are shown in the figure 16.

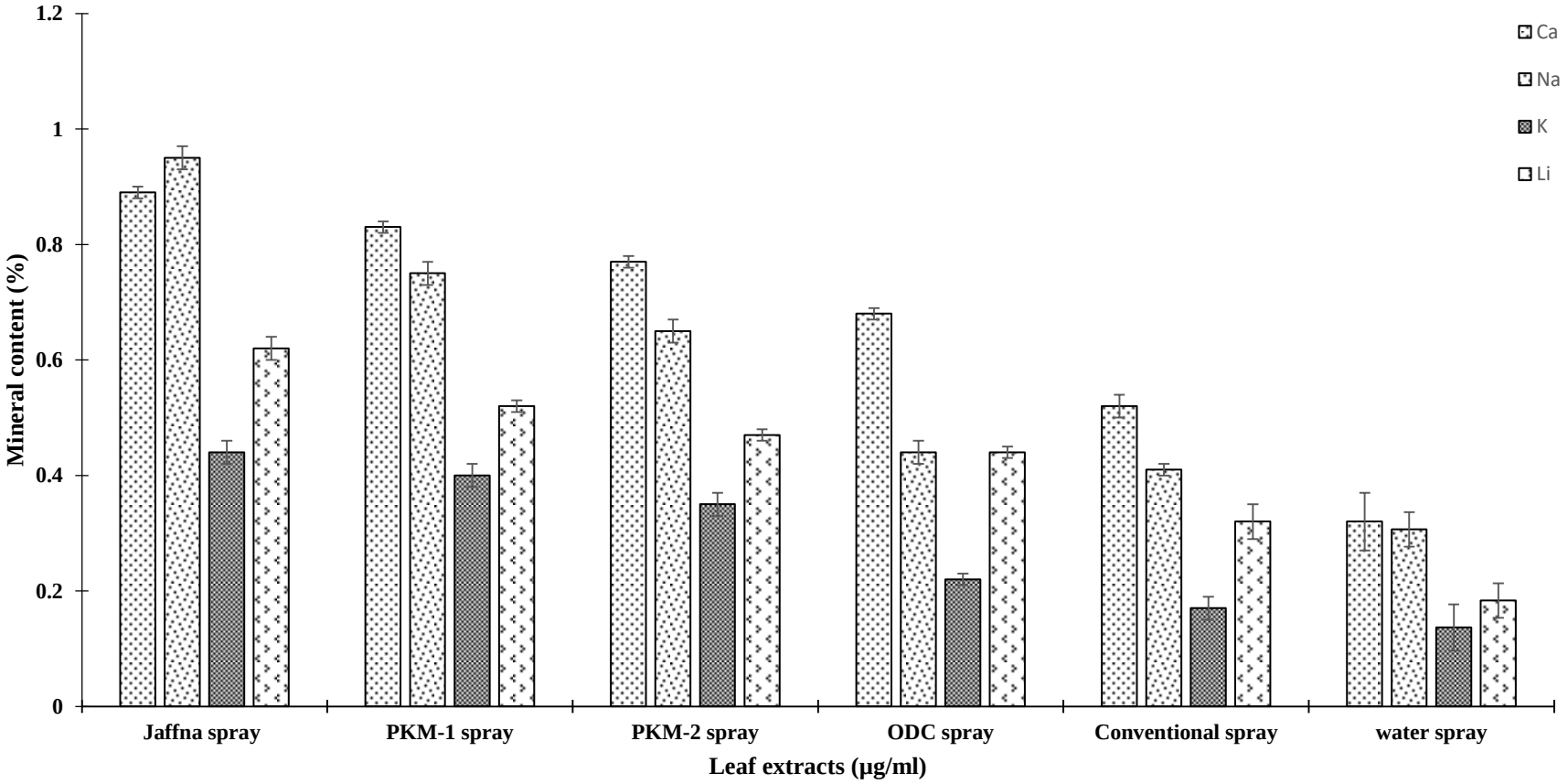

Figure 16: Mineral content of treated *Stevia rebaudiana*

Table 16: Mineral composition of *Moringa oleifera* treated *S. rebaudiana*

Varieties*	K^+	Na^+	Li	Ca^{2+}
JSS	0.44±0.02	0.95±0.02	0.62±0.02	0.89±0.01
P1SS	0.40±0.02	0.75±0.02	0.52±0.01	0.83±0.01
P2SS	0.35±0.02	0.65±0.02	0.47±0.01	0.77±0.01
OSS	0.22±0.01	0.44±0.02	0.44±0.01	0.68±0.01
CSS	0.17±0.02	0.41±0.01	0.32±0.1	052±0.02
WSS	0.13±0.04	0.30±0.03	0.18±0.03	0.32±0.05

6.10. MTT assay

Leaf extracts of all five varieties were evaluated for their cytotoxic activity against HepG2 cancer cell line, through MTT assay. 5-FU (5-Fluorouracil) was used as positive control (0.5, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 Mm). IC_{50} values of 5-FU was 0.87 Mm/ml. Table 17 reveals the percentage (%) cytotoxicity of different concentrations of the leaf extracts (0.25, 0.5, 1, 2, 3, 4, and 5 mg/ml) of five varieties. The leaf extracts of all varieties exhibited dose-dependent cytotoxic activity against the cancer cell line. The trend observed for cytotoxicity on the basis of IC_{50} values was: Conventional (1.22 mg/ml) > ODC (0.90 mg/ml) > PKM-2 (0.65 mg/ml) > PKM-1 (0.35 mg/ml) > Jaffna (0.15 mg/ml). Jaffna leaf extract showed a strong cytotoxic activity as compared to the other four varieties. Thus, it can be used as novel 'alternative and complementary' therapeutic agent against in cancer treatment regime.

Cancer cells quickly divide and thus anticancer agents are essential to stop their growth by targeting them. Interestingly, *M. oleifera* leaf extract performs a wide collection of biological activities, and even target numerous proteins and biomolecules to retard cancer progression (Tiloke et al., 2013; Vasanth et al., 2014). It contains glucosinolates that have the ability to induce the apoptosis and are very effective against the cancer (Jung et al., 2015). Berkovich et al., (2013) reported that *M. oleifera* leaf extract inhibits the pancreatic cancer cell growth; Thorneloe et al., (2019), breast cancer cell growth and Sadek et al., (2017), liver cancer (tempted by the diethyl nitrosamine in rats).

M. oleifera also inhibits the cell viability of myeloid leukaemia, liver carcinoma, and lymphoblastic (Cai et al., 201). These activities of Moringa can be attributed to the presence of different classes of anticancer compounds such as kaempferol, β-sitosterol-3-O-β-D-glucopyranoside, niazimicin, and 4-(α-L-rhamnosyloxy) benzyl isothiocyanate (Jung et al., 2015). These targets the cell cycle by accumulating the cells at sub-G1 phase. According to the Tiloke et al., (2013), hot water leaf extract of *M. oleifera* exhibits antiproliferative activity against A549 lung cancer cells. The reason behind the fact is that it increases the reactive oxygen species (ROS), which leads to the cleavage of PARP-1, induction of caspases, and P53 initiates apoptosis of cancer cell line. Moringa leaf extract up-regulates the apoptotic markers which leads to the death of cancer cells and down-regulates the NFKB pathway which further decreases the expression of P-IKB α, IKB α, and P65 proteins. These together induce cytotoxicity in cancer

cells (Ferlay et al., 2015). The current study reports the cytotoxic potential of five different varieties of *M. oleifera* on HepG2 cell line. Jaffna variety showed highest cytotoxic effect on cancer cell line as compared to the other varieties. The reason for the strong cytotoxic effect of the Jaffna extract may be due to the presence of high amounts of the aforementioned secondary metabolites.

Different varieties of *M. oleifera* leaf extracts were evaluated for their cytotoxic activity against HepG2 cancer cell line, through MTT assay. The trend observed for cytotoxicity on the basis of IC_{50} values are listed in table 17.

Table 17: MTT assay using leaf extracts of five varieties of *Moringa oleifera*

Varieties	**Cytotoxicity at different concentrations (mg/ml)**							**IC50 (mg/ml)**
	0.25	**0.5**	**1**	**2**	**3**	**4**	**5**	
Jaffna	48.37	56.97	63.86	67.01	68.64	72.27	74.28	0.15
PKM-1	43.97	45.94	48.66	50.38	56.02	67.20	71.22	0.35
PKM-2	36.13	40.63	46.94	48.66	53.25	63.09	68.64	0.65
ODC	27.53	35.37	41.49	46.36	52.10	60.80	63.19	0.90
Conventional	22.37	32.31	35.37	40.63	51.62	54.58	57.83	1.22

Table 18: Summary of the trends observed for the tested parameters

S.No.	**Parameter**	**Trend**
1	Seed germination	ODC > PKM-1 > PKM-2 > Jaffna > Conventional
2	Plant height	PKM-2 > PKM-1 > ODC > Jaffna > Conventional

3	Plant spread	PKM-1 > PKM-2 > ODC > Jaffna > Conventional
4	No. of primary branches	PKM-1 > PKM-2 > Jaffna > ODC > Conventional
5	No. of leaves	PKM-1 > PKM-2 > ODC > Jaffna > Conventional
6	DPPH	Jaffna > PKM-1 > PKM-2 > ODC > Conventional
7	ABTS	Jaffna > PKM-1 > PKM-2 > ODC > Conventional
8	FRAP	Jaffna > PKM-1 > PKM-2 > ODC > Conventional
9	TPC	Jaffna > PKM-1 > PKM-2 > ODC > Conventional
10	TFC	Jaffna > PKM-1 > PKM-2 > ODC > Conventional
11	Total sugar content	Jaffna > PKM-1 > PKM-2 > ODC > Conventional
12	Protein content	Jaffna > PKM-1 > PKM-2 > ODC > Conventional
13	Chlorophyll content	Jaffna > PKM-1 > PKM-2 > ODC > Conventional
14	Mineral content	Jaffna > PKM-1 > PKM-2 > ODC > Conventional
15	HPLC of bioactive compound	Jaffna > PKM-1 > PKM-2 > ODC > Conventional
16	Antibacterial activity	Jaffna > PKM-1 > PKM-2 > ODC > Conventional
17	MTT assay	Conventional > ODC > PKM-2 > PKM-1 > Jaffna
18	Enhancement in growth attributes of *S. rebaudiana* by *M. oleifera* foliar spray	Jaffna > PKM-1 > PKM-2 > ODC > Conventional
19	Effect of Moringa leaf extract on stevioside and zeatin content of *S. rebaudiana*	Jaffna > PKM-1 > PKM-2 > ODC > Conventional
20	Mineral content of treated *Stevia rebaudiana*	Jaffna > PKM-1 > PKM-2 > ODC > Conventional

Chapter 6

Conclusions

This is the first report wherein a comparison of antibacterial, antioxidant, anticancer and growth promoting activities of five different varieties of *M. oleifera* namely as; ODC, Conventional, Jaffna, PKM-1, and PKM-2) has been achieved, to suggest the best variety among the five varieties. Ethanolic extracts of *M. oleifera* varieties showed higher antioxidant and antibacterial activity as compared to the aqueous extracts, the reason being the presence of the secondary metabolites (phenols and flavonoids) of plant extract are more soluble in ethanol than in aqueous. The high flavonoid and phenolic contents positively correlates to its antioxidant, antibacterial, and anticancer activity. Countless industries are concerned with the fabrication of synthetic antioxidants so as to cure the health issues and these varieties of *Moringa* can be the alternative to substitute the toxic synthetic chemicals. The strong antibacterial potential of Jaffna variety against the different bacterial strains such as *P. aeruginosa* (MTCC 2453), *B. subtilis* (MTCC 168), *E. coli* (MTCC 443) and *S. aureus* (MTCC 3160) suggests a promising natural alternative to the noxious artificial drugs. Jaffna leaf and seed extracts can be certified as a prophylactic agent against dreadful and infectious bacterial diseases. Current study also revealed that the *M. oleifera* leaf extract of different varieties improved the growth of *S. rebaudiana* as compared to the control. This cause behind this is that the *Moringa* is rich source of macro and micro nutrients, phytohormones such as gibberellins, zeatin, and auxin. The highest performance in all growth characteristic features was observed by using the Jaffna foliar spray. *Moringa* leaf extracts can be utilized as a biofertilizer or biostimulant for defensible cultivation of *S. rebaudiana* and even for other profitable crops as well in order to search their functional-food and nutraceutical potential, for value addition. *Moringa* leaves extract can be a potent substitute to chemical fertilizer or inorganic so as to handle with the global issues of soil pollution and also to raise a premium in the agriculture market.

The purpose of our research was to recommend a natural source of plant growth enhancer which is affordable and ecological friendly. We were blessed enough to get a single variety namely as Jaffna with combination of numerous traits. Jaffna variety can be explored further for different

trails to be involved in reforestation programmes and health care. Thus, the current study recommends Jaffna variety to be used commercially as an environment friendly and economically viable plant growth enhancer. So, we suggest that everybody should have a *Moringa* tree in their backyard.

The present study reveals that the Moringa leaf extracts of different varieties of *M.oleifera* such as ODC, Conventional, Jaffna, PKM-1, and PKM-2 have improved the growth parameters [zeatin and stevioside, mineral contents calcium (Ca), sodium (Na), potassium (K), and lithium (Li)] of *Stevia*. Jaffna foliar spray displayed the best results as compared to water foliar spray on *Stevia*. Due to the existence of larger amount of macro nutrients and micro nutrients, phytohormones (zeatin, gibberellins, and IAA) in Jaffna variety, it has improved the productivity and quality of *S. rebaudiana*. Thus, the application of Jaffna foliar spray could be utilized to enrich the crop productivity as well as the productivity of bioactive compounds and essential nutrients in *S. rebaudiana*. Additionally, MLE can be used as biofertilizer or biostimulant for sustainable cultivation of *S. rebaudiana* and other important crops as well in order to explore nutraceutical potential to avail the maximum benefits from the plants. It can also be a powerful substitute of inorganic or chemical fertilizer which helps in diminishing the major global problem of environmental pollution. Moreover, it is commercially economical and environmentally friendly. Thus, the present study suggests that *M. oleifera*'s superior variety named Jaffna can be used by the farmers as a cost-effective fertilizer.

In conclusion we can say that *M. oleifera* is an angel tree, due to its extraordinary therapeutic and nutritional values. It is considered as an outstanding and inexpensive alternative to good health due to its immense medicinal properties. The present study showed the comparison of five different varieties of *M. oleifera* namely ODC, Conventional, Jaffna, PKM-1, and PKM-2 on the bases of different parameters [(nutritional values- chlorophyll, sugar and protein content), quantification of anti-cancer compounds (β-sitosterol, quercetin, khaempherol, and moringin), and FRAP activity]. By assessing these parameters, it became possible to conclude that Jaffna

variety has proved to be the most beneficial variety in terms of nutrition, anticancer bioactive compounds, and in anticancer activity. In Jaffna variety, the experimental values of all the parameters appeared to be significantly higher than other four varieties. Thus, Jaffna has proved to be the best variety for cultivation in Punjab. However, this variety needs to be explored more so that the people may fully capitalize on its amazing benefits.

Chapter 7

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