Comparison of antioxidant and antibacterial activities of *Moringa oleifera* varieties

Dissertation report

Submitted

By

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In partial fulfilment of M.Sc. in Botany

Under the guidance of

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Declaration

Myself, KM Shalvi Pandey, student of M.Sc. Botany, School of Bioengineering and Biosciences, Lovely Professional University, Punjab, hereby declare that all the information furnished in this dissertation report entitled "**Comparison of antioxidant and antibacterial activities of *Moringa* varieties**" is based on my own intensive research and is genuine.

This report does not contain any work for the award of any degree or diploma either of this university or any other university.

Date: **KM Shalvi Pandey**

Registration no. 11506943

Certification

This is to certify that KM Shalvi Pandey is undergoing her project titled "**Comparison of antioxidant and antibacterial activities of *Moringa oleifera* varieties**" under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation. No part of the project has ever been submitted for any other degree in any university.

The project is a necessary requirement for the award of the degree of M.Sc. Botany (Hons.).

Date:

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Date: **(KM Shalvi Pandey)**

Place: Phagwara

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Abstract

Moringa oleifera L. is a multipurpose plant and it is grown in tropic and subtropical regions of the world. It is an excellent source of phytonutrients and is rich in vitamin A, B, C, protein, calcium, potassium etc. The plant root, stem, bark, leaf, flower and seeds possess anti-oxidant, anti-bacterial, anti-inflammatory, anti-pyretic, anti-tumour, anti-cancer, anti-diabetic, anti-fungal, anti-ulcer and anti-hypertensive properties. Anti-oxidants play a vital role to provide protection to humans and animals against infections and degenerative diseases. In the present study, methanolic leaf extracts of *Moringa oleifera* varieties such as 'PKM-1', 'Jaffna' and 'Conventional' has been used to analyse the free radical scavenging effect on DPPH (2, 2-diphenyl-2-picryl hydrazyl) and ABTS (2, 2-azinobis,3-ethyl-benzothiazolin-6-sulfonic acid) assay**.** Methanolic leaf extract of PKM-1 variety showed higher free radicals scavenging activity as compare to other varieties. The trend observed with total phenolic content was found to be, PKM1 > Jaffna > Conventional and total flavonoid content was also found in same manner PKM1 > Jaffna > Conventional. The anti-bacterial activity (disc diffusion method) of the aqueous leaf extract of the three varieties on *Escherichia coli, Pseudomonas aregenosa, Bacillus subtilis* and *Staphylococcus aureus* showed a trend of Jaffna > PKM1 > Conventional while, with methanolic extracts the trend was Jaffna > PKM1 > Conventional, and the trend observed with ethanolic extract was Jaffna > PKM1 > Conventional. Meticulous phytochemical and pharmacological studies on *Moringa* species could yield reliable bioactive compounds of pharmacological importance, for better healthcare.

Keywords: *Moringa oleifera*, anti-oxidant, anti-bacterial, free radicals, scavenging activity.

Abbreviations

ABTS	:	2, 2-azinobis-3-ethyl-benzothiazolin-6-sulfonic acid
$AlCl_3$	:	Aluminium chloride
BHT	:	Butylated hydroxytoluene
$C_2H_3KO_2$	:	Potassium acetate
DPPH	:	2, 2-Diphenyl-2-picryl hydrazyl
EtOH	:	Ethanol
FCR	:	Folin-ciocalteau reagent
G	:	Grams
GAE	:	Gallic acid equivalence
H	:	H
$K_2S_2O_8$	:	Potassium per sulphate
M. oleifera	:	*Moringa oleifera*
Na_2CO_3	:	Sodium carbonate
Psi	:	Pound per square inch

Introduction

In recent time Medicinal plants play an important role for maintaining the human health and provide the proper treatment (Patwardhan 2014). By using medicinal plants many industries produce different types of herbal products which contain different types of bioactive compounds used mainly for the medicinal purposes. Demand of herbal products are increasing day by day as there is no side effect (Cragg et al. 1997; Negi 2010; Gosh 2003; Fransworth et al. 1995).

It has been reported that more than 60% of synthetic drugs are also derived from the plant sources and so also leads to the exploitation of biodiversity (Cragg and Newman 2013, Patwardhan 2014). Therefore, adequate production and conservation of the medical plant germplasm is a prerequisite so as to heal the community from various illnesses (Bannerman et al. 1983; Anon 1994). At the same time proper standardization of herbal drugs is necessary in order to increase their efficacy towards a specific illness like diabetes, cancer, hepatoprotective and urinary system illnesses. Various urological disorders have been reported, among which 'urolithiasis' is third most commonly reported illness. It is a disorder which is related to both genetic as well as environmental factors (Adriano et al. 2000; Devuyst and Pirson 2007; Vitale et al. 2008; Asha Jyothi et al. 2016).

Oxidation is a type of chemical reaction and it leads to the chain reaction. Oxygen atom has higher capacity to react with other biomolecules and produce the unpaired electrons called as free radicals which may cause the cell damages (loss of cell structure and their function) and different types of diseases (Percival 1998; Gopalakrishnan et al. 2016).

Plants produces abundant amount of anti-oxidants that inhibit the production of free radicals. Free radicals are also produced in human body in the form of O_2^-, hydroxide ion (OH^-) and hydrogen peroxide (H_2O_2). Free radicals are harmful to the different kinds of biomolecules such as DNA, protein, lipid etc. Due to the cell damages many types of disease occur in human body including: Cancer, arthritis, diabetes, cardiovascular disease, brain dysfunction etc. Anti-oxidants are very effective against these diseases (Sreelatha et al. 2009).

Generally three types of major anti-oxidants are found such as phytochemicals (polyphenols, flavonoids, carotenoids etc.), enzymes (superoxide dismutase (SOD), catalase, glutathione peroxidase etc.) and vitamins (A, C, E and β-carotene). The anti-oxidant provide a protection against free radicals and a large number of anti-oxidant properties are found in the plants (Razis et al. 2014).

There has been an increase in the number of life threatening infections caused by the pathogenic micro-organisms which is becoming an important cause of mortality in the developing countries (Al-Bari et al. 2006). The most common cause of food poisoning is the bacteria (De Doughari 2007). The primary sources of natural products for maintaining the health of animals and human beings are the plants which are considered to have large varieties of chemical substances that act in the process of prevention and curative therapies. There has been a large amount of work done on the antimicrobial potentiality of different medicinal plants (Arora et al. 2009); but only a few are conducted in a systematic way. *Moringa oleifera* is a medicinal plant which belongs to family Moringaceae (order Brassicales), which is considered to be a complete food as it has not only medicinal use but also has a high nutritional value as well. It is also used in the traditional medicinal system because of its therapeutic remedy for various diseases (Anwar 2007). The biological properties are found in the extract of the leaves which vary with the type of solvent used for extracting the active components. Antimicrobial properties are also reported in the plants which explains the reason for its wide use in the treatment of a large number of diseases.

Moringa oleifera L. commonly known as "Drumstick," is an extremely valuable medicinal plant, distributed in the tropical and subtropical regions of the world (Ramchandran et al. 1980). Apart from having high nutritional value it possess several medicinal properties. Various parts of this tree contain protein, vitamins, carotenoids, glucosinolates, flavonoids, oxalates, amino acids, anti-oxidants, tannins, minerals and phenolic compounds (Rotruck et al. 1973; Faize et al. 1994; Fahey et al. 2001; Rodriguez 2003; Adedapo et al. 2009). The flowers, immature pods, seeds, leaves, roots and bark exhibits several biological activities such as anti-bacterial, anti-fungal, anti-pyretic, anti-inflammatory, anti-oxidant, anti-spasmodic, anti-hypertensive, anti-tumor, anti-ulcer, anti-epileptic, diuretic, anti-diabetic, cholesterol lowering and cardiac activities (Pong 2003; Fahey 2005; Anwar et al. 2006) and it is used for the treatment of many kinds of

diseases (Uda et al. 1997; Perry et al. 1999; Kumar et al. 2010). *Moringa* plays a beneficial role in several industrial and economical point of view and it is also used in re-forestation package, as a bio-sorbent for heavy metals and as a fodder for livestock, water treatment, lubricants, skin lotions and as a biodiesel (Kakkar et al. 1972; Chandler et al. 1993; Kohen et al. 2000; Blois 2002; Sabate 2003; Sathya et al. 2010; Koul et al. 2015).

Moringa is a perennial, short to medium-sized, draught resistant, straight stem tree which attains a height of 1.5 – 2 m and bears tripinnate compound leaves with green to dark green elliptical leaflets. Its branches grow in a disorganized manner to form umbrella shaped canopy. It grows well in dry to moist tropical and subtropical climates with yearly rainfall of 760-2500 mm and temperature from 18-28 °C (Morton 1991; Chang et al. 2003). It grows in almost all soil types but better growth has been observed in heavy clay with a pH range of 4.5-8. 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, pointed at both ends, 9 ribbed with nearly 20 seeds embedded in the pith. Seeds are almost round with brown semipermeable seed hull with 3 papery wings (Anwar et al. 2003; Prabhu et al. 2011).

Along with the Conventional variety of *Moringa* various other varieties have also been developed in India in order to improve the growth characteristics and yield. The variety 'has been developed at Horticultural Research Station, Tamilnadu Agricultural University (TNAU), Periyakulam, and Tamilnadu which possess improved pod production. Another variety, 'Jaffna' bears soft and tasty fruits (pods). These varieties are extensively grown in southern parts of India and the flowers and pods are routinely used in making several delicacies. The present study may help to find the best variety of *M. oleifera* on the basis of their anti-oxidant and anti-bacterial activity (Harbon 1973; Habig et al. 1984; Gutteridge 1995). The same variety may be promoted for cultivation in Punjab and this may help these people to enjoy multiple health, medicinal and economic benefits from this 'miracle tree'.

Problems background

- *M. oleifera* possess excellent nutritional properties and so it is a ‘miracle tree’ to combat malnutrition among the young populations of the world who are devoid of daily nutritious diet.
- People living in Punjab specially Malwa region have been largely victimized by diseases like cancer and diabetes. Several studies have confirmed anti-cancerous and anti-diabetic properties of *M. oleifera*.
- In present time people are facing many types of diseases like eye disease, muscular pain, bacterial and fungal infection, fevers, inflammation, piles, hypertension, tumors, ulcers, epilepsy etc. and *M. oleifera* has the potential in treating of these kinds of diseases.
- But, its fruitful plantation has not been done in Punjab and its benefits too are not availed.

Review of Literature

Moringa distribution

Moringa oleifera Lam. belongs to the Moringaceae family (Santos et al. 2005). In the Moringaceae family about 33 species have been reported and among these 13 species are well-known and found worldwide (Koul et al. 2015). *Moringa oleifera* is a reputed member of this family and is a multipurpose softwood-tree. It is native of sub-Himalayan regions of North West India and South America and 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

Hybrid varieties of *Moringa oleifera* are also found and out of these varieties some varieties such as PKM-1 and Jaffna are described.

Jaffna:

Jaffna contain long and slender pods with soft fruit and good taste. Jaffna, firstly introduced from Ceylon (Srilanka). It is a perennial tree and it can grow on drought and waste land. Flowering start in the month of February-March and pods are harvested in June- July.

PKM-1:

PKM-1 is a hybrid variety of *Moringa oleifera*. It is a superior variety of Moringa with high production. It grows different types of soil. Fruit quality and oil production of this variety is good. It is busy in nature, with dark green leaves and long pods. Pods having better test than other hybrid varieties. Flowering starts so fast after plantation.

Conventional variety:

It is a natural variety. Conventional seeds are also called natural *Moringa* seeds and this variety is pollinated openly and suitable for the wild production. It contains huge amount of vitamin C and vitamin A. It is also contain high level of nutritional value and the conventional seeds are used in anti-biotic, anti-inflammatory properties. These seeds are very effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria and these bacteria are responsible for the skin-infections. It is also used for water purification

Oldest records

Moringa plant is famous from the ancient time due to many beneficial properties. In African languages, it is called 'Nebedaye' which means never die. About 2000 BC, it is described as a medicinal plant for the first time in North India. An ancient time, the Egypt people used Moringa's oil for skin protection against rough weather. After sometime Greek people found medicinal properties in Moringa and they introduced Moringa's properties to the Roman people.

Taxonomic position:

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

Botanical description

Moringa belongs to the Moringaceae family and it is commonly known as 'Drumstick'. 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.

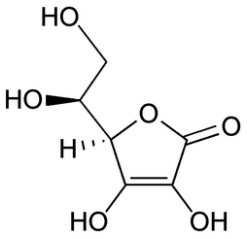
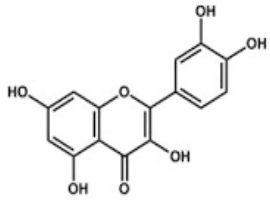
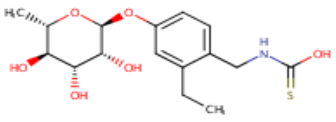
Nutritional properties

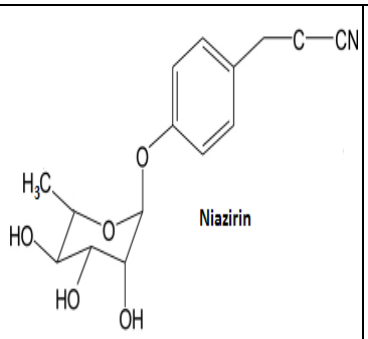
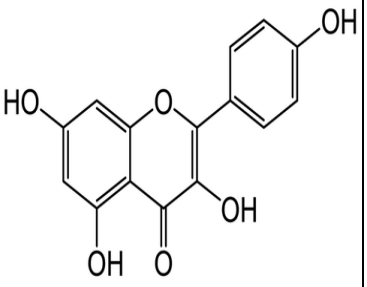
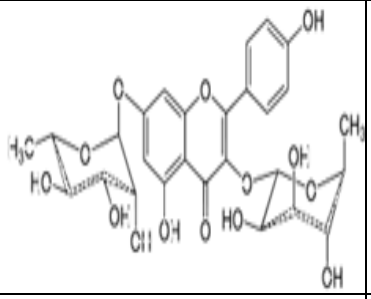
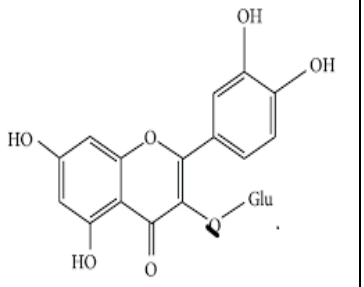
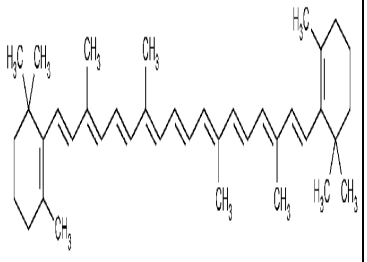
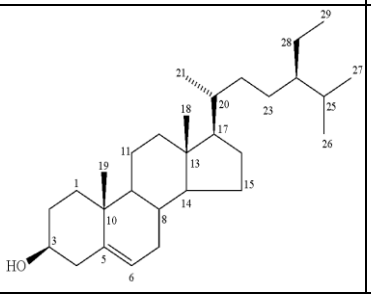
Moringa plant parts are eatable. It is also containing a huge amount of nutrients such as manganese, phosphorus, iron, β-carotene, vitamin B and C etc. It is very beneficial for the human health benefits. Common people use the Moringa leaves as vegetables and they crushed the leaves and prepare the different type of food such as soups, juice, sauces etc. *Moringa* fruit are also used as vegetable and it contain high amount of vitamin C and same as vitamin B also.

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 (Verma et al. 1997; Caceres et al. 1992; Freiberger et al. 1998; Marchioli et al. 1976; Bharali et al. 2003).

Table 1: List of bioactive compounds reported in *Moringa oleifera*:

Bioactive compounds	Properties	Chemical formula	Solubility	Structure	References
Ascorbic acid	Anti-oxidant activity	$C_6H_8O_6$	In water		Anwar et al. 2007
Moringine	Anti-cancer activity, anti-inflammatory, cardiac stimulant	C_7H_9N	In acetone, benzene		Ruckmani et al. 1998, Anwar et al. 2007, Varmani et al. 2014.
Quercetin	Anti-oxidant, hepatoprotective activity		In ethanol, DMSO and dimethyl formamide		Fernandes et al.2015, Anwar et al. 2007.
Niazimicin	Anti-bacterial activity, anti-cancer activity, anti-tumour activity, blood pressure lowering effect	$C_{14}H_{17}NO_5$			Anwar et al. 2007

Niazirinin	Hypotensive activity	$C_{16}H_{19}NO_6$	In water		Anwar et al. 2007
Kaempherol	Anti-oxidant, anti-bacterial, anti-diabetes and anti-viral activity	$C_{15}H_{10}O_6$	In ethanol, ethers, DMSO		Anwar et al, 2007
Kaempferitin	Anti-inflammatory, anti-oxidant, anti-microbial activity	$C_{16}H_{12}O_5$			Anwar et al. 2007
Isoquercetin	Anti-oxidant	$C_{21}H_{20}O12$	In water		Anwar et al 2007
β-carotene	Anti-oxidant, cholesterol lowering				Anwar et al. 2007
β-sitosterol	Anti-cancer activity, reduce blood cholesterol level	$C_{29}H_{50}O$	In alcohol		Anwar et al 2007

Medicinal properties

Each and every part of the *Moringa* possess a medicinal properties. Leaves used as antipyretic, antioxidant, hepatic tonic, diuretic, etc. and it is also used for thyroids disorders, diarrhoea, dysentery (Anwar et al. 2007; Kodia et al. 2014; Caceres et al. 1992; Dahot et al. 1988).

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. (Gupta et al. 2013, Fahey et al. 2009).

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 inbiogas also.

***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.

***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.

Future prospects of *Moringa*

M. oleifera is the most widely cultivated tree and all its parts such as flowers, fruits, seeds, young leaves, and roots are very useful in the medicinal field (Rosenberg 1992; Farber 1994; Morimitsu et al. 2000; Chitra et al. 2002).

M. oleifera flowers have been used for medicinal as well as nutrional value and is consumed as a salad (Farber 1994; Sharma et al. 2011). A large 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 activity and total phenolic activity of tender leaves and mature leaves of *Moringa*. Among the two types of leaves (mature and tender), the mature leaves were found to contain a higher 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. 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.

Koul et al. (2015) and 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.

Siddiq et al. (2005) evaluate the free radical scavenging activity of different solvent extracts of *Moringa* leaves by using enhanced aging of 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.

Mohammed et al. (2015) conducted a study in which they determined the *invitro* anti-oxidant by DPPH assay , cytotoxicity by MTT assay, phytochemical screening of ethanolic extract of leaves of *Moringa oleifera.* By using DPPH they tested anti-oxidant screening for free radical scavenging properties while for the standard anti-oxidant propyl galate was used, beside using it for phytochemical screening and cytotoxicity by MTT

assay at various concentration (125ppm, 250ppm, and 500ppm) and compare it to reference control (tritron-100). The anti-oxidant activity of ethanol extract of leaves of *Moringa* was 75±0.01RSA % as compare to control propyl galate levels which was 88±0.07 RSA %. It was found that alkaloids, glycosides, triterpenes, tannins, flavonoids and saponins were present in the leaves of *Moringa* tree by preliminary phytochemical testing. Also the safety of the tested extract was confirmed with an IC_{50} less 100 μg/ml by comparing cytotoxicity with tritron which was used as control. Alkaloids showed negatively results showed that Moringa can be used in treating disease which are related to free radicals due to its anti-oxidant properties.

Mishra et al. (2011) conducted a study that provides important information about *Moringa 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 niazirin and niazirinin (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 new. By using spectroscopic method like appropriate 2D nmr experiments and chemicals reactions, structural determination was obtained.

Nantachit (2006) 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 observed against *E. coli, S. aureus, P.areginosa,* and *K. Pnemonia* by the methanolic crude extract. Antibacteria was observed by isolated parts from column chromatography and purified dichloromethane extract.

Anwar et al. (2005) analysed *Moringa* seed oil grown in temperate regions of Pakistan. The hexane extracted oil content ranged 38-42%. Protein, fibres, and ash contents were found to be 26.50-32.00, 5.80-9.29, and 5.60-7.505, respectively. Tocopherols (α, γ, and δ) were found to be sufficient amount along with high levels of oleic acid followed by

palmitic, stearic, behenic and arachidic acid up to levels of 7.00, 7.50, 5.99, and 4.21 respectively. Many parameters of *Moringa* oil indigenous to Pakistan were comparable to different edible vegetable oils.

Siddhuraju et al. (2003) analysed the anti-oxidant properties of various solvent extracts of phenolic constituents from different agro climatic origin of *Moringa*. The main bioactive compounds of phenols were found to be flavonoid groups such as Quercetin and kaemopherol. *Moringa* leaves found to be a great source of natural anti-oxidants. Health promoting secondary metabolites (glucosinolates, phenols and flavonoids) were also found in Moringa leaves for human health benefits.

Pari and Kumar (2003) evaluated the hepatoprotective effect of ethanolic extract of *M. oleifera* leaves on liver damage induced by antitubercular drugs such Isoniazid (INH), Rifampicin (RMP), and Pyrazinamide (PZA) in rats. Leaves extract of *Moringa* showed a significant protective action to the liver sections. The results of this study showed that the treatment with *M. oleifera* leaves extract gives the protective effect from hepatic damage.

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

Jayaprakasam et al. (2005) studied insulin secretion by bioactive anthocyanins and anthocyanidins present in *Moringa* fruits which have been found to be implicated in a decrease in coronary herat disease and in anti-diabetic preparations. Pelargonidin-3-galactoside is one of the major anthocyanins and its Aglycone, pelargonidin, caused by a 1.4 fold increase in insulin secretion at 4 mM glucose concentration. The result confirmed its role in the cure of diabetes.

Bose (2007) studied possible role of *Moringa* oleifera Lam. Roots in epithelial ovarian cancer. A hormonal etiology of epithelial ovarian cancer has long been suspected and the role of FSHR has also been demonstrated. The results verified the role of Moringa oleifera Lam. In the treatment of epithelial ovarian cancer worth investigating.

K. Suzuki et al. (2007) reported anti-diabetic effects of *Moringa* oleifera leaves on glucose tolerance in Goto-Kakizaki and Wistar rats. *Moringa* induced significant decrease in the blood glucose levels of Wistar rats. The overall action of *Moringa* leaves was found greatsr in Goto-Kakizaki rats than Wistar rats.

Shanker et al. (2007) used reverse phase high-performance liquid chromatography (HPLC) method to determine bioactive nitril glycosides-niaziridin and niazirin in the leaves, pods, and bark of *Moringa* oleifera. Niaziridin (0.015% and 0.039%) and niazirin (0.038% and 0.033%) were found to be present in leaves andpods respectively. Niaziridin and niazirin were not detected in the bark whereas a relatively higher amount of niazirin was found in leaves than the pods while niaziridin content was found almost three times higher in the pods than the leaves.

Parvathy et al. (2007) investigated cytotoxic effect of various extracts of *Moringa oleifera* leaves on human multiple myeloma cell lines. Methanolic extracts of *Moringa* leaves exhibited least viability at highest dose.

Heredia et al. (2008) studied azo dye removal from *Moringa oleifera* seed extracts. The study was particularly focused on testing the removal of an azo dye such as Chicago sky Blue 6B (CSB). Lower CSB percentage removal is achieved by increasing the initial dye concentration but an optimum relationship between dye amounts removed and *Moringa oleifera* extract amount has been established.

Agrawal et al. (2008) conducted clinical trials to confirm the role of *Moringa oleifera* in curing bronchial asthma. It is reported to elicit good clinical response in persons, suffering from upper respiratory tract infection asthma, chronic rheumatism and other allergic disorders. An alkaloid extracted from *Moringa* plant called moringine closely resemble ephedrine in action and is found useful in the treatment of asthma by causing relaxation of bronchioles.

Karadi et al. (2008) reported that anti-urolithiatic property from the aqueous, methanolic and ethanolic extract of the root bark of *Moringa oleifera*. U urinary excretion and kidney retention levels of oxalate, calcium, and phosphate were significantly lowered by these extracts. Moreover *Moringa* extracts significantly reduced the elevated serum levels of urea nitrogen, creatinine and ureic acid.

Heredia et al. (2009) tested *Moringa* seed extract for carmine indigo dye. The kinetics of high potential of this coagulant agent to treat waste water from dye stuff has been revealed. *Moringa oleifera* has been found to work well in coagulation and flocculation process and it achieves an average level of removal up to 80%. Temperature has a negative influence and coagulant process does not affect by the pH. By increasing initial dye concentration, lower dye percentage removal is achieved.

Jaiswal et a. (2009) reported anti-diabetic activity of aqueous extract of *Moringa oleifera* leaves on glycaemic control, haemoglobin, total protein, urine sugar, urine protein and body weight.

Rastogi et al. (2009) reported that anthelmintic activity of ethanolic extract of *Moringa oleifera* against Indian earthworm *Pheritima posthuma*. Results expressed in terms of the time for paralysis and the time for the death of worms under various concentration of *Moringa* extracts. Distilled water was used as control group where Piperazine citrate (10 mg/ml) was used as a reference standard.

Amagloh et al. (2009) investigated the role of mature dried *Moringa* seeds in water purification. Completely randomized design with 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 g/l treatment of *Moringa* and 10and 12 g/l alum treatment were quite acceptable as per WHO guidelines.

Thurber et al. (2009) reported the significant role of *Moringa 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.

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 as well as *in vitro* anti-oxidant potential. Its methanolic extract contained chlorogenic acid, rutin, Quercetin glucoside and kaemopherol rhamnoglucoside, where in the root and stem barks, several procyanidin peaks were detected. The high anti-oxidant effects observed for differents prt of *Moringa*

provide justification for their widespread therapeutic use in traditional medicine in different continents.

Patel et al. (2010) reviewed medicinal and economic importance of *Moringa*. The study focused on various parameters including medicinal uses, phytochemical composition along with pharmacological properties of different parts of *Moringa* tree. 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 uses andphytochemicals of *Moringa 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 tannins, steroids, triterpinoids, flavonoids, Saponins, anthraquinones, alkaloids and reducing sugar in the leaf extracts indicate preventive and curative properties of *Moringa* leaves.

Amalog et al. (2010) studied phytochemicals in different tissues from vegetative and reproductive parts of *Moringa oleifera* grown in Ghana. Rhamnose and acetyl-rhamnose substituted glucosinolates were found in all the 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 palmitic and oleic acid whereas stems were found rich in palmitic acid, potassium, magnesium and calcium were found in all the tissues whereas traces of selenium were found in whole seeds only.

Prabhu (2011) evaluate the larvicidal and pupicidal potential of methanolic extracts from *Moringa oleifera's* seeds against malaria vector Anophelese stephensi. The phytochemicals derived from its seed extracts were found to be effective mosquito vector control agents and the plant extracts may be used for pest management programs.

Luqman et al. (2012) investigated that the effect of *Moringa's* leaves and fruit extracts on markers of oxidative stress, its toxicity evaluation, and correlation with anti-oxidant properties using in vitro and in vivo assays. The ethanolic extract of fruit shoed highest phenolic content, strong reducing power and free radical scavenging capacity. The anti-

oxidant capacity of ethanolic extract of both fruit and leaf was higher in vitro assay compared to aqueous extract which showed higher potential in vivo. Result support the potent anti-oxidant activity of Moringa oleifera which adds one more positive attribute to its known pharmacological importance.

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 mg/kg) and selenium (363 mg/kg). 17 fatty acid s were also observed 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.

Rationale and scope of the study

In an ancient time *Moringa* leaves were considered as 'poor man's vegetables' but now health care workers and scientists have worldwide named it as 'Miracle tree' or 'Nature's medicine cabinet' due to the presence of vitamins, minerals and other bioactive compounds which can be used to cure several diseases.

In India the research on *M. oleifera* has not gained much importance yet. It is important to exploit the new trends of this plant for different purposes. It has great anti-cancer anti-diabetic anti-bacterial, anti-fungal, anti-pyretic, anti-inflammatory, anti-oxidant, anti-spasmodic, anti-hypertensive, anti-tumor, anti-ulcer, anti-epileptic, diuretic, cholesterol lowering and cardiac activities properties. Moreover, it is promisingly used for biosorption and water purification, as plant growth enhancer, as biopesticides and for the production of biodiesel and biogas. However, more research is needed to determine its primary mechanism as anti-cancer and ant-diabetic agents. 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, its plantation can be an alternative support to the farmers in bad weathers. *Moringa* plant parts have potential applications in developing herbal tea, health supplements, cooking oil, cosmetics etc. Multiple products from the same plant may lead to explore many economic ventures for youth seeking employment in Punjab.

Therefore, a comparison of the antioxidant and anti-bacterial activities of the leaf extracts of three varieties of *M. oleifera* (PKM1, Jaffna and Conventional) shall reveal the best variety. This best variety can be grown for the aforementioned purposes in Punjab.

Objectives of the study

- To compare the anti-oxidant activities of leaf extracts of three varieties of *M. oleifera* (PKM1, Jaffna and Conventional).

- To compare the anti-bacterial activities of three varieties of *M. oleifera* against selected bacterial strains (*E. coli*, *B. subtilis*, *P. aeruginosa* and *S. aurens*) using leaf extracts (aqueous, methanolic, and ethanolic).

Materials and methods

Plant materials

Seeds of three varieties of *M oleifera* L. named PKM-I, Jaffna and Conventional varieties were procured from Tamilnadu Agricultural University.

Chemicals

The chemicals used in the present study were procured from Hi-media and Lobachemie. Several chemicals like DPPH (2,2-Diphenyl-2-picryl hydrazyl), ABTS (2, 2-azinobis (3-ethyl-benzothiazolin-6-sulfonic acid) Gallic acid, Quercetin, Butylated hydroxytoluene, Folin-ciocalteau reagent, Sodium carbonate, Aluminium chloride , Potassium acetate, Potassium persulphate, Methanol and Ethanol were used in the present study.

Bacterial strains

The bacterial strains like: *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* that are to be used in the present study were procured from MTCC, IMTECH Chandigarh.

Table 2: Various major and minor equipments used during the experiments:

S. No.	Equipment	Specifications	Use
1.	Autoclave	Ambient to150 °C, upto 30 psi	For sterilization of nutrient media glassware, decontamination of biological waste
2.	Electronic balance	Weighs from 0.02–310 g, with Mono Bloc technology	For precise weighing of compounds, media components, plant growth regulators etc.
3.	Hot air oven	Ambient to 80 °C; 0–10 rpm	For drying the glassware
4.	Laminar flow cabinet	HEPA and coarse filters	For providing contamination free work environment
5.	pH meter	pH range from 0–14 with temperature sensor	For adjusting the pH of MS media components, reagents etc.

6.	Refrigerator (4 ºC)	Temperature (4 ºC)	To preserve the growth hormones
7.	Soxhlet	Water extract (80ºC)	Plant extraction
8.	Spectrophotometer	Samples	Absorbance

Seed germination

Seeds of three varieties of *M. oleifera* (PKM-1, Jaffna and Conventional) were sown (2 inches deep into the soil) in pots. The seeds of PKM-1 variety was treated with hot water for 2 min before sowing. The seeds of PKM-1 variety germinated within 2 weeks of sowing but the seeds of Jaffna variety took almost 18 days for the same (table.1)

Preparation of *M. oleifera* leaf extract

Take 13 g of fresh leaf of *M. oleifera* varieties (Jaffna, PKM-1, and Conventional) and dried the leaves in the shade for one week at room temperature and ground it. Prepared the leaf extract in 300 ml methanol by soxhlet apparatus at 45˚C for 9 h. After soxhlet extraction, allow the extract to evaporate by using rotary evaporator at 40-45 ˚C. Then 100 mg (0.1 g) leaf extract dissolved in 40 ml methanol and placed under refrigerator at 4 ˚C for the further analysis (Sreelatha et al. 2009; Anwar et al. 2005).

Determination of antioxidant activity using DPPH free radical (Scavenging Activity)

Principle

The antioxidant reacts with DPPH and neutralize the stable free radicals. A purple colour solution converts into the light yellow colour. It indicates the free radicals are stable now (Sreelatha et al. 2009).

Reagents used

DPPH- 0.1mM: Weigh 0.00394 g DPPH in 100 ml methanol.

Gallic acid 10mg/10ml: Weigh 0.01g of gallic acid and dissolve in 10 ml of distilled water.

Procedure

This assay was carried out by employing the procedure defined by Brand-Williams et al. (1995) with some modification. DPPH assay is performed by spectrophotometer analysis and scavenging activity of extract is also measured in terms of hydrogen donating ability

using the stable free radical DPPH. 0.00394 g of DPPH was dissolved in 100 ml of methanol and kept in dark for 30 min. Different concentrations of the leaf extract were prepared (25-100µg/ml) in triplicates and 2 ml of DPPH solution was added in each test tubes so as to evaluate the scavenging percentage. The aliquots were incubated in dark for 30 min. The colour changes from purple to yellow which indicates the scavenging effect. Then, the absorbance was taken at 520 nm. The DPPH scavenging activity is calculated by the following equation:

$$\% \text{ scavenging activity} = \frac{A_{520}(\text{control}) - A_{520}(\text{sample})}{A_{520}(\text{control})} \times 100$$

ABTS (2, 2-azinobis (3-ethyl-benzothiazolin-6-sulfonic acid) Radical Scavenging Assay

Principle

The principle of ABTS is based on the generation of ABTS radical when it mixed with potassium per-sulphate 16-24 h in dark. This reaction produce a blue green complex which can reduced by antioxidants.

Reagents used

7mM ABTS, 2.45 mM Potassium Per-sulphate, Butylated hydroxytoluene and 50% methanol

Procedure

This procedure followed the method Re et al. 1999. ABTS was dissolved to a 7mM concentration. ABTS radical cations was produced by the reaction ABTS stock solution with 2.45mM potassium per-sulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The kept solution was diluted with 50% methanol at the ratio of 1:25. 2ml of ABTS was added to test tubes which were set in triplicates, followed by varying concentrations of leaf extracts 25-100µg/ml. Butylated hydroxyl toluene was used as standard. After incubation at room temperature the absorbance were recorded at 745nm. The inhibition percentage was calculated based on the following formula-

$$\% \text{ scavenging activity} = \frac{A_{520}\,(\text{control}) - A_{520}\,(\text{sample})}{A_{520}\,(\text{control})} \times 100$$

Determination of total phenolic content (TPC)

Principle

Phenols react with follin's ciocalteu reagent which contain phosphomolybdic acid and produce blue coloured complex in alkaline medium which was measured at 765 nm, (Mallick & Singh, 1980).

Reagents used

- Folin reagent 10%: Measured 10 ml of follin's reagent and dissolved in 90 ml of distilled water.
- 7.5% Sodium carbonate: Weighed 7.5 g of sodium carbonate and dissolved in 100 ml of distilled water.
- 70% methanol: Measured 70 ml methanol and mixed with 30 ml of distilled water.
- Gallic acid 10mg/10ml: Weighed 0.01g of Gallic acid and dissolved in 10 ml of distilled water.

Procedure

The folin ciocalteu method of Singleton et al. (1999) with slight modification was used to determine the total phenolic contents of the leaf extract. Briefly, 2.5 ml of follin ciocalteu reagent (FCR) was added into aliquot of the various leaf extracts. The mixture was shaken well, 2 ml of sodium carbonate (Na_2CO_3) was added after 5-10 minutes of incubation period at room temperature. Absorbance was taken at 745 nm. All the tests are carried out in triplicates. A standard curve was established using gallic acid at concentration 100-500 µg/ml and the total phenolic contents of the extract was expressed as gallic acid equivalent (GAE) in mg/ml.

Determination of total flavonoid content (TFC)

Principle

The total flavonoid content is determined by using aluminium chloride ($AlCl_3$) colorimetric method. The formation of acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols in addition with aluminium chloride. Aluminium chloride also forms acid labile complexes with the ortho – dihydroxyl group in the A- or B-ring of flavonoids.

Reagent preparation:

- Aluminium chloride (10%): Weigh 10 g of aluminium chloride and dissolve in 100 ml of distilled water.
- Potassium acetate (1M): Weigh 9.82 g of potassium acetate and dissolve in 90 ml of distilled water.
- Gallic acid 10 mg/10 ml: Weigh 0.01 g of gallic acid and dissolve in 10 ml of distilled water.
- Absolute methanol

Procedure

The assay was determined according to the method of Chang et al. (2002). For developing the standard curve, quercetin was used as a standard material (at concentration 100-500 µg/ml). Different concentrations of Quercetin solution is use to build a standard curve in order to evaluate the total flavonoid content. 10 g of Aluminium chloride dissolved in 100 ml of distilled water. Then 0.5 ml of sample solution were mixed with 1.5 ml methanol (70%) and also added 0.1 ml of aluminium chloride solution and potassium acetate. After this added 2.5 ml of distilled water, mixed it gently and incubate for 1 h. Then take the absorbance at 415 nm. The flavonoid contents of the leaves extracts was expressed as quercetin equivalent (QE) mg/ml.

Determination of antibacterial activity

Step wise procedure for anti-bacterial of *Moringa oleifera* varieties:

Preparation of leaf extract

- 12.5g of leaf powered was dissolved in 62.5ml of solvent (distilled water, ethanol, and methanol) in a conical flasks.
- Conical flask was shaken on a rotatory shaker for 48 h at 30˚C
- The extract was filtered by using the whatman no.1 filter paper
- Allowed the extract to evaporate by using rotary evaporator at 40˚C and collected in labelled vials.
- The crude extracts obtained were then stored in a refrigerator at 4°C to check the antibacterial activity (Moyo et al. 2012).

Sterilization of glassware

First of all the glasswares are collected and washed properly with detergent and rinse with distilled water. Then keep these glasswares in hot air oven for dry sterilization at 160ºC for 60-80 min. Wrap them with brown paper and keep in autoclavable bags. After that keep the glasswares, media, distilled water, and others required equipments in autoclave for steam sterilization at 15 psi for 20 min at 121ºC temperature.

Surface sterilization of laminar air flow hood

- Before starting the work in Laminar Air Flow the UV tube was kept on for 30 min so as to remove all the contaminants that may be Virus, Fungi and Bacteria.
- Then the UV is switched off.
- Then the surface of the Laminar Air Flow is wiped with 70% Ethanol so as remove all the physical impurities.

Culture preparation

15 µl of pure bacterial culture was added to 5 ml of nutrient broth, and then allowed to shake in rotatory incubator for 24 h at 120 rpm. After 24 h 50 µl of bacterial culture was added to 25 ml of nutrient broth and allowed it to shake for 24 h again.

Media preparation for bacteria strain

- 14 g of Nutrient Agar was dissolved in 250 ml of distilled water in a conical flask.
- Adjust the pH to 7.4 ± 2 with 1 N NaOH or 1 N HCl.
- Plug the conical flask with cotton cap and wrap it with brown paper
- Autoclave it at 15 psi for 20 min at 121^{o}C.
- Media was dispensed inside the Laminar Air Flow in autoclaved culture vessels.

Antibacterial activity

Antibacterial activity of Jaffna, PKM-1, and Conventional variety of Moringa extracts were determined by using a modified disc diffusion method.

Broth culture was spreaded (500 µl each) on the nutrient agar media in petriplates under lab conditions and kept in bacterial incubator for 24 h at 37˚C. 5mm filter paper discs were soaked in different concentrations of aqueous, ethanolic, and methanolic extracts (200, 175, 150, and 100mg/ml) which were used to test on different bacterial strain. Streptomycin (10mcg/disc) was used as a positive and distilled water as negative control (Kalpana *et al* 2013).

Results and discussion

Seed germination:

Seeds of the three varieties were sown in pots containing LPU, garden soil.

Table 3: The seed germination percentages are shown below

Varieties	Seeds sown	Seed germination	Seed germination percentages (%)
PKM-1	20	19	95.0
Jaffna	20	13	65
Conventional	20	13	65

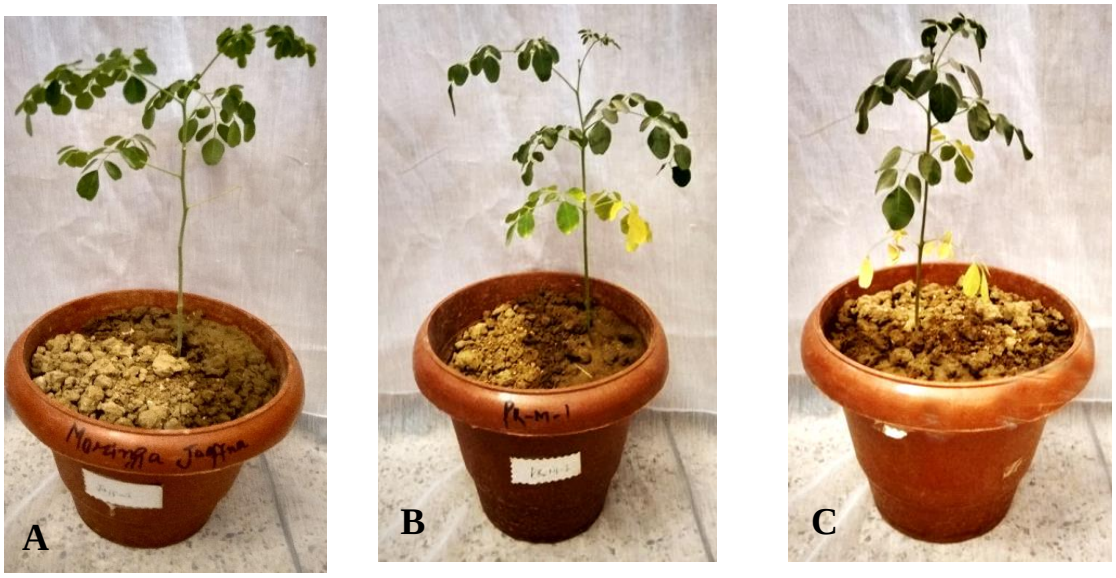


Fig. 1: *M. oleifera* varieties **(A)** Jaffna **(B)** PKM1 **(C)** Conventional

DPPH free radical scavenging activity

DPPH solution was prepared in an amber colored bottle by dissolving 0.00394 g of DPPH in 100 ml methanol and mixed it gently and kept in dark for 30 minutes. Then 0.100 g of crude leaf extract of *M. oleifera* varieties (Jaffna, PKM-1 and Conventional varieties) were dissolved in 40 ml of methanol. Then different concentrations of the each sample extract were pipetted in test tubes i.e. 25µl, 50 µl, 75 µl and 100 µl. Then add 2 ml of DPPH solution in each test tube and incubated in dark for 30 min. For each dilution three replicates were taken. The absorbance was taken at 520 nm on

spectrophotometer. The absorbance of control (DPPH solution) was also taken at 520 nm which was 0.936. The results obtained are given in table 4.1-4.3 and represented by a line graph (fig. 2). The scavenging percentage was calculated according to the formula as shown below:

$$\textbf{\% scavenging activity} = \frac{\mathbf{A_{520}\,(control) - A_{520}\,(sample)}}{\mathbf{A_{520}\,(control)}} \times \mathbf{100}$$

DPPH scavenging activity has become a routine assay for estimation of the antioxidant activity of bioactive compounds. The initial concentration of DPPH is decreased by 50% with a sample amount which is generally used in measuring activity of anti-oxidant. In this study the DPPH scavenging activity of three varieties of *M. oleifera:* PKM-1 (25.569 to 79.282), Jaffna (28.510 to 78.840) and Conventional (22.753 to 76.740) were found respectively at 25-100 µg/ml. Gallic acid was taken as standard which was prepared in similar manner the DPPH free radical scavenging activity of standard was not so strong as compared to *M. oleifera* leaf varieties. Scavenging percentage of all three varieties are mentioned.

Table 4.1: Scavenging activity of *M. oleifera* (PKM-1) against DPPH.

O.D. of control (0.790)	**Concentrations (µg/ml)**			
Sample replicates	25	50	75	100
1	0.588	0.369	0.257	0.155
2	0.585	0.360	0.254	0.170
3	0.583	0.366	0.255	0.166
Average	0.585	0.365	0.255	0.163
% inhibition	25.569	53.797	67.679	79.282

Table 4.2: Scavenging activity of *M. oleifera* (Jaffna) against DPPH.

O.D. of control (0.857)	**Concentrations (µg/ml)**			
Sample replicates	25	50	75	100
1	0.615	0.486	0.288	0.180
2	0.610	0.482	0.282	0.183
3	0.613	0.485	0.287	0.181
Average	0.612	0.484	0.285	0.181
%inhibition	28.510	43.485	66.666	78.840

Table 4.3: Scavenging activity of *M. oleifera* (Conventional) against DPPH.

O.D. of control (0.855)	**O.D. at concentrations (µg/ml)**			
Sample replicates	25	50	75	100
1	0.664	0.436	0.301	0.199
2	0.660	0.433	0.299	0.201
3	0.662	0.435	0.302	0.198
Average	0.662	0.434	0.300	0.199
% inhibition	22.753	49.280	64.916	76.740

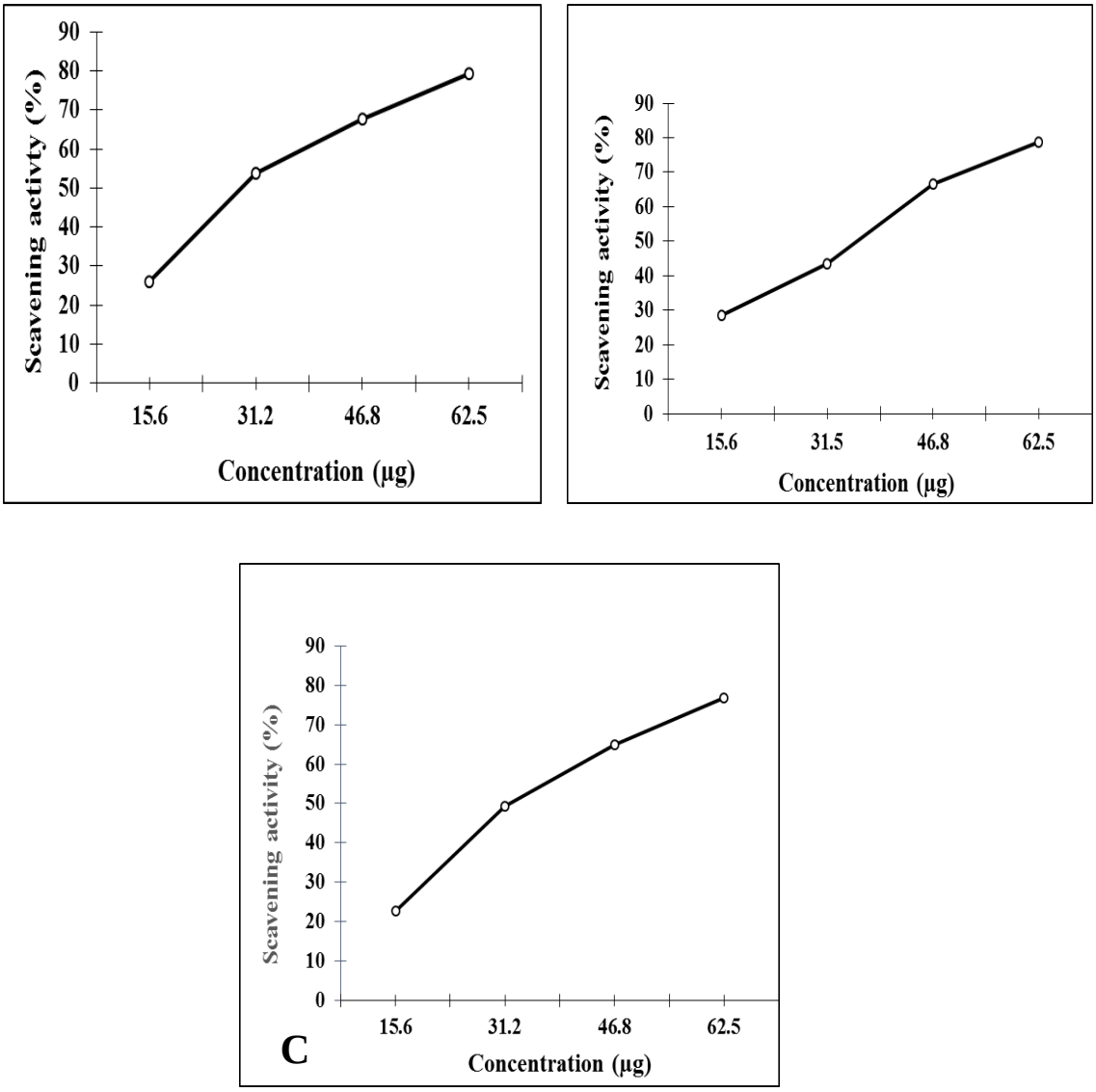


Fig. 2: Graph showing scavenging activity of three varieties against DPPH. **(A)** PKM-1, **(B)** Jaffna and **(C)** Conventional.

ABTS (2, 2-azinobis (3-ethyl-benzothiazolin-6-sulfonic acid) Radical Scavenging Assay

This procedure followed the method Re et al. 1999. ABTS was dissolved to a 7mM concentration. ABTS radical cations was produced by the reaction ABTS stock solution with 2.45mM potassium per-sulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The kept solution was diluted with 50% methanol at the ratio of 1:25. 2ml of ABTS was added to test tubes which were set in triplicates, followed by varying concentrations of leaf extracts 25-100µg/ml. Butylated hydroxyl toluene was used as standard. After incubation at room temperature the absorbance were recorded at 745nm. The inhibition percentage was calculated based on the following formula:

$$\textbf{\% scavenging activity} = \frac{\textbf{A}_{520}\textbf{ (control)} - \textbf{A}_{520}\textbf{ (sample)}}{\textbf{A}_{520}\textbf{ (control)}} \times \textbf{100}$$

Methanolic leaf extracts of Moringa varieties were very effective against ABTS free radicals. The scavenging percentage of three varieties were found in PKM-1 (18.743 TO 59.743), Jaffna (14.697 to 58.052) and Conventional (16.373 to 48.531). At 25-100 µg/ml concentration, PKM-1 showed higher scavenging activity against ABTS free radical as compared to other varieties. BHT was taken as standard which was prepared in similar manner the DPPH free radical scavenging activity of standard was not so strong as compared to *M. oleifera* leaf varieties. The results obtained are given in table 5.1-5.3 and represented by a line graph (fig. 3).

Table 5.1: Scavenging activity of *M. oleifera* (PKM-1) against ABTS.

O.D. of control (0.987)	**O.D. at different concentrations (µg/ml)**			
Sample replicates	25	50	75	100
1	0.800	0.715	0.602	0.394
2	0.805	0.710	0.599	0.400
3	0.801	0.709	0.598	0.398
Average	0.802	0.711	0.599	0.397
% inhibition	18.743	27.929	39.243	59.743

Table 5.2: Scavenging activity of *M. oleifera* (Jaffna) against ABTS.

O.D. of control (0.987)	**O.D. at different concentrations (µg/ml)**			
Sample replicates	25	50	75	100
1	0.810	0.700	0.597	0.398
2	0.807	0.695	0.599	0.395
3	0.809	0.699	0.595	0.400
Average	0.808	0.698	0.597	0.397
% inhibition	14.697	26.371	37.025	58.052

Table 5.3: Scavenging activity of *M. oleifera* (Conventional) against ABTS.

O.D. of control (0.987)	**O.D. at different concentrations (µg/ml)**			
Sample replicates	25	50	75	100
1	0.804	0.732	0.622	0.433
2	0.810	0.737	0.618	0.494
3	0.807	0.735	0.623	0.497
Average	0.807	0.734	0.621	0.496
% inhibition	16.373	23.868	35.647	48.531

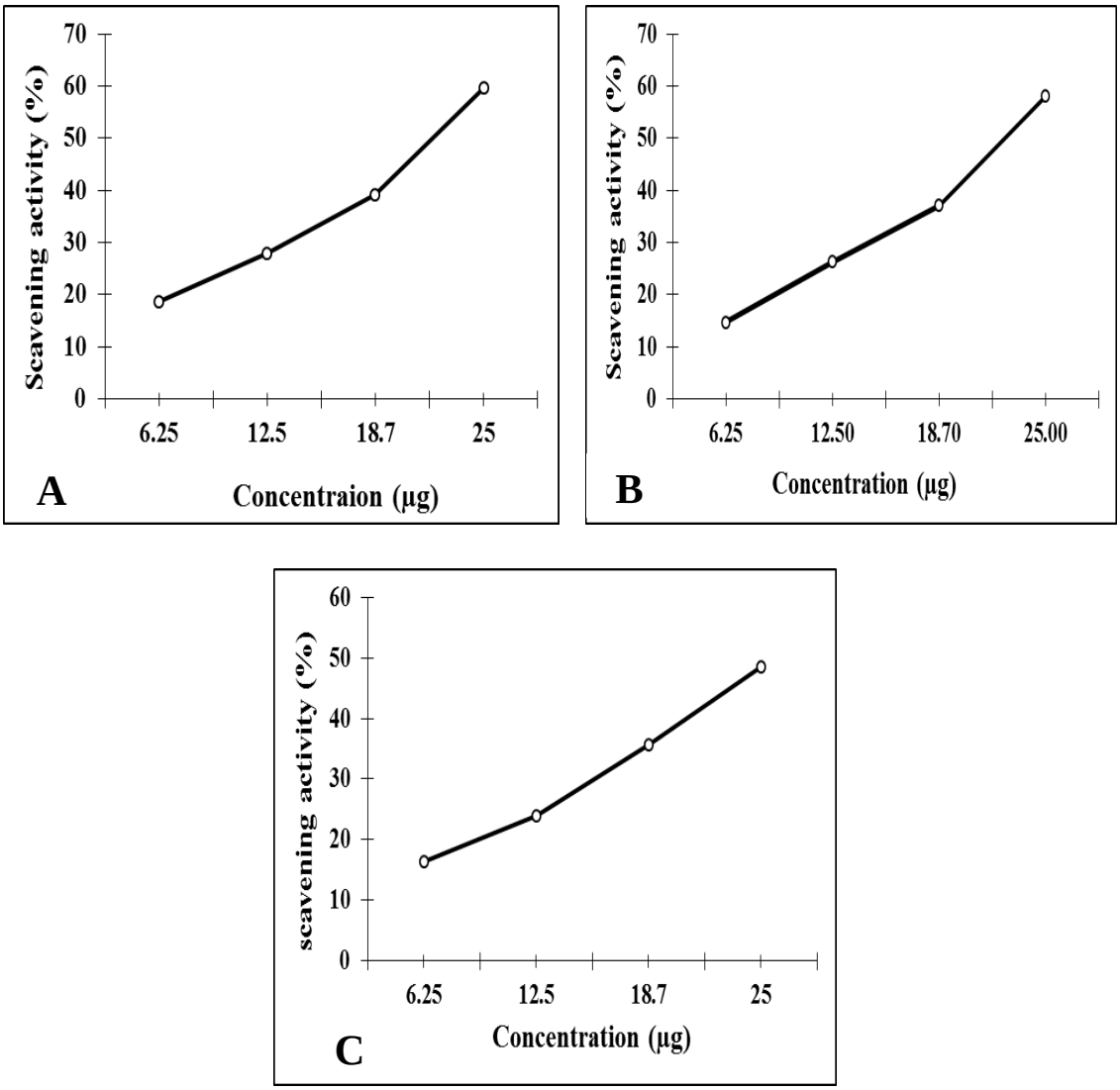


Fig. 3: Graph showing scavenging activity of three varieties **(A)** PKM-1 **(B)** Jaffna and **(C)** Conventional against ABTS

Determination of total phenolic contents (TPC)

The follin ciocalteu method of Singleton et al. (1999) with slight modification was used to determine the total phenolic contents of the leaf extract. Briefly, 2.5 ml of follin ciocalteu reagent (FCR) was added into aliquot of the various leaf extracts. The mixture was shaken well, 2 ml of sodium carbonate (Na_2CO_3) was added after 5-10 minutes of incubation period at room temperature. Absorbance was taken at 745 nm. All the tests are carried out in triplicates. A standard curve was established using gallic acid at concentration 20-120 µg/ml and the total phenolic contents of the extract was expressed as gallic acid equivalent (GAE) in mg/ml. The results obtained are represented by a line graph in (fig. 4).

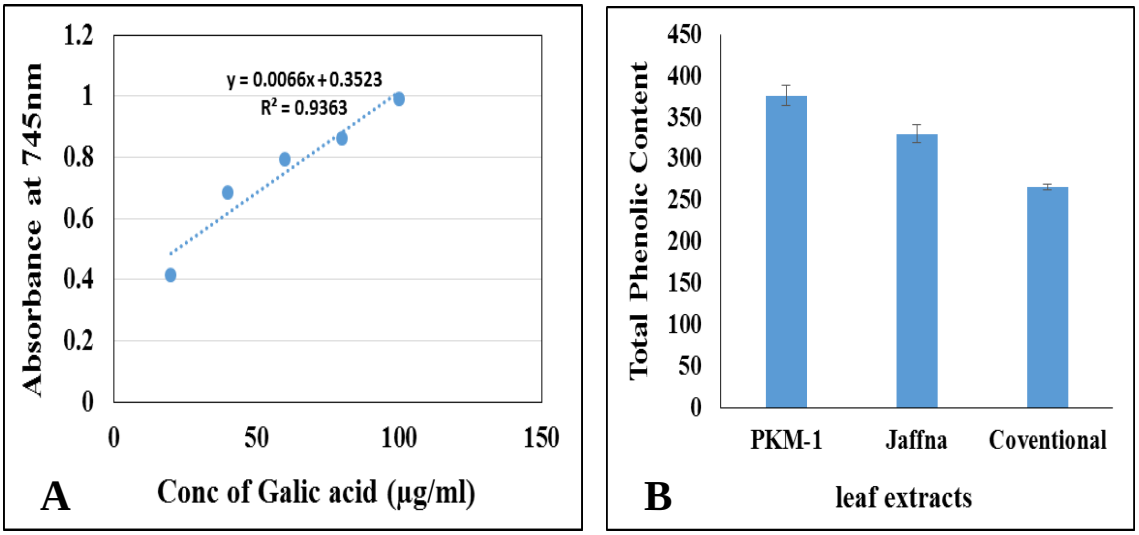


Fig. 4: Graph showing (**A**) standard curve of Gallic acid and (**B**) total phenolic content

After the observation of Total Phenolic Content, methanolic leaf extract of PKM-1 (376.494 mg/ml) variety showed higher phenolic content and Conventional variety (266.080 mg/ml) showed less phenolic content. Jaffna variety also contain 330.323 mg/ml phenolic content.

Determination of total flavonoid content (TFC)

For developing the standard curve, Quercetin was used as a standard material (at concentration 100-500 µg/ml). Different concentrations of Quercetin solution is use to build a standard curve in order to evaluate the total flavonoid content. 10 g of Aluminium

chloride dissolved in 100 ml of distilled water. Then 0.5 ml of sample solution were mixed with 1.5 ml methanol (70%) and also added 0.1 ml of aluminium chloride solution and potassium acetate. After this added 2.5 ml of distilled water, mixed it gently and incubate for 1 h. Then take the absorbance at 415 nm. The flavonoid contents of the leaves extracts was expressed as Quercetin equivalent (QE) mg/ml. The results obtained are represented by a line graph in (fig. 5).

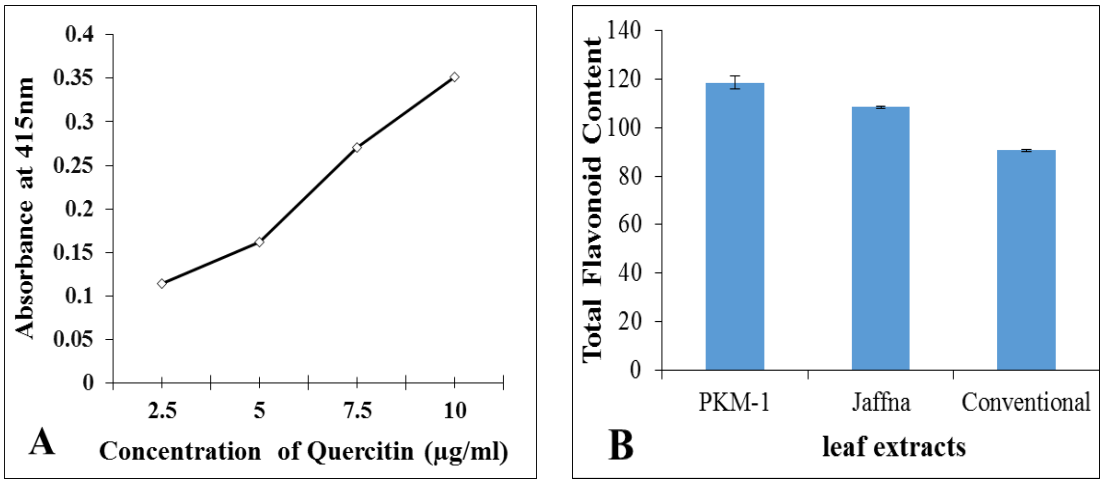


Fig. 5: Graph showing **(A)** standard curve of Quercitin and **(B)** total flavonoids content

In this observation, higher flavonoids content was found in PKM-1 (118.5 mg/ml) and less value occurred in Conventional variety (90.583 mg/ml). Jaffna variety contain (108.375) mg/ml total flavonoid content.

Determination of anti-bacterial activity

Antibacterial activity of aqueous, methanolic and ethanolic leaf extracts was conducted using disc diffusion method. The bacteria used in the study are *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa*.

The aqueous, methanolic and ethanolic leaf extracts of Jaffna variety showed the highest anti-bacterial activity compared to PKM-1 and Conventional variety, against all the bacterial strains under test. Among the three, the ethanolic extract exhibited the highest antibacterial activity as indicated by the maximum zone of inhibition that is 25, 22, 28 and 24 mm against *E.coli*, *B.subtilis*, *S. aureus* and *P. aeruginosa* respectively, followed by PKM-1 (22, 20, 28 and 23 mm) and Conventional (20, 19, 20 and 22mm).

The zone of inhibition with methanolic extracts of Jaffna variety was observed to be 25, 23, 28 and 20 mm against *E.coli*, *B.subtilis*, *S. aureus* and *P. aeruginosa* respectively followed by PKM1 (20, 22, 20 and 18 mm) and conventional (12, 17, 16 and 15mm). The zone of inhibition with aqueous extracts of Jaffna variety was observed to be 18, 14, 17 and 20 mm against *E.coli*, *B.subtilis*, *S. aureus* and *P. aeruginosa* respectively followed by PKM-1 (13, 12, 16 and 15 mm) and conventional (9, 8, 9 and 7mm).

The antibacterial activity increased significantly with the increasing concentration, 200 mm being the maximum concentration used. Hence, the present study was done to investigate the role of aqueous, methanol and ethanol extracts of conventional, PKM-1 and Jaffna varieties of Moringa leaves as a potential antibacterial agents like *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria. Aqueous extract of all the varieties exhibited the least anti-bacteria activity as compared to the ethanolic and methanolic extracts. Our results are in agreement with the previous reports (Kalpana et al. 2013, Rehman et al. 2009). Reasons behind that, it may be attributed to the low solubility of bioactive compounds in water. The results obtained are given in table 6.1-6.9 and represented by graphs (fig. 6.1-6.9).

Table 6.1: Aqueous extraction of PKM-1.

	Zone of inhibition (ZOI) (mm)			
Conc.	***P. aeruginosa***	***B. subtilis***	***E. coli***	***S. aureus***
200mg	13	12	16	15
175mg	12	11	15	14
150mg	11	11	14	12
100mg	10	9	12	11

Table 6.2: Aqueous extraction of Jaffna.

	Zone of inhibition (ZOI) (mm)			
Conc.	***P. aeruginosa***	***B. subtilis***	***E. coli***	***S. aureus***
200mg	18	14	17	20
175mg	14	13	14	17
150mg	13	12	14	15
100mg	11	10	12	12

Table 6.3: Aqueous extraction of Conventional variety:

	Zone of inhibition (ZOI) (mm)			
Conc.	***P. aeruginosa***	***B. subtilis***	***E. Coli***	***S. aureus***
200mg	9	8	9	7
175mg	7	7	8	6
150mg	7	5	5	6
100mg	6	5	4	5

Table 6.4: Methanolic extraction of PKM-1.

	Zone of inhibition(ZOI(mm)			
Conc.	***P. aeruginosa***	***B. subtilis***	***E. coli***	***S. aureus***
200mg	20	22	20	18
175mg	22	24	23	16
150mg	15	22	22	14
100mg	15	20	20	12

Table 6.5: Methanolic extraction of Jaffna.

	Zone of inhibition (ZOI) (mm)			
Conc.	***P. aeruginosa***	***B. subtilis***	***E. coli***	***S. aureus***
200mg	25	23	28	20
175mg	18	20	26	18
150mg	16	19	24	17
100mg	16	17	21	15

Table 6.6: Methanolic extraction of Conventional variety.

Conc.	**Zone of inhibition (ZOI) (mm)**			
	P. aeruginosa	***B. subtilis***	***E. coli***	***S. aureus***
200mg	12	17	16	15
175mg	15	15	15	14
150mg	15	14	15	14
100mg	13	12	12	12

Table 6.7: Ethanolic extraction of PKM-1.

	Zone of inhibition(ZOI) (mm)			
Conc.	***P. aeruginosa***	***B. subtilis***	***E. coli***	***S. aureus***
200mg	22	20	28	23
175mg	20	18	27	20
150mg	17	16	22	18
100mg	14	14	20	14

Table 6.8: Ethanolic extraction of Jaffna.

	Zone of inhibition (ZOI) (mm)			
Conc	***P. aeruginosa***	***B. subtilis***	***E. coli***	***S. aureus***
200mg	25	22	28	24
175mg	24	20	27	20
150mg	20	19	25	19
100mg	19	18	20	15

Table 6.9: Ethanolic extraction of Conventional variety.

	Zone of inhibition (ZOI) (mm)			
Conc.	***P. aeruginosa***	***B. subtilis***	***E. coli***	***S. aureus***
200mg	20	19	20	22
175mg	18	17	17	20
150mg	15	15	15	19
100mg	13	13	12	17

Conclusions

- Among the three *M. oleifera* varieties PKM-1 exhibited the best anti-oxidant property.
- While, the highest anti-bacterial property was shown by Jaffna followed by PKM-1 and conventional variety.
- On the whole PKM-1 proved to be a better performer than the other varieties under test as its seeds showed a higher germination percentage, higher germination rate and the leaf extracts exhibited a higher anti-oxidant activity and a reasonable anti-bacterial activity.
- Therefore, PKM-1 variety can be used for plantations throughout Punjab and its plantation can be an alternative support to the farmers in bad weathers and unprecedented rainfall conditions.

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