Volume-3, Issue-7, July-2019: 24-34
International Journal of Recent Innovations in Academic Research **ISSN: 2635-3040**

A Comparative Studies on Antimicrobial Activity and Antioxidant Activity on Different Extracts of Leaf, Bark and Root of *Moringa oleifera* Lamk (Drumstick tree)

Dr. Mi Mi Yee*

E-mail: drmimiyee.chem2017@gmail.com

Received: June 22, 2019; **Accepted:** June 29, 2019; **Published:** July 3, 2019

Abstract: Medicinal plants were and still continue to be an important therapeutic aid and for alleviating aliment of human kind. All parts of trees are considered medicinal and used in the treatment of ascites, rheumatism, venomous bites and as cardiac and circulatory stimulant. The preliminary phytochemical screening test has shown the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, saponins, flavonoids, α- amino acids, starch and tannins but reducing sugar was absent in the sample. Antibacterial activity of five extracts (PE, EtOAc, EtOH, MeOH and H_2O) of *Moringa oleifera* Lamk (leaf, bark and root) were studied on five microorganisms which include *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumalis* and *Escherichia coli*. Among these five crude extracts, EtOAc extract showed the inhibition zone diameters in the ranged of 35-45mm has highest antimicrobial activity than the other extracts. Screening of antioxidant activity by using DPPH assay of watery extracts of *Moringa oleifera* Lamk (leaf, bark and root) compared with antioxidant ascorbic acid. Radical scavenging of watery extract of *Moringa oleifera* Lamk leaf (IC_{50} = 4.30µg/mL), bark (IC_{50} = 4.99µg/mL) and root (IC_{50} = 8.46µg/mL) were less radical scavenging than standard ascorbic acid (IC_{50} = 1.17µg/mL).
Keywords: Antioxidant Activity, Antimicrobial Activity and *Moringa oleifera*.

Citation: Mi Mi Yee. 2019. A Comparative Studies on Antimicrobial Activity and Antioxidant Activity on Different Extracts of Leaf, Bark and Root of *Moringa oleifera* Lamk (Drumstick tree). International Journal of Recent Innovations in Academic Research, 3(7): 24-34.

Introduction

Moringa oleifera is a small, graceful, deciduous tree, fast growing, drought resistance, which can reach a maximum height of 7-12 m and a diameter of 20-40 cm at chest height. *Moringa oleifera* Lamk is distributed in many countries of the tropics [1]. It grows in all types of soil, except stiff clays and thrives best under the tropical insular climate of South India. The tree can be propagated by seeds or from cuttings, and cuttings are preferred. Furthermore, cuttings of fairly large size planted in moist soil, strike root readily and grow to sizeable trees within a few months [12].The leaf of this plant contain betacarotene, riboflavin, nicotinic acid, ascorbic acid, alpha- tocopherol, calcium, iron and phosphorus [5]. Amazingly, it was found that the leaf is an excellent source of protein containing nine essential amino acids [6]. It contains quercetin – 3-O-glucoside and quercetin -3-O-(6-malonyl- glucoside) and lower amounts of kaempferol -3-O- glucoside and kaempferol -3-O(6-malonyl- glucoside).

It also contained 3-caffeoylquinic and 5- caffeoylquinic acid. EtOH extract of moringa oleifera leaf found 4-(4´-O- acetyl- alpha- L- rhamnosyloxy) benzylisothiocyanate and niaziminin as new compound [10]. The roots contain pterygospermin, moringine and spirochin[4]. Moreover, roots of M. oleifera have high concentration of both 4-(L-rhamnopyranosyloxy)-benzylglucosinolate and benzylglucosinolate. Medicinal plants were and still continue to be an important therapeutic aid and for alleviating aliment of human kind. Thousands of years ago, effective medicinal plants were selected by the process of trial and error, empirical reasoning, and even by experimentation. These efforts are recorded in history by the none discovery of 'medicine' [7] and [8].

The barks contain moringine, moringinine alkaloids and β-sitosterol.The pods contain leucine, threonine , methionine, phenylalanine, tryptophan and lysine [13]. The seeds of moringa oleifera Lamk. contain cis -9 octadecenoic (oleic acid), cis-11-eicosenoic acid, cis -11- octadecenoic acid (vaccenic acid [[10], palmitic acid, β- sitosterol, stigmasterol, compesterol, alpha–gamma and delta tocopherols [10]. The stems contain 4-hydroxymellein, vanillin, β- sitosterone, octacosanic acid and β- sitosterol.

The flowers and fruits contain amino acid such as alanine, arginine, glycine, serine, threonine, valine, glutamic acid and aspartic acid. Lysine is found in the flower. The flowers contain both sucrose and d- glucose, whereas the fruits show the presence of sucrose only. All parts of trees are considered medicinal and used in the treatment of ascites, rheumatism, venomous bites and as cardiac and circulatory stimulant.

The leaf are rich in vitamins A and C and are considered useful in scurvy and catarrhal affection. A paste of the leaf is used as an external application for wounds. A leaf –juice was dropped into the eyes in fainting fits due to nervous delibity, spasmodic affection of the bowels, hysteria and flatulence [3]. The root bark is use for heart complaints, eye diseases, fevers, inflammation, dyspepsia and enlargement of spleen. The roots are bitter, act as a tonic to the body and lungs, laxative, expectorant, diuretic, emmenagogue, enriches the blood, good for inflammations of throat, chest wound, bronchitis, piles, loss of appetite, cures stomatitis, urinary discharges, abstinate asthama, stimulants in paralytic afflictions, epilepsy and hysteria. The fresh root of the young tree is administrated in case of intermittent fever. Moringa fresh root is vescicant and rubefacient [3].

According to [9], leaf, flower, root and seed are used for tumours. Medicinal plants and herbs contain substances known to modern and ancient civilization for their healing properties. Seeds crushed to a powder are used to clarify turbid, dirty water. The cleansing take place by a process of electrical charges established between the muddy particles suspended in the water and the pulverized seed and gradually after about an hour, the muddy particles are pulled to the bottom of the water by the force of gravity. Even today, plants are the most exclusive source of drugs for the majority of the world's population.

In industrialized countries, medicinal plants research has had its up and down during the last decades. Traditional medicine is defined as the therapeutic that have been in existence, often for hundreds of year, before the development and spread of modern medicine and are still in use today (WHO, 1991). In 1998, WHO reviewed that medicinal plants are for pharmacological research and drug development, not only when plant constitutents are used directly as therapeutic agents, but also as starting materials for the synthesis of drug or as models for pharmacologically active compounds (WHO, TRM,1998). Hence safety and efficacy data play an important role for the plants, their exacts and active in gradients and the

preparations of them. Thus, assurance of the safety, quality and efficacy of medicinal plants and herbals products has now become a key issue in industrialized and in developing countries [11].

The Government of Myanmar has initiated a national programme for the development of Traditional Medicine System in combating six major types of diseases, namely, malaria, tuberculosis, diarrhea, dysentery, diabetes and hypertension. Scientists have also established that every single part of the Moringa tree has been of great use not only to the human beings in terms of their health in one form or the other but also for their livestock. It makes a great fodder for cattle.

The antimicrobial activities of leaves, bark and seeds were investigated in vitro against bacteria, yeast, dermatophytes and helminthes pathogenic to man. Aqueous extract from seeds inhibits the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Materials and Methods

Collection and Preparation of Sample

Leaf, Bark and Root of *Moringa oleifera* Lamk (Drumstick tree) were collected from Mingalar taung Nyunt Township, Yangon Division, 2019.The collected plant was identified and confirmed as *Moringa oleifera* Lamk at Botany Department, Dagon University. The collected plant samples were washed thoroughly with water. After cleaning, the sample were cut into small pieces and air dried at room temperature for three weeks. The dried samples were powdered by using grinding machine and stored in air- tight container and labeled systematically to prevent moisture changes and other contamination.

Chemicals

All chemicals used in this work were from British Drug House Chemical Ltd., Poole, England. All standard solutions and other diluted solutions throughout the experimental runs were prepared by using distilled water. In all the investigations the recommended methods and standard procedures involving both conventional and modern techniques were employed. All other chemicals and reagents used were of analytical grade.

Preliminary Phytochemical analysis

Qualitative phytochemical analyses were performed in Preliminary Phytochemical analysis were performed in extraction of Leaf, Bark and Root of *Moringa oleifera* Lamk (Drumstick tree).Preliminary phytochemical test were carried out according to determine the presence of phytochemicals the alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, α- amino acids, starch and tannins were found to be present but reducing sugar was absent in leaf ,bark and root sample.as described by standard procedure.

Test organism

Screening of antimicrobial activity of various crude extracts such as 95% EtOH, MeOH and watery extract of *Moringa oleifera* Lamk. Sample was investigated by Agar Well Diffusion Methods. In the present work, the test microorganisms were *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus pumilus, Candida albicans* and *Escherichia coli.*

Preparation of inoculum

The microorganisms were inoculated into nutrient broth and rose Bengal broth for bioassay and incubated For 24 and 48 h at 37°C.The turbidity of the medium indicates the growth of organisms.

Antimicrobial studies

The agar well diffusion method was employed for the determination of antimicrobial activity of extracts. Lawn culture of *E.coli*, *Candida albican*, *Bacillus puimilus, psrudomonus aeruginosa, Staphylococcus aureus and Bacillus subtils* were spread on nutrient agar and *A. niger* & *A. flavus* spread on rose bengal agar using sterile cotton swabs. The wells (6mm in diameter) were cut from the agar plates using a cork horer.30µlof the extracts (7mg/ml) were poured into the well using a sterile micro pipette. The plates were incubated at 37±2°C for 24 hours for bacterial activity and 48 hours for fungal activity. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

DPPH (2, 2-Diphenyl-1-picryl-hydrazyl) radical scavenging activity

The ability of the extract to scavenge DPPH radical was determined according to the method described by Mensor et al.(2001).One ml of a 0.3 m M DPPH methanol solution was added to a solution of the extract or standard (250µg/ml,2.5ml) and allowed to read at room temperature for 30 min. The absorbance of the resulting mixture was measured at 518nm and converted to percentage antioxidant activity (AA%).Methanol (1.0 ml) plus extract solution (2.5ml) was used as a blank 1 ml of 0.3 mM DPPH plus methanol (2.5ml) was used as a negative control .Solution of gallic acid served as positive control.

Results and Discussions

Preliminary phytochemical test were carried out according to determine the presence of phytochemicals the alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, saponins, α- amino acids, starch and tannins were found to be present but reducing sugar was absent in Drumstick leaf ,bark and root samples. In the present work, antibacterial activities of five extracts (PE, EtOAc, MeOH, 95% EtOH and H_2O) obtained from plant were investigated on 5 strains of bacteria which include *Bacillus Subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis* and *Escherichia coli* by agar disc diffusion method.

The diameter of agar well was 10 mm. When comparing different antibacterial agents to known concentration, the inhibitory zone diameter is taken as a measure of antibacterial activity. The larger the diameter, the higher the antibacterial activity of test agents. The results of the antibacterial activity on five crude extracts of plant tested against selected organisms were presented in Table 1 and Figure 2,3,4,5,6,7.8,9,10 and 11. In the present work, antifungal activity of five extracts (PE, EtOAc, MeOH, 95% EtOH and H_2O) obtained from plant were investigated on *Candida albican* by agar disc diffusion method. The inhibition zone diameters for five extracts against *Candida albicans* species are shown in Table 2 and Figure 12, 13. MeOH, EtOAc and 95% EtOH extracts of all samples showed the inhibition zone diameters of 13 ~ 45 mm. PE and H_2O extracts were not active against *Candida albicans.*

The antioxidant activity was studied on the watery extracts from selected plants sample by DPPH free radical scavenging assay method. DPPH (1, 1- diphenyl-2, picrylhydrazyl) method is the most widely reported method for screening of antioxidant activity on many plant drugs. This method is based on the reduction of coloured free radical DPPH in ethanolic solution by different concentration of the samples. The antioxidant activity was expressed as 50% oxidative inhibitory concentration (IC_{50}).The present study was carried out to investigate the radical scavenging activity of the plant samples using crude extract such as water extract by using DPPH assay according to the spectrophotometric method. In this experiment, five

different concentrations (1.25 µg/mL, 2.5 µg/mL, 5µg/mL, 10µg/mL and 20µg/mL) of each crude extract in ethanol solvent were used.

Vitamin C used as standard and ethanol without crude extract was employed as control. Determination of absorbance was carried out at wavelength 517 nm using UV-visible spectrophotometer. Each experiment was done triplicate. The percent oxidative inhibition values of crude extracts measured at different concentrations and the results are summarized in Table 3,4 and Figure 14 , 15.

From these experimental results, it was found that as the concentrations were increased, the absorbance values were decreased, ie. increase in radical scavenging activity of crude extracts usually expressed in term of % inhibition. From the average values of % inhibition, IC_{50} (50% inhibition concentration) values in µg/mL were calculated by linear regressive excel program. The IC_{50} values were found to be 4.30 µg/mL for leaf, 4.99 µg/mL for bark and 8.46 µg/mL for root. Among these extracts, since the lower the IC_{50}, the higher the free radical scavenging activity, the leaf extract was found to be more effective than both of bark extract and root extract in free radical scavenging activity.

However, it was observed that all of these extracts have the lower antioxidant activity than standard Vitamin C (IC_{50} = 1.29µg/mL).Therefore it can be deduced that the antioxidant activity was found in the order of (leaf) water extract (IC_{50} = 4.30µg/mL) > (bark) water extract (IC_{50} = 4.99µg/mL) > (root) water extract (IC_{50} = 8.46µg/mL).

Conclusion

From the present research work on "Preliminary Phytochemical Investigation and Antibacterial Activity and the Antioxidant Activity of Crude Extracts from Leaf, Bark and Root of *Moringa Oleifera Lamk*. the following inferences may be deduced. The preliminary phytochemical screening test has shown the presence of alkaloids, carbohydrates, glycosides, phenolic compounds, saponins, flavonoids, α- amino acids, starch and tannins but reducing sugar was absent in the sample. Antibacterial activity of five extracts (PE, EtOAc, EtOH, MeOH and H_2O) of *Moringa Oleifera Lamk* (leaf, bark and root) were studied on five microorganisms which include *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumalis* and *Escherichia coli.* Among these five crude extracts, EtOAc extract showed the inhibition zone diameters in the ranged of 35-45mm has highest antimicrobial activity than the other extracts.

H_2O extracts of all samples did not show antibacterial activity but PE extract of bark showed some antibacterial activity.Antifungal activity of five extracts (PE,EtOAc, EtOH, MeOH and H_2O) of *Moringa oleifera* Lamk (leaf, bark and root) were studied on *Candida albican*. MeOH, EtOAc and 95% EtOH extracts of all samples showed the inhibition zone diameters of 13 ~ 45 mm. Among these five crude extracts, EtOAc extract showed the inhibition zone diameters in the ranged of 39-45 mm has highest antifungal activity than the other extracts.

H_2O and PE extracts were not active against *Candida albican.*Screening of antioxidant activity by using DPPH assay of watery extracts of *Moringa oleifera* Lamk (leaf, bark and root) compared with antioxidant ascorbic acid. Radical scavenging of watery extract of *Moringa oleifera* Lamk leaf (IC50 = 4.30µg/mL), bark (IC50 = 4.99µg/mL) and root (IC50 = 8.46µg/mL) were less radical scavenging than standard ascorbic acid (IC50 = 1.17µg/mL). Among these three watery extracts, leaf (IC50 = 4.30µg/mL) was higher antioxidant activity than bark and root extracts.

(a) Tree

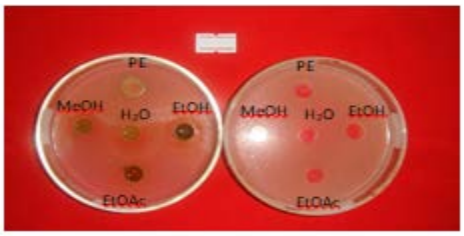
(b) Leaf

(c) Flower

(d) Bark

Figure 1. (a) Plant, (b) Leaf, (c) Flower and (d) Bark of *Moringa oleifera* Lamk. (Drumstrick)

Leaf



Bark

Root

Figure 2. Effect of *Moringa oleifera* Lamk leaf, bark and root extracts on *Bacillus subtilis*

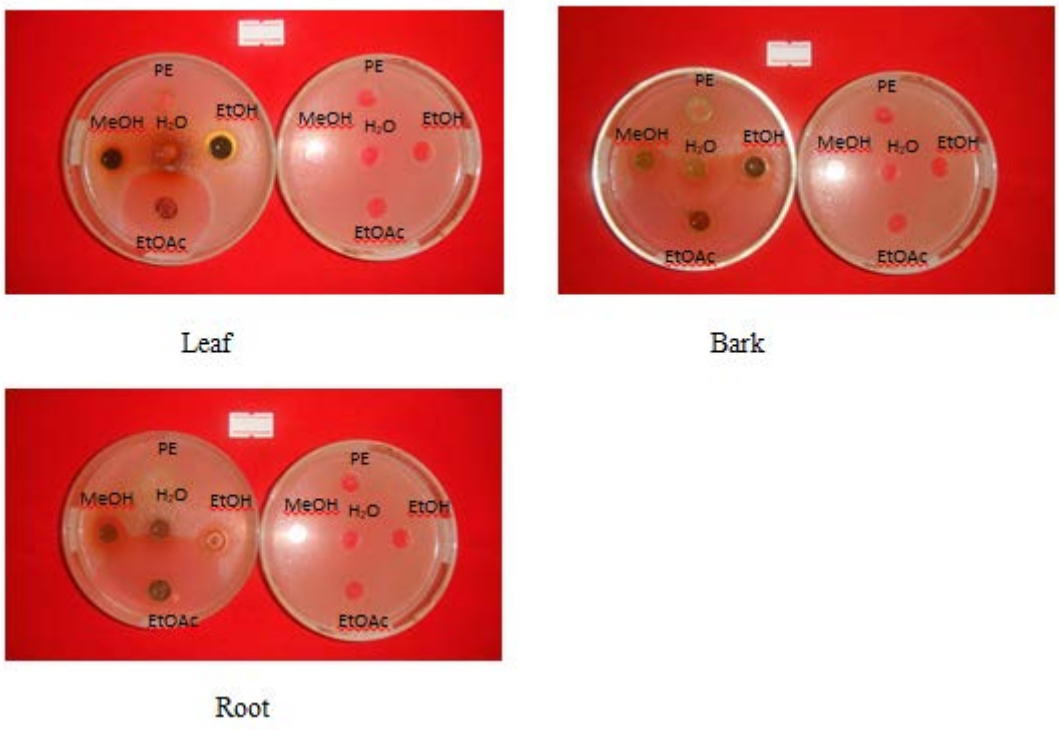


Figure 3. Effect of *Moringa oleifera* Lamk. leaf, bark and root extracts on *Staphylococcus aureus*

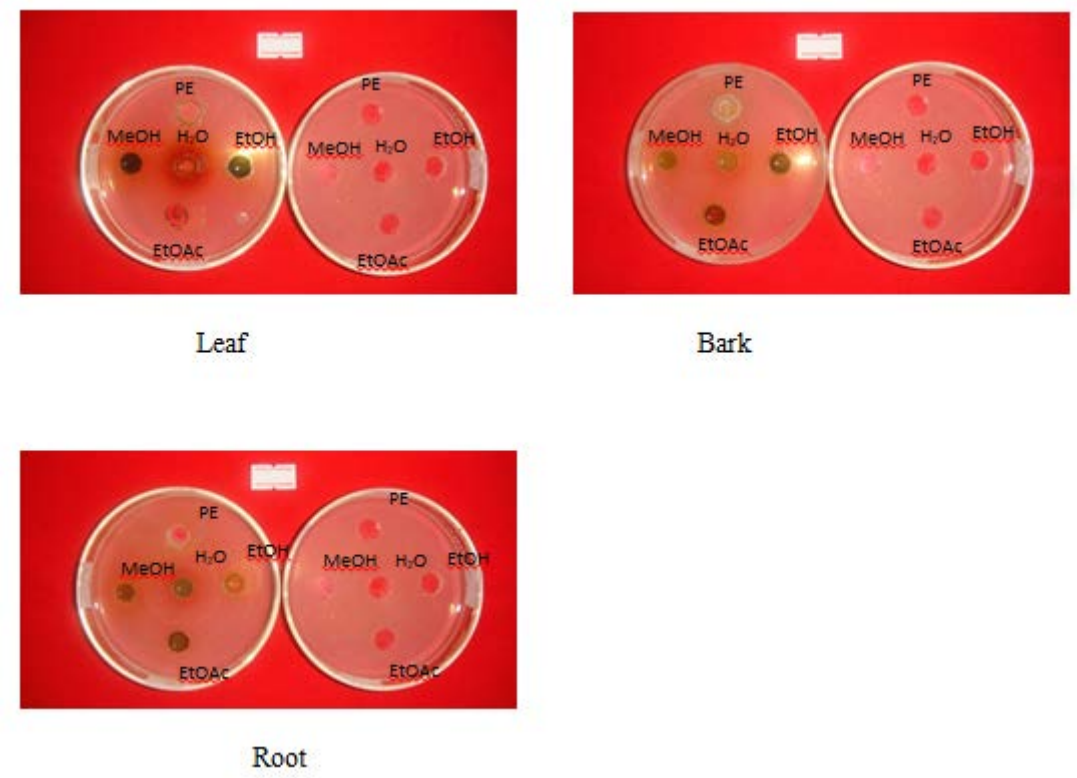


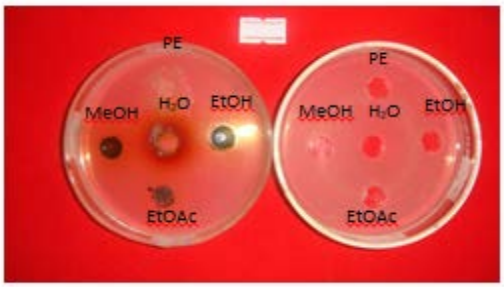
Figure 4. Effect of *Moringa oleifera* Lamk. leaf, bark and root extracts on *Pseudomonas areuginosa*

Leaf

Bark

Root

Figure 5. Effect of *Moringa oleifera* Lamk. leaf, bark and root extracts on *Bacillus pumalis*



Leaf

Bark

Root

Figure 6. Effect of *Moringa oleifera* Lamk. leaf, bark and root extracts on *Escherichia coli*

Table 1. Results for Antibacterial Activity of crude Extracts of leaf, bark and root from *Moringa oleifera* Lamk (Well diameter = 10 mm)

No.	Type of bacteria	Sample	Inhibition zone diameters (mm)				
			I	II	III	IV	V
1	*Bacillus subtilis*	Leaf	-	14mm (+)	35mm (+++)	14 mm (+)	-
		Bark	15mm (++)	19mm (+++)	45mm (+++)	19mm (++)	-
		Root	-	15mm (++)	45mm (+++)	15mm (++)	-
2	*Staphylococcus aureus*	Leaf	-	15mm (++)	40mm (+++)	17mm (++)	-
		Bark	14mm (+)	20mm (+++)	45mm (+++)	20mm (+++)	-
		Root	-	18mm (++)	45mm (+++)	19mm (++)	-
3	*Pseudomonas areuginosa*	Leaf	-	-	-	-	-
		Bark	-	-	-	-	-
		Root	-	-	-	-	-
4	*Bacillus pumalis*	Leaf	-	15mm (++)	40mm (+++)	17mm (++)	-
		Bark	13mm (+)	20mm (+++)	45mm (+++)	20mm (+++)	-
		Root	-	16mm (++)	45mm (+++)	18mm (+++)	-
5	*Escherichia coli*	Leaf	-	-	-	-	-
		Bark	-	-	-	-	-
		Root	-	-	-	-	-

I = PE extract Agar well; II = MeOH extract10mm ~14mm (+) III = EtOAc extract15mm ~19mm (++); IV = 95% EtOH extract20mm~above (+++); V = H_2O extract

Table 2. Results for Antifungal Activity of crude Extracts of leaf, bark and root from *Moringa oleifera* Lamk. (Well diameter = 10 mm)

No.	Type of fungus	Sample	Inhibition zone diameters (mm)				
			I	II	III	IV	V
1	*Candida albicans*	Leaf	-	13mm (+)	39mm (+++)	17 mm (++)	-
		Bark	-	20mm (++)	45mm (+++)	20mm (++)	-
		Root	-	14mm (++)	45mm (+++)	20mm (++)	-

Agar well 10mm ~14mm (+),15mm ~19mm(++), 20mm~above (+++)
I=PE extract; II =MeOH extract; III = EtOAc extract; IV =95% EtOH extract; V = H_2O extract

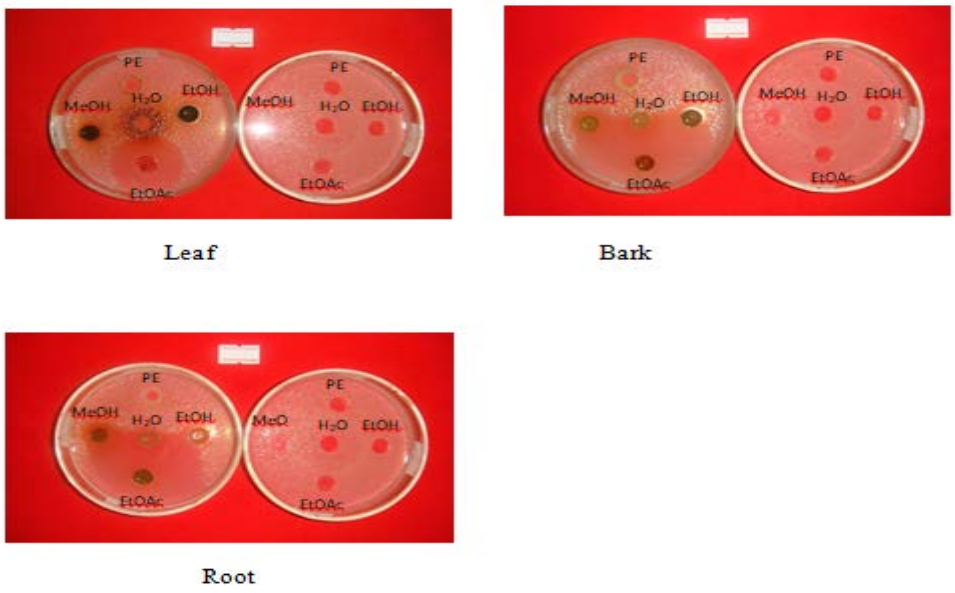


Figure 7. Effect of *Moringa oleifera* Lamk. leaf, bark and root extracts on *Candida albicans*

Table 3. Oxidative inhibition % and IC_{50} values of watery Extracts from Leaf, Bark and Root of *Moringa oleifera* Lamk. and Standard Ascorbic Acid

Extracts	% Oxidative inhibition (Mean ± SD) (µg/mL)					IC_{50} (µg/mL)
	1.25	2.5	5	10	20	
H_2O (Leaf)	35.23± 0.66	44.39± 0.52	52.14 ± 0.75	69.25 ± 0.61	73.32 ± 0.60	4.30
H_2O (Bark)	29.53 ± 0.48	32.99 ± 0.13	50.10 ± 0.41	60.69 ± 0.37	71.28 ± 0.73	4.99
H_2O (Root)	25.25 ± 0.74	31.98 ± 0.09	41.96 ± 0.12	53.57 ± 0.73	58.25 ± 0.61	8.46
Ascorbic acid	25.20 ± 0.40	53.58 ± 0.48	65.53 ± 0.13	74.82 ± 0.59	83.32 ± 0.78	1.17

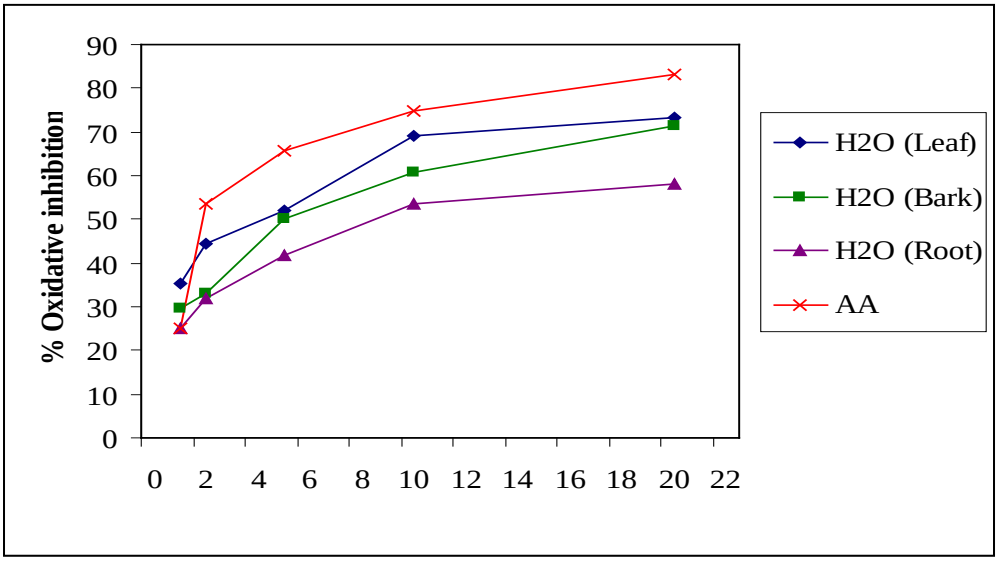


Figure 8. Oxidative inhibition % vs different concentrations of watery extract from leaf, bark and root of *Moringa oleifera* Lamk.and standard ascorbic acid

Table 4. Comparing the IC_{50} values of H_2O extracts from leaf, bark and root of *Moringa oleifera* Lamk.and standard Ascorbic Acid

Extract	IC_{50} (µg/mL)
H_2O(Leaf)	4.30
H_2O(Bark)	4.99
H_2O(Root)	8.46
A.A	1.17

References

1. Akinmoladun, A.C., Ibukun, E.O., Afor, E., Obuotor, E.M. and Farombi, E.O. 2007. Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum.* Scientific Research and Essays, 2(5): 163-166.
2. AOAC, 2002. Official Method of Analysis. International Food Research Journal, 17: 426−432.
3. Chopra, R.N., Nayer, S.L. and Chopra, I.C. 1965. Glossary of India Medicinal Plants. Council of Scientific & Industrial Research, New Delhi, India, 79-80 pp.
4. Das, B.R., Kurup, P.A. and Narasimha, P.R. 1957. Antibiotic principle from *Moringa pterygosperma*. VII. Antibacterial activity and chemical structure of compounds related to pterygospermin. Indian Journal of Medical Research, 45(2), 191-196.
5. Fugile, L.J. 1999. The Miracle Tree: *Moringa oleifera*: Natural Nutrition for the Topics, Church World Service, Dakar, 1-68 pp.
6. Fugile, L.J. 1999. The Miracle Trees *Moringa oleifera*: Natural Nutrition for the topics. New York, 2: 152-173 pp.
7. Fahey, J.W. 2005. *Moringa oleifera*: a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Trees for life Journal, 1(5): 1-15.
8. Kirtikar, K.R., Basu, B.D. and an I.C.S. 1956. Indian Medicinal Plants. Volume 3, 2nd Edition, 2003-2004.
9. Kirtikar, K.R. and Basu B.D. 1975. Indian Medicinal Plants. International Book Distribution Book Sellers, New Delhi, 2, 1495-1496 pp.
10. Robinson, T. 1983. The Organic Constituents of Higher Plants. 5th Edition, Cordus Press, North Amberst, 285-286 pp.
11. Cáceres, A., Saravia, A., Rizzo, S., Zabala, L., De Leon, E. and Nave, F. 1992. Pharmacologie properties of *Moringa oleifera*. 2: Screening for antispasmodic, antiinflammatory and diuretic activity. Journal of Ethnopharmacology, 36(3): 233-237.
12. Wealth of India, 1948. The Council of Scientific and Industrial Research. New Delhi, 140-142, 438 pp.
13. Wealth of India, 1948. A dictionary of Indian Raw Materials and Industrial Products. Council of Scientific and Industrial Products, New Delhi, 1, 366-367 pp.
14. Ashokkumar, R. and Ramaswamy, M. 2013. Determination of DPPH free radical scavenging Oxidation effects of methanolic leaf extracts of some Indian medicinal plant species. Journal of Chemical, Biological and Physical Sciences (JCBPS), 3(2): 1273-1278.