



BRIEF REVIEW: Study of Antibacterial properties of *Moringa oleifera* leaves

*Ms. Snnehaa Bhosle**, *Ms. Santoshi Shivdas*, *Ms. Nidhi Naralkar*, *Dr. Pranati Tilak*, *Dr. Meera Deshmukh*

Lokmanya Tilak Institute of Pharmaceutical Sciences, Pune-411037

ABSTRACT

M. oleifera plant have high nutritional and medicinal values. Different parts of this plant such as flowers, leaves, immature pods and fruit have been found to be very useful and used in many countries as a highly nutritive vegetable. The plant belongs to Moringaceae family that is a monogenetic family and consists of about thirty three species. Recently, it is cultivated in many areas mainly in India, Srilanka, Africa, Pakistan, South America and Mexico. It is used traditionally to treat inflammation, diarrhea, skin infection, cough, diabetes, headache and fever. The most used part in the plant is leaves as these are enriched with protein, carotenoids, ascorbic acid, antioxidant and phenolic. In this research crude protein, crude fat, total ash and carbohydrates were determined in the dry leaves powder beside mineral content which also was determined. The aim of the present study was to evaluate the antibacterial activity of *Moringa oleifera* leaf extracts, *Escherichia coli* and *Klebsiella* were used. The bioactive compounds extracted from *M. oleifera* leaves by using many solvents, ethanol, ethyl acetate, water and methanol. The qualitative phytochemical analysis of *M. oleifera* leaves were carried out using standard procedures to identify the constituents of bioactive compounds (Alkaloids, Flavonoids, Tannins and Phenols) This study is to focus on the cultivation, nutritional values, medicinal potential for commercial use and pharmacological properties of this unique plant.

INTRODUCTION

Moringa oleifera is commonly known as —drum stick or the —horse radish. It is cultivated all over the country as it can withstand both severe drought and mild frost conditions. It is a good source of proteins, vitamins, fatty acids, micro-macro minerals and other essential phytochemicals. The pharmaceutical effects of *Moringa oleifera* makes it useful in therapeutic remedy in traditional medicinal system.

Each part of the tree is useful in one form or another due to its high nutritional and medicinal value. It is also used as anti-inflammatory, anticancer, antioxidant, antidiabetic, cardiovascular hepatoprotective, anti-ulcer and antimicrobial agent. [1]

Genus: The *Moringa* genus comprises of 14 species namely *Moringa arborea*; *Moringa longituba*; *Moringa borziana*, *Moringa pygmaea*; *Moringa hildebrandtii*; *Moringa drouhardii*; *Moringa longituba*; *Moringa peregrina*; *Moringa stenopetala*; *Moringa rivae*; *Moringa ruspoliana*; *Moringa Ovalifolia*; *Moringa Concanensis* and *Moringa oleifera* [2].

Scientific name: *Moringa oleifera* **Family:** Moringaceae

Order: Brassicales **Kingdom:** Plantae

From the Moringaceae family, *Moringa oleifera* is the commonly known studied and used species [2, 3] and it is also the most commonly cultivated plant. It is extremely nutritious with a variety of uses and it is an effective remedy for malnutrition. *Moringa oleifera* is an indigenous plant of Indian subcontinent and now has become naturalized in many tropics and subtropics regions worldwide [4] *M. oleifera* is referred to as —**The Miracle Plant or Tree of Life** [5].

There are several reasons why the issues and challenges of malnutrition and under nutrition still prevail and are unresolved. There are many reasons including food insecurity, lack of access and affordability to the modern health care, lack of availability of nutritional food supplements to certain class of people [6]

One solution to resolve these issues is to find alternative and cost effective ways of producing nutritional food supplements. This could reduce the chances of people getting ill and thus reducing their expenses on medical treatments. The best choice for healthy and nutritional food is from fruits and vegetables. There are only few trees that grow in almost all climatic conditions, one such tree is *Moringa oleifera*. *Moringa oleifera* can survive in all climates - humid or dry hot, and can grow even in poor soils, it is found in all regions irrespective of the climatic condition. Almost all parts of moringa are used in diverse culinary ways [7,8]. All parts of this plant, namely leaves, flowers, seeds, pods, bark and roots have enormous nutritional content and are in traditional medicine to treat numerous pathologies. *Moringa oleifera* leaf has been used as an alternative food resource to combat malnutrition especially among children and infants.

Traditional healers and health consultants prescribe different parts of *Moringa oleifera* for treatment of skin diseases, respiratory illness, hypertension, diabetes, cancer treatment, ear and dental infections and have considered its use as a nutrient condensed food source.

NUTRITIONAL PROPERTIES OF MORINGA *OLEIFERA*

Moringa Oleifera tree is rich in a number of nutrients such as proteins, fibers, minerals, flavonoids content and essential phytochemicals present in its leaves, pods and seeds that play important role in human nutrition and cosmetic industry [9, 10].

Moringa oleifera
10 times more vitamin A than carrots
7 times more vitamin C than oranges
17 times more calcium than milk
9 times more protein than yoghurt
15 times more potassium than bananas
25 times more than iron spinach

As Moringa oleifera is easily cultivable and cheaper supplement compared to other products, it has become a sustainable remedy for malnutrition. It is rich in iron content and its powder is used in the treatment for anemia [11]. The bioactive flavonoids in the leaves of Moringa oleifera have excellent anti-viral and anti-estrogenic activities that make it an appropriate for nutritional and pharmaceutical supplementation.

CULTIVATION OF MORINGA LEAVES

M. oleifera can be grown in any tropical and subtropical regions of the world with a temperature around 25–35 °C. It requires sandy or loamy soil with a slightly acidic to slightly alkaline pH and a net rainfall of 250–3000 mm. The saplings are placed in plastic bags containing sandy or loamy soil. After it grows to about 30 cm, it can be transplanted. In commercial cultivation, spacing is important as it helps in plant management and harvest. *M. oleifera* differs in nutrient composition at different locations [12].

Soil is an important factor that defines nutrient content and strength of the plant showed that fertilizers when applied solely or in combination with others resulted in different nutrient compositions on plant parts. NPK fertilizer, poultry manure and organic base fertilizer was provided to study the effect on the nutrient content and found that poultry manure gave the best results than phosphorous, potassium, sodium and manganese. [13, 14]

The overall nutrient attributes of the plant remains same albeit nutrient variability. This makes moringa viable as a potential nutraceutical anywhere in the world.

Nutrient Content

The study on the nutrient content of these leaves showed that they are a valuable source of both macro- and micronutrients. In addition to these nutrients, they also have significant amounts of vitamins like beta-carotene of vitamin A, vitamin B such as folic acid, pyridoxine and nicotinic acid, vitamin C, D and E [15, 16]. These nutrients when combined with a balanced diet may have immunosuppressive effects [17]. Moringa leaves also have low calorific value and can be used in the diet of the obese.

Studies have reported that among all species of Moringa, Moringa oleifera has the highest amount of β -carotene, ascorbic acid (Vitamin C), α -tocopherol (Vitamin E) and iron.

Table 1. The Nutritional analysis of Fresh and Dry leaf powder, seeds and pods per 100 g of edible portion.

Contents	Value
trans-lutein	approx. 37 mg/100 g
trans- β -carotene	approx. 18 mg/100 g
trans-zeaxanthin	approx. 6 mg/100 g
ascorbic acid	271 mg/100 g
tocopherols	36.9 mg/100 g

Nutrients	Fresh leaves	Dry leaves	Leaf powder	Seed	Pods
Calories (cal)	92	329	205	–	26
Protein (g)	6.7	29.4	27.1	35.97 \pm 0.19	2.5
Fat (g)	1.7	5.2	2.3	38.67 \pm 0.03	0.1
Carbohydrate (g)	12.5	41.2	38.2	8.67 \pm 0.12	3.7
Fibre (g)	0.9	12.5	19.2	2.87 \pm 0.03	4.8
Vitamin B1 (mg)	0.06	2.02	2.64	0.05	0.05
Vitamin B2 (mg)	0.05	21.3	20.5	0.06	0.07
Vitamin B3 (mg)	0.8	7.6	8.2	0.2	0.2
Vitamin C (mg)	220	15.8	17.3	4.5 \pm 0.17	120
Vitamin E (mg)	448	10.8	113	751.67 \pm 4.41	–
Calcium (mg)	440	2185	2003	45	30
Magnesium (mg)	42	448	368	635 \pm 8.66	24
Phosphorus (mg)	70	252	204	75	110
Potassium (mg)	259	1236	1324	–	259
Copper (mg)	0.07	0.49	0.57	5.20 \pm 0.15	3.1
Iron (mg)	0.85	25.6	28.2	–	5.3
Sulphur (mg)	–	–	870	0.05	137

Table 1***Antibacterial activity of the leaves extract:***

Antimicrobial components of *Moringa oleifera* have been proved to be inhibitory against several microorganisms. Research studies have shown that aqueous extracts of *Moringa oleifera* was found to be inhibitory against many pathogenic bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Mycobacterium phlei* and *B. subtilis* and by growth of fungi *Basidiobolushapto sporus* and *Basidiobolusranarums*[18](Saadabi and Abu Zaid, 2011, [19]Eilert et al.,1981, [20]Nwosu and Okafor, 1995).

Infections such as urinary tract infections and respiratory tract infections are a serious health problem and are treated with classical antibacterial drugs which becomes less effective nowadays, because of emergency of many resistant or multi resistant causative pathogenic microorganisms. In addition to this, synthetic antibiotics have high costs and adverse side effects. So, there is a need to search for alternative therapeutic options.

Plants are the oldest pharmacologically active compounds and have provided human kind with various medically useful compounds for centuries

[21]and also nowadays the plants are considered as an alternative source of antimicrobials as they are safe and cheap. [22] CJ et al., [23] [24]Doughari JH et al., 2007; [25] Jasmine R et al., 2013 Mbwambo ZH et al., 2007 and [26]Belguith H et al., 2010). The medicinal plants consists of many secondary metabolites such as alkaloids, Flavonoids, phenolic compounds which possesses antimicrobial activities. [27](Silver LL et al., 1993 and [28](Liu Y et al., 2018).

The studies showed that the leaf extracts of *Moringa oleifera* contain proteins that have various antibacterial activities on many bacterial species such as *E. coli*, *K. aerogenes*, *S. aureus* and *Bacillus subtilis* [29](Isitua, 2016, [30] Caceres A et al., 1999; Doughari JH et al., 2007; [31]Kekuda N et al., 2010 and [32] Jamil A et al., 2007 and [33]Dahot MU et al., 1998), whereas Kiran singh showed that this extract was more effective than traditional antibiotics to combat this pathogenic bacteria [34] (Kiran singh GM et al., 2014). However, no extensive work on its antimicrobial properties has been done. Hence, the present study was an attempt to examine the role of leaf extracts of *M. Oleifera* as a potential antimicrobial agent against some human pathogenic by using the Libyan ecotype of *Moringa oleifera*.

Antipyretic properties

As *Moringa* bioactive constituents give anti-inflammatory action, it might also have antipyretic properties. A study was carried out on rats to assess antipyretic effect of ethanol, petroleum ether, solvent ether and ethyl acetate extracts of *Moringa oleifera* seeds using yeast induced hyperpyrexia method. Paracetamol was used as control in the study. Ethanol and ethyl acetate extracts of seeds showed significant antipyretic activity [35]. From all above observations, it can be concluded that the seeds possess promising antarthritic property.



Extraction of *M. oleifera* leaves

The experimental plant *M. oleifera* was collected from a farm at Tripoli Libya, the leaves were healthy and uninfected. They were air dried at room temperature and grinded to powder form [36] (Rios, Villar 1987). 150 grams of powdered leaves were extracted with 600 ml of methanol. The extract was filtered using Whitman's no.1 filter paper. Then the extract was concentrated in vacuum using a rotatory evaporator at 40 C. the remaining methanol in the extract was removed by placing it at room temperature overnight to give a residue weighing 8g.

Preliminary Phytochemical Screening of Successive Extracts of *M. oleifera* Pods

Qualitative phytochemical analysis of *M. oleifera* leaves was carried out using standard procedures to identify the constituents: Alkaloids, Flavonoids, Tannins and Phenols as described by (Patel et al., 2014).

Test for alkaloids:

1% of HCL prepared and added to the *Moringa Oleifera* extract in test tube and heat it for 20 min with continuous shaking, then leave it to cool for a bit. Take 1 ml of the extract and add few drops of Wagner's reagent. You will observe creamy brown colour which indicates the presence of alkaloids.

Test for flavonoids:

3 ml of *Moringa Oleifera* extract is added to 10ml of distilled water and mix it well. You will notice yellow color which indicates the presence of Flavonoid.

Test for tannins:

2ml of *Moringa Oleifera* extract in a test tube and gently heat it for 2min. Add 3 drops of Ferric chloride. You will notice orange color which indicates the presence of Tannin.

Test for phenols:

3ml of *Moringa Oleifera* extract is added to 5ml distilled water then add few drops of 5% Ferric chloride. You will notice dark green color which indicates the presence of phenols.

METHODOLOGY**Chromatographic purification:**

TLC was carried out to isolate the principle components that were present in most effective extracts of plants. The TLC was performed using different solvent systems.

Solvent phase: Chloroform: methanol: ethanol (1:1:1), Chloroform: methanol: ethanol (2:2:0.5) Chloroform: Glacial acetic acid: methanol (4:5:1).

The plant extract was treated with 4 different solvents, each extract applied on pre-coated TLC plate by using capillary tubes. Draw a light line on the plate and dots to know the place of each extract applied on the plate. After using each mobile phase, the TLC plates were air dried and observed under ultra violet light. They were later sprayed with iodine vapors for the development of the separated bands. The movement was expressed by its Retention factor (Rf) values were calculated for different samples.

Preparation of Bacterial Isolate

Two different types of bacterial strains were obtained from the medical laboratories which were *Escherichia coli* and *Klebsiella*.

Screening of antimicrobial activity**Media for test organisms**

36g of Muller Hinton Agar was added to 1000 ml of sterile distilled water and autoclaved at 121°C for 30 minutes at 1.5 lbs. After cooling both the agar were poured into sterile petri plates for approximately 4mm and allowed to set at ambient temperature.

Sterile Mueller Hinton agar plates were inoculated with the test culture by surface spreading using sterile wire loops and each bacterium evenly spread on the entire surface of the plate to obtain uniformity of the inoculum. The culture plate then had at most 4 holes of 7 mm diameter and 5 mm depth made into it using a sterile agar glass borer. The density of suspension inoculated onto the media for susceptibility test was determined by comparison with 0.5 McFarland standard of Barium sulphate solution.

Inhibition Activity of Different Concentration of *Moringa oleifera* Extracts

This was carried out using agar well diffusion method. 200 µl of different concentration of the ethanoic extracts (25mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml) of *Moringa oleifera* were dispensed separately in wells already seeded with the test isolates and incubated at 37°C for 24 h. After incubation, the inhibitory activity of the minimum concentration of the extracts against the test organisms was determined by measuring the clear zones around the wells in diameter. Standard antibiotic discs were used as a positive control to compare the antibacterial activity. The discs loaded with test extracts, and the standard antibiotic were placed with help of sterile forceps carefully with adequate spacing between each other. After incubation, the antibacterial activity of the extracts against the test organisms was determined by measuring the clear zones around the wells in diameter.

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration of the crude leaves extract of *Moringa oleifera* was determined using the method of Greenwood (1989) as described by Geidam et al., (2007). Serial dilution of the extract at the concentrations of 25, 30, 35, 40, 45, 50, 100 and 200 mg/ml. Where 18 mg of the Muller Hinton Agar media was prepared in 500 ml of distilled water and autoclaved at 121 C and 5lb for 30 minutes then cooled, the media filled in tubes, each tube contains 17 ml. A standard inoculum for each bacterial strain was prepared to give an inoculum size approximately 10⁵ in 5 tubes each tubes contain 10 ml of distilled water. Each extract concentration poured in tube containing 3ml of distilled water and mixed properly then taken off by a sterile syringe and filtered by filter paper and poured to the prepared M.H.A broth and mixed properly then add 100µl of bacterial isolate and mixed again then put them in autoclaved petri dishes and move the dishes in different directions to homogenize the plant extract. The control sample containing only the bacteria without extract. Then all dishes kept at 37 C for 24 hrs. In incubator. Then determine minimum inhibitory concentration and recorded as the least concentration of the extract that completely inhibited the growth of the organisms.

RESULT**Phytochemical Screening of Sequential Extracts of *M. oleifera* leaves**

Phytochemical screening of the sequential extract of *M. oleifera* leaves shows the presence of various bioactive components which are phenol, alkaloids, flavonoids and tannins which are the most prominent components and the result of phytochemical test is presented in Table 1. Among these

phytochemical tests, ethanol and ethyl acetate extract were found to contain maximum of alkaloids, flavonoids, tannin and phenol in comparison with other solvents. All these phytochemicals possess good antioxidant activities and has been reported to exhibit multiple biological effects including anti-inflammatory and antitumor activities.

Table 1: Qualitative phytochemical screening of sequential extracts of *Moringa oleifera* leaves

Solvent used	Alkaloids	Flavonoids	Tannin	Phenol
Methanol	++	+++	++	+++
Ethanol	+++	+++	+++	+++
Ethyl acetate	+++	+++	+++	+++
Water	++	+++	+++	+++

Chromatographic Purification: TLC

Chloroform: Methanol: Ethanol (1:1:1) TLC of pet methanol extract of *M. oleifera* leaves revealed the presence of 4 compounds having Rf values of 0.01, 0.89, 0.92 and 0.93 respectively when a solvent phase was used. With ethanol extract by same solvent showed 5 bands having Rf values of 0.046, 0.66, 0.83, 0.90 and 0.93 respectively. With ethyl acetate, extract shows 4 bands having R_f of 0.046, 0.50, 0.56, 0.89. However, with water the extract shows no bands with chloroform: methanol: ethanol (2:2:0.5). With methanol extract shows 3 bands having Rf values of 0.090, 0.090, 0.81. With ethanol, extract shows 3 bands having Rf values of 0.090, 0.8, 0.81. With ethyl acetate, extract shows no bands with water extract show 2 bands having Rf values of 0.63, 0.76 Chloroform: Glacial acetic acid: methanol (4:5 :1) with methanol extract shows 2 bands having Rf values of 0.45, 0.78. While, Ethanol extract show no bands and with ethyl acetate extract shows 3 bands having Rf values of 0.4, 0.76, 0.83. However, with water extract show no band.

The present study was concentrated on determining antibacterial activity by using agar well diffusion method by measuring the inhibition zone in mm against two bacterial strains *E. coli* and *Klebsiella* species and to determine the phytochemical screening in Leaves of *Moringa oleifera* with different solvents like water, 70% ethanol, 80% methanol and petroleum ether. The extract used in this study was the methanol extract.

Antibacterial activity of the leaves extracts:

By using a different concentration of the leaves extract on the bacterial isolate, the results shown in Table2, where the extract is active against the isolates. However, the inhibitory effect of the isolate is depending on the dose, where higher activity was clear by dose 200 mg/L. Also, the sensitivity of the bacterial isolate to the extract differs. *Klebsiella* is more sensitive to the extract with average zone 3.73mm while *E. coli* is less sensitive by average zone of inhibition 3.47mm at a maximum concentration 200mg/Lin comparison with a control.

Table 2: Antibacterial Activity of *Moringa oleifera* leaves Extract against bacteria (*E.coli* and *Klebsiella*) concentration (mg/mL)/ Zone of inhibition (mm)

Organism	25	50	100	200	Control
<i>E.coli</i>	2.40 ± 0.40	2.68 ± 0.29	3.13 ± 0.06	3.47 ± 0.06	4.40 ± 0.00
P-value	0.001	0.000	0.000	0.000	
Organism	25	50	100	200	Control
<i>Klebsiella</i>	2.33 ± 0.29	2.40 ± 0.69	3.10 ± 0.17	3.47 ± 0.06	4.40 ± 0.00
P-value	0.000	0.007	0.000	0.038	

The minimum inhibitory concentration (MIC) of methanol extract of *Moringa oleifera* against *Klebsiella*. According to Table 3 the MIC value of methanol extract treated on *Klebsiella* was found to be 45 mg%.

Table 3: MIC of methanolic *Moringa* leaves extract for *Klebsiella*

Concentration of extract	Number of colonies
200	No
100	No
50	No
45	No
40	187
35	177
30	304
25	121

DISCUSSION

The rising prevalence of pathogenic microorganisms' resistance to the newer antibiotics has been expressed in the last three decades [37]. Current study exhibits that *Moringa oleifera* leaves extract shows phytochemical bioactive compounds like flavonoids, tannins, alkaloids, saponins and phenols in methanol extract shows in Table 1 that they exhibit antibacterial activity.

Alkaloids are natural bioactive compounds containing basic nitrogen atoms. They also have pharmacological effect and are used as herbal medications [38].

Flavonoids promote the effect of vitamin C and act as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes [39].

Tannins have revealed to have potential Antiviral, Antibacterial and Anti-parasitic effects. Saponins cause hemolysis of red blood cells [40].

Antibacterial activity of *Moringa oleifera* was seen against several bacteria namely *Escherichia Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus vulgaris*, *Streptococcus mutants*, *Bacillus subtilis* and *Staphylococcus epidermidis* [41] (Napolean P, 2009). The methanol extract showed antibacterial effect against both *E. coli* and *Klebsiella*(Table 3). The results showed that increasing the concentration of the methanol extracts increased the zone of inhibition. The medicinal plant *Moringa oleifera* exhibits good antibacterial activity against, *Klebsiella*, and *E. coli* in this study. However, many previous results revealed the strains *E. coli*, *P. aeruginosa* and *S. enteritidis* (IH) were resistant to many treatments [42] (Jackson et al., 2011). This resistance observed was matched from a study on the antibacterial properties of Indian plants showing *Moringa* extracts to be ineffective against *E. coli*. Thus, the results in current study revealed significant inhibitory effect of methanol on leaves extract of *Moringa oleifera* on the *E. coli* and *Klebsiella* by using 200 mg% concentration with inhibitory zone about 3.47 ± 0.06 and 3.47 ± 0.06 respectively in comparison with the control where the zone of inhibition was 4.40 ± 0.00 '

CONCLUSION

This study has revealed that the methanol extracts of *Moringa oleifera* leaf possess some degree of antibacterial effects where the leaf extracts are observed to contain bioactive compounds with a clear antibacterial activity, capable of inhibiting the growth of gram negative bacteria, *E. coli* and *Klebsiella*. More and further studies can achieve that *Moringa oleifera* can be used to discover which bioactive compounds are responsible for the antibacterial activity.

REFERENCES

- [1] Abdull Razis, A. F., Ibrahim, M. D., &Kntayya, S. B. (2014). Health benefits of *Moringa oleifera*. *Asian pacific journal of cancer prevention*, 15(20), 8571-8576.
- [2] Rani A, Husain K, Kumolosasi E (2018) *Moringa* genus: a review of phytochemistry and pharmacology. *Front Pharmacol* 9(108): 1–26.
- [3] Olson ME, Sankaran RP, Fahey JW, Grusak MA, Odee D, Nouman W (2016) Leaf protein and mineral concentrations across the —Miracle Tree genus *Moringa*. *PLoS ONE* 11(7):1–17.
- [4] Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J., & Bertoli, S. (2016). *Moringa oleifera* seeds and oil: Characteristics and uses for human health. *International Journal of Molecular Sciences*, 17(12), 2141.
- [4] Palada M.C., (1996). *Moringa (Moringa oleifera Lam.): a versatile tree crop with horticultural potential in the subtropical United States*. *HortScience*. 31:794–797
- [5] Fuglie, L., (1999). *The Miracle Tree: Moringa oleifera : Natural nutrition for the Tropics*. Church World Services. Dakar, pg 172.
- [6] West KP, Jr., Caballero B, Black RE. (2006). *International Public Health: Diseases, Programs, Systems, and Policies*. Jones and Barlett Publishers; Sudbury, Massachusetts. 187 – 272.
- [7] Chumark, P., Khunawat, P., Sanvarinda, Y., Phornchirasilp, S., Morales, P.N., Phivthongngam, L., Ratanachamngong, P., Srisawat, S., Pongrapeeporn, K. S. (2008) *Thein vitro and ex vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of the water extract of Moringa oleifera Lam. leaves*. *J. of Ethnopharmacology.*, 116: 439-446.
- [8] Iqbal, S., Bhangar, M.I., (2006). Effect of season and production location on antioxidantactivity of *Moringa oleifera* leaves grown in Pakistan. *J. of Food Comp. and Anal.*19: 544-551
- [9] Jongrungruangchok, S., Bunrathep, S., Songsak, T., (2010). Nutrients and minerals content of eleven different samples of *Moringa oleifera* cultivated in Thailand *J. Health Res.*, 24; 123- 127
- [10] Moyo, B., Masika, P., Hugo, A., Muchenje. V., (2011). Nutritional characterization (*Moringa Oleifera Lam*) leaves. *Afr.J. Biotechnol.* 10. 12925-12933.
- [11] Fuglie, L., (1999). *The Miracle Tree: Moringa oleifera : Natural nutrition for the Tropics*. Church World Services. Dakar, pg 172.
- [12] M.D. Thurber, J.W. Fahey

Adoption of *Moringa oleifera* to combat under-nutrition viewed through the lens of the diffusion of innovations theory Ecol. Food Sci. Nutr., 48 (2010), pp. 1-13

[13] M.F. Aslam, R. Anwar, U. Nadeem, T.G. Rashid, A. Kazi, M. Nadeem Mineral composition of *Moringa oleifera* leaves and pods from different regions of Punjab, Pakistan Asian J. Plant Sci., 4 (2005), pp. 417-421

[14] S.O. Dania, P. Akpansubi, O.O. Eghagara Comparative Effects of different fertilizer sources on the growth and nutrient content of moringa (*Moringa oleifera*) seedling in a greenhouse trial Pharma. Clin. Res., 5 (2014), pp. 67-72

[15] Hekmat, S., Morgan, k., Soltani, M., Gough, R (2015). Sensory evaluation of locally- grown fruit purees and inulin fibre on ptpbiotic yogurt in mwanza, Tanzania and the microbial analysis of probiotic yogurt fortified with Moringa Oleifera. J. Health popul. Nutr. 33: 60-67.

[16] M. Mbikay, M., (2012). Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: a review Front. Pharmacol, 3 : 1-12.

[17] DanMalam, H., Abubakar, Z., Katsayal, U., (2001). Pharmacognosostic studies on the leaves of Moringa Oleifera Nig J. Nat. Prod. Med., 5: 45-49.

[18] Saadabi AM, Abu ZAI (2011). An in vitro antimicrobial activity of Moringa Oleifera L. Seed extracts against different groups of microorganisms. Asian J. Basic Appl. Sci. 5: 129-134.

[19] Eilert U, Wolters B, Nahrstedt A (1981). The antibiotic principle of seeds of Moringa Oleifera and Moringa stenopetala. Planta Med. 42: 55-61.

[20] Nwosu MO, Okafor JL (1995).Preliminary studies of the antifungal activities of some medicinal plants against Basidiobolus and some other pathogenic fungi. Mycoses 38: 191- 195.

[21] Mbwambo, Z. H., Moshi, M. J., Masimba, P. J., Kapingu, M. C., & Nondo, R. S, (2007). Antimicrobial activity and brine shrimp toxicity of extracts of Terminalia brownie roots and stem. BMC Complement Alternative Medicine, 30, 7-9.

[22] Pretorius, C. J., & Watt, E. (2001). Purification and identification of active components of *Carpobrotus edulis* L. J. Ethnopharm, 76, 87-91.

[23] Sharif, M. D. M., & Banik, G. R. (2006). Status and utilization of medicinal plants in Rangamati of Bangladesh. Res. J. Agric. Biol. Sci, 2(6), 268-273.

[24] Doughari, J. H., Pukuma, M. S., & De, N. (2007). Antibacterial effects of *Balanites aegyptiaca* L. Drel. and *Moringa oleifera* Lam. on *Salmonella typhi*. African Journal of biotechnology, 6(19), 2212- 2215.

[25] Jasmine, R., Aishwarya, S., SenthamilSelvi, S., Chitra S., & Selvakumar, B. N, (2013). Assessing the curative property of *Moringa oleifera* and investigating its mechanism of action against urinary tract infection. International Journal of Pharmacy and Technological Research, 5(2): 562- 567.

[26] Belguith, H., & Kthiri, F. (2010). Inhibitory effect of aqueous extract of *Allium sativum*. African J Microbiol Res, 4(5), 328-338.

[27] Silver, L. L., & Bostian, K. A. (1993). Discovery and development of new antibiotics: the problem of antibiotic resistance. Antimicrobial agents and chemotherapy, 37(3), 377-383.

[28] Liu, Y., Wang, X. Y., Wei, X. M., Gao, Z. T., & Han, J. P. (2018). Values, properties and utility of different parts of *Moringa oleifera*: An overview. Chinese Herbal Medicines, 10(4), 371- 378.

[29] Isitua, C. C., Ibeh, I. N., & Olayinka, J. N. (2016). Antibacterial Activity Of *Moringa oleifera* Lamk. Leaves on Enteric Human Pathogens. Indian Journal of Applied Research, 6(9), 553-556.

[30] Caceres, A., Cebrea, O., Morales, O., Miollinedo, P., & Mendia P. (1999). Pharmacological properties of *Moringa oleifera*: Preliminary screening for antimicrobial activity. Journal of Ethno pharmacology, 33, 213-216.

[31] Kekuda, T. P., Mallikarjun, N., Swathi, D., Nayana, K. V., Aiyar, M. B., & Rohini, T. R. (2010). Antibacterial and Antifungal efficacy of steam distillate of *Moringa oleifera* Lam. Journal of Pharmaceutical Sciences and Research, 2(1), 34-37.

[32] Jamil, A., Shahid, M., Khan, M. M., & Ashraf, M. (2007). Screening of some medicinal plants for isolation of antifungal proteins and peptides. Pakistan Journal of Botany (Pakistan), 39, 211-221.

[33] Dahot, M. U. (1998). Antimicrobial activity of small protein of *Moringa oleifera* leaves. Journal of Islamic Academy of Sciences, 11(1), 27-32.

-
- [34] Singh, K., & Tafida, G. M. (2014). Antibacterial activity of *Moringa oleifera* (Lam) leaves extracts against some selected bacteria (No. RESEARCH). *International Journal of Pharmacy and Pharmaceutical Science*, 6(9).
- [35] Hukkeri VI, Nagathan CV, Karadi RV, Patil BS (2006). Antipyretic and wound healing activities of *Moringa Oleifera* Lam. In rats. *Ind. J. Pharm. Sci.* 68: 124-126.
- [36] Rios, J. L., Recio, M. C., & Villar, A. (1987). Antimicrobial activity of selected plants employed in the Spanish Mediterranean area. *Journal of ethnopharmacology*, 21(2), 139-152.
- [37] Valarmathy, K., Babu, P., & Abhilash, M. (2010). Antimicrobial activity of ethanolic extracts of various plant leaves against selected microbial species. *Electronic Journal of Environmental, Agricultural & Food Chemistry*, 9(8), 1378-1382.
- [38] Rhoades, D. F. (1979). Evolution of Plant Chemical Defense against Herbivores. In: Rosenthal, editors. *Herbivores: Their Interaction with Secondary Plant Metabolites*. New York, USA: Academic Press, 41.
- [39] Van Wyk, B. E., & Wink, M. (2004). *Medicinal plants of the world: An illustrated scientific guide to important medicinal plants and their uses*. South Africa: Briza Publications, Korkina.
- [40] Winter, W. P., Mason, K. T., & Ford, T. D. (1993). Mechanism of saponin-induced red cell hemolysis: reexamination. *Blood*, 82(1), 461.
- [41] Napoleon P, Anitha J, Emilin RR, (2009). Isolation, analysis and identification of phytochemicals of antimicrobial activity of *Moringa oleifera* Lam. *Current Biotica*;3(1):33-37.
- [42] Peixoto, J. R. O., Silva, G. C., Costa, R. A., Vieira, G. H. F., Fonteles Filho, A. A., & dos Fernandes Vieira, R. H. S. (2011). In vitro antibacterial effect of aqueous and ethanolic *Moringa* leaf extracts. *Asian Pacific journal of tropical medicine*, 4(3), 201-204.