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Analysis of Phytochemicals, Antibacterial and Antioxidant activities of *Moringa oleifera* Lam. Leaf extract- an in vitro study

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Abstract:
The leading important things about utilizing plant-derived medication are relatively less dangerous than artificial drugs and provide deep restorative benefits. In this regard, *Moringa oleifera* plant was evaluated for its nutritional effects. The phytochemical study, antibacterial activity and the *in vitro* antioxidant activity of aqueous, chloroform and petroleum ether extracts of *Moringa oleifera* leaves were investigated. Phytochemical analysis revealed the presence of alkaloids, flavonoids, steroids, tannins, saponins and glycosides as major components. The Petroleum ether extracts of *Moringa oleifera* contains the high content of bioactive compounds such as phenol and flavonoids. The extracts were screened for *in vitro* antibacterial potential against enteric pathogenic bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, E. coli and Vibrio cholera* by disc diffusion method. *Moringa oleifera* showed potent antibacterial activity against several gram negative and gram positive bacteria. Antioxidants are shown to play an important position in your body immune system next to Reactive oxygen species (ROS). The enzymatic and non-enzymatic activities of SOD, Catalase, Peroxidase, Vitamin C, Carotenoids, and reduced Glutathione were high in petroleum ether extracts of *Moringa oleifera*. It shows high scavenging activity against DPPH, Nitrous Oxide and Hydroxyl Radical scavenging assays. As a result, *Moringa oleifera* possesses a good antioxidant, it has a scavenging property against ROS and also it has good antibacterial properties. Thus, *Moringa oleifera* can be used to synthesize a new drug preparation against various diseases responsible for severe illness.

Keywords: *Moringa oleifera,* phytochemicals, antibacterial activity, antioxidant, free radical scavenging activity.

Introduction

The intensity of life-threatening microbe infections brought on by pathogenic microbes has elevated world-wide and it is becoming a significant cause of morbidity and mortality within immune sacrificed affected individuals within acquiring places [(1)]. Totally free radicals play a vital function in the pathogenesis involving various human diseases, for instance cancers, rheumatoid arthritis, as well as cardiovascular diseases [(2)]. Natural antioxidants contained in foods from plant origin involving control most of these radicals and so are therefore essential methods inside acquiring as well as preserving a healthy body [(3, (4)]. The "Moringa" tree is considered one of the world's most useful trees, as almost every part of the Moringa tree can be used for food or has some other beneficial properties [(5)]. *Moringa oleifera* also known as drumstick in India belongs to family Moringaceae is a well-documented world renowned plant herb for its extraordinary nutritional and medicinal properties. It is a natural antihelmintic, antibiotic, detoxifier, outstanding immune builder and is used in many countries to treat malnutrition and malaria. In Senegal and Haiti, health workers happen to be healing malnutrition within babies, pregnant and also

nursing ladies together with *Moringa* leaf powdered ingredients [6]. Moringa leaves are known to have a high content of essential amino acids, proteins, minerals and vitamins, hence an ideal nutritional supplement [7]. Many evidences exposed in which *M. oleifera* experienced several drug actions including antibacterial [8], antifungal, anti-inflammatory and diuretic activities [9]. The antioxidant activity of various extracts of *M. oleifera* leaf has been reported by several other authors [10, 11, 12]. Mutagenic activity of *M. oleifera* was proposed by Villasena et al [13]. The plant was shown to possess antimicrobial activity against wide array of pathogens [14]. Therefore, this current article ended up being undertaken exclusively to examine the role connected with aqueous, chloroform and petroleum ether extracts of *M. oleifera* Lam. leaves as a potential anti-oxidant and antimicrobial agent against some human pathogenic bacteria.

MATERIALS AND METHODS:

Solvent extraction

One kg of leaves was collected, washed thoroughly, shade dried at room temperature and then powdered with the help of the blender. The powdered material was kept in airtight bottles until further use. Dry powdered plant material (200g) was extracted with the solvents petroleum ether, chloroform and aqueous extract with soxhlet extractor for 8 hours. After extraction, the solvent was removed using rotary vacuum evaporator to give a concentrated extract at 60º C in a water bath, which was then frozen and free dried at -20º C until use.

QUALITATIVE PHYTOCHEMICAL ANALYSIS

The freshly prepared leaf extracts of *Moringa oleifera* were subjected to standard preliminary phytochemical analysis for the presence of various known secondary plant metabolites [15]. All three solvent extracts of *Moringa* were exposed to phytochemical assay using colour reactions according to several protocols as follows:

Detection of Alkaloids

Wagner's test: A little amount of the extract was added with Wagner's reagent (1.27g of iodine and 2g of KI along with 100ml of distilled water) and observed for the formation of reddish brown coloured precipitate.

Detection of Phenolic Compounds

a. *Ferric chloride test*: The extract added to 5% $FeCl_3$ reagent and in addition the formation of deep blue colour was appeared.
b. *Lead acetate test*: The extract along with 10% lead acetate solution mixed and seen for the formation of white precipitate.

Detection of Flavonoids

a. *Aqueous sodium hydroxide test: The extract was treated along with aqueous NaOH solution and observed for the formation of yellow-orange colour.*
b. *H2SO4 test: The extract added to concentrated H2SO4 and observed for the formation of orange colour.*

Test for Sugars

a. *Fehling's test*: The extract was treated with Fehling's solution, heated in a boiling water bath and observed for the formation of a brick red precipitate.
b. *Benedicts test*: The extract was treated with Benedict's solution, heated in a boiling water bath and observed for the formation of red precipitate.

Test for Saponins

a. *Foam test*: A fraction of the extract was vigorously shaken with water and observed for the persistent foam.

The results of the qualitative analyses showed the presence of alkaloids, phenols, flavonoids, sugars and saponins in the leaves of *Moringa oleifera.*

ANTIBACTERIAL ACTIVITY

AGAR WELL DIFFUSION METHOD

PREPARATION OF INOCULUM:

Stock cultures were maintained at 4° C on slant of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated at 24hrs at 37º C. The Assay was performed by agar disc diffusion method.

Antibacterial activity of sample was determined by disc diffusion method on Muller Hinton agar (MHA) medium. The Muller Hinton Agar medium was weighed as 3.8gms and dissolved in 100ml of distilled water and add 1gm of agar. Then the medium is kept for sterilization. After sterilization the media was poured in to sterile petriplates and were allowed to solidify for thirty minutes. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. Add 20 µl of sample (concentration: 1000 µg, 500 µg, 250 µg, 125 µg & 62.5 µg), negative control- add 20 µl of Petroleum ether and positive control- add 10 µl (10 µg) streptomycin on respective disc and placed on MHA plates. These plates were incubated for 24 hrs at 37ºC. Then the microbial growth was determined by measuring the diameter of zone of inhibition [16].

ANTIOXIDANT ACTIVITY

Rapid screening by Dot plot assay: Aliquots (3µl) of *Moringa oleifera* leaves extracts were spotted on a Whattman filter paper and allowed to dry. The paper displaying the actual dry locations was placed inverted for 10 seconds in the solution of DPPH. The locations exhibiting radical scavenging antioxidant activity appeared as yellow spots in a violet background. The intensity of the yellow colour depends on the total amount and nature of the radical scavenger present in the location.

DPPH Scavenging Capacity:

Antioxidant activity of *Moringa oleifera* was calculated by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity. An exact total (0.5ml) of the methanolic solution with DPPH was added with 20 µl in the *Moringa oleifera* leaves (corresponding to 4mg) and 0.48ml of methanol, and permitted to stand in a dark place at room temperature. After half an hour, the absorbance was measured at 517 nm. Methanol served as the blank. The particular radical-scavenging capacity of the extracts was calculated with the following equation and expressed as DPPH percent of inhibition

$$\text{Scavenging activity (\%)} = \frac{A_{518}\,(\text{Sample}) - A_{518}\,(\text{Blank})}{A_{518}\,(\text{Blank})} \times 100$$

ENZYMIC ANTIOXIDANTS ACTIVITY:

Assay of SOD: This particular assay mixture contains 1.2ml with salt pyrophosphate buffer, 0.1ml with PMS, 0.3ml with NBT, 0.2ml with enzyme preparation as well as normal water in a total volume of 2.8ml. The particular reaction has been initiated up from the addition of 0.2ml of NADH. Immediately after incubation at 30º C pertaining to 90 seconds, reaction has been arrested the inclusion of 1.0ml connected with glacial acetic acidity. The mix has been stirred intensely using

4ml of n-butanol, allowed to stand for 10 minutes, then centrifuged. The particular intensity with the chromogen from the butanol layer has been assessed at 560 nm against butanol as blank. The reaction mix without the need for enzyme functioned as control. One unit of enzyme activity is defined as the enzyme reaction that gave 50% inhibition of NBT reduction in one minute.

Assay of *Peroxidase*: Pyrogallol solution (3.0ml) and enzyme extract (0.2ml) were being pipetted into a cuvette. The spectrophotometer was adjusted to maintain 'zero absorbance' at 430 nm. H_2O_2 (0.5ml) was added to the test cuvette. The actual change in absorbance was recorded for every 30 seconds up to 3 minutes. One unit of peroxidase activity is defined as the change in absorbance per minute at 430 nm.

Assay of *Catalase*: An aliquot (3.0ml) of hydrogen peroxide in phosphate buffer was drawn in a quartz cuvette and the baseline was adjusted at 240nm in a spectrophotometer. The enzyme extract (40 µl) was rapidly added and mixed thoroughly. Enough time interval required for a decrease in absorbance by 0.05 units were recorded at 240nm. The concentration of H_2O_2 was determined using the extinction coefficient 0.036 per µM cm^{-1}. One unit is the amount of enzyme activity required to reduce the absorbance at 240nm by 0.05 units.

NON ENZYMIC ANTIOXIDANTS ACTIVITY:

Reduced Glutathione: A definitive aliquot (0.1 ml) from the supernatant has been comprised to 1.0 ml along with 0.2 M sodium phosphate barrier. Freshly prepared DTNB solution (2.0 ml) has been added and the yellow-coloured shade has been examined with 412 nm in a spectrophotometer immediately after 10 min's. A standard curve with GSH has been well prepared involving the concentrations of 2 to 10 nmoles. The values are expressed seeing that nmoles GSH/g leaf.

Carotenoids: Leaves was homogenized and saponified for approx 30 minutes in a shaking water bath at 37ºC with a specific volume of 12% alcoholic KOH. The saponified extract was drawn into a separating funnel containing 10 to 15ml of petroleum ether (40-60º C) and mixed well. The lower aqueous phase was transferred to another separating funnel and collected the upper petroleum ether containing the carotenoid pigment. The extraction was repeated until the aqueous phase was colourless. To the petroleum ether extract, a small quantity of anhydrous sodium sulphate was added to remove turbidity. The absorbance of the extract at 450 nm was noted in a spectrophotometer using petroleum ether as blank.

Ascorbic acid: Aliquots of 0.5 - 1.0ml of the supernatant had been taken and 0.2 to 1.0ml of standard ascorbate has been made up to 2.0ml with 4% TCA. 0.5ml of DNPH reagent was added to all the tubes, followed by two drops of 10% thiourea solution. The contents were mixed and incubated at 37˚C for 3 hours. The osazones formed were dissolved in 2.5ml of 85% sulphuric acid, in cold. Towards the blank alone, DNPH reagent and thiourea were added after the addition of sulphuric acid. Immediately after incubation for half an hour at room temperature, the absorbance was read spectrophotometrically at 540nm. From the standard curve produced on an electronic calculator set to the linear regression mode, the concentration of ascorbate in the samples were calculated and expressed as mg ascorbate/g leaf.

RESULT

Solvent extraction

Fig 1: Soxhlet extraction procedure and the concentrated extract

Phytochemical analysis of *Moringa oleifera*

Test	*Moringa oleifera*		
	Pet. Ether	Aqueous extract	Chloroform
Phenols	+	+	-
Alkaloids	+	+	-
Flavonoids	+	+	-
Sugar	+	+	+
Tannins	+	+	-
Saponins	+	+	-

Antibacterial activity of *Moringa oleifera* leaf extracts

Fig 2: Antibacterial Disc diffusion assay plates

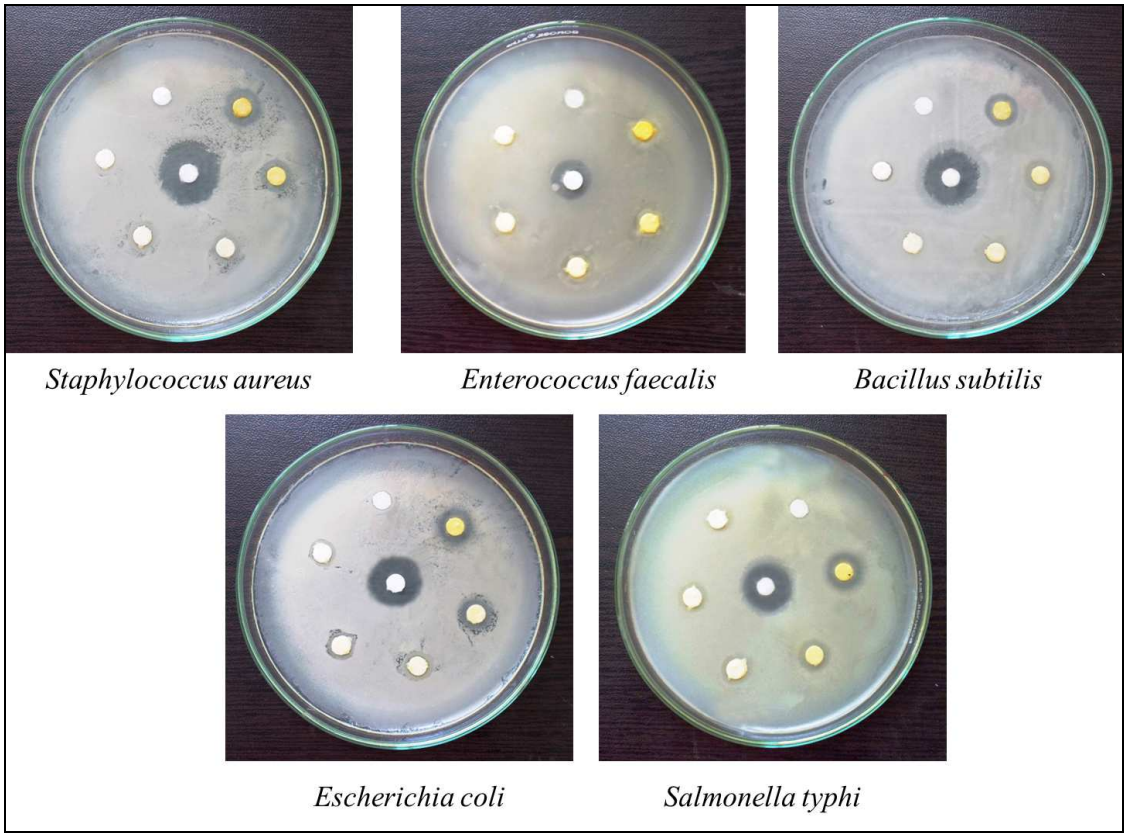


Table Zone of inhibition in mm

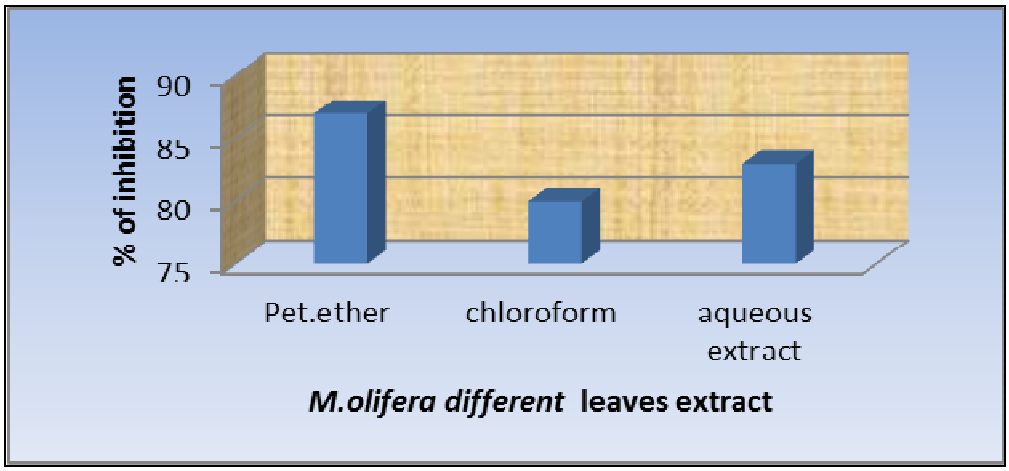
S. No	Microorganisms	Zone of Inhibition in mm						
		1000µg	500µg	250µg	125µg	62.5µg	DMSO	Streptomycin 10µg
1	*Staphylococcus aureus*	11	10	8	-	-	-	16
2	*Enterococcus faecalis*	-	-	-	-	-	-	14
3	*Bacillus subtilis*	11	8	2	-	-	-	15
4	*E. coli*	11	10	9	8	7	-	15
5	*Salmonella typhi*	10	9	7	7	7	-	15

DOT PLOT ASSAY for *Moringa oleifera*

Fig 3: Dot plot assay for Petroleum ether > Chloroform > Aqueous extracts

DPPH Assay

Fig 3: Graph representing the percentage inhibition of radicals



Activities of enzymic antioxidants in *Moringa oleifera* leaf extract

S. No	Parameters	*Moringa oleifera*
1	SOD (U/g)	13.97 ± 0.124
2	CAT (U/g)	109.29 ± 1.96
3	POD (U/g)	65.42 ± 0.748

Non-enzymic antioxidants in *Moringa oleifera* leaf extract

S. No	Parameters	*Moringa oleifera*
1	Ascorbic acid (mg/g)	2.91 ± 0.032
2	Total carotenoids (mg/g)	8.75 ± 0.086
3	Reduced glutathione (nmoles/g)	32.0 ± 1.000

Discussion

Phytochemicals are present in virtually all plant tissues of *Moringa oleifera* e.g. leaves, roots, stem and fruits [17]. The antimicrobial things to do associated with phytochemical compounds may perhaps require numerous modes of actions for example oil degrades this cell wall, retaining wall, interact with this composition and break up cytoplasmic membrane layer [18] and harm membrane layer healthy proteins, obstruct membrane layer bundled minerals, cause leakage associated with cellular ingredients, coagulate cytoplasm, deplete proton motive force, alter fatty chemical and phospholipid constituents, transform nutrient uptake and

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electron transportation, impair enzymatic mechanism for energy etc [19, 20] revealed the prominent presence of alkaloids, phenolics, flavonoids and tannins in hydro-ethanolic extract of *Moringa oleifera* pods. In present study alkaloids, tannins, saponins and flavonoids are detected in *Moringa oleifera*. This is in analogy with other workers [21]. The antimicrobial action of *M. oleifera* leaves is due to the presence of an array of phytochemicals. Free radical scavenger is a significant mechanism for the inhibitory activity towards lipid peroxidation and in addition excellent marker for antioxidant activity [22]. The free-radical scavengers (antioxidants) have potential to prevent, delay or restructure many of human chronic and ageing diseases such as cancer, diabetes, heart disease, stroke, malaria, rheumatoid arthritis symptoms.

Conclusion

In the present circumstances of emergence of multidrug resistance to help human pathogenic infections, it has become very necessary to look for new antimicrobial substances from other sources such as plants. *M. oleifera* is extremely valued plant, with impressive range of medicinal uses and high vitamins and minerals. A surplus of conventional medicine references attest to its healing power, and scientific validation of these popular uses is developing to support at least some of the claims *Moringa* product known to have antibiotic, hypotensive, antiulcer, anti-inflammatory, hypocholesterolemic, and hypoglycemic activities as cited in the scientific literature. Further purification of compounds can be done and the compounds may be subjected for animal studies to carry out more pharmacological studies.

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