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PHYTOCHEMICAL STUDIES AND EFFECT OF METHANOL LEAF EXTRACT OF MORINGA OLEIFERA AND ITS ANTIMICROBIAL ACTIVITY

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Abstract:
The South Asian tree Moringa oleifera Lamarck, often called Moringa pterygosperma Gaertner, is a member of the Moringaceae family and grows close to the Himalayan mountains, from Northwest Pakistan to North India. The deciduous treesgrows swiftly, even on unfavorable soils, is well-suited to dry spells, and may reach a height of up to 15 meters. At chest height, its width is 20 to 40 centimeters.
Here we found the moringa olifera methanolic extract show immense potential as anti-microbial agent against several gram positive as well as gram negative bacteria.

Keywords: Moringa oleifera Lamarck, Moringa pterygosperma Gaertner

Introduction

It creates three-sided dried natural materials that make seed dispersal by wind easier to manage It is distributed around the planet, with populations in the Americas (Brazil, Mexico, Peru, the Caribbean Islands, Afghanistan, Bangladesh, and Sri Lanka) and Africa, West Asia, Afghanistan, Bangladesh, and Sri Lanka. Vegetable tree Moringa oleifera Lam. has several potential applications and is very nutritious and versatile. Numerous regional names, such as benzolive, drumstick tree, kelor, marango, mulangay, nébéday, saijhan, mooringai, and sajna, are used to refer to this sub-exotic animal variety.

The many bioactive combinations may explain the pharmacological characteristics of Moringa Oleifera leaves. These pharmacological characteristics have been confirmed by several in vitro and in vivo tests. While the Moringa Oleifera plant's entire body has healing qualities, the leaves are particularly beneficial to diet, with higher levels of nutrients c and a, potassium, proteins, calcium's, & irons. Expanded leaves contain abundant amounts of amino acids, including cystine, lysine, methionine, and tryptophan, as well as phytochemicals including carotenoids, alkaloids, and flavonoids. MoringaOleifera is use in theconventional therapy of hepatic, cardiovascular, and diabetes mellitus.

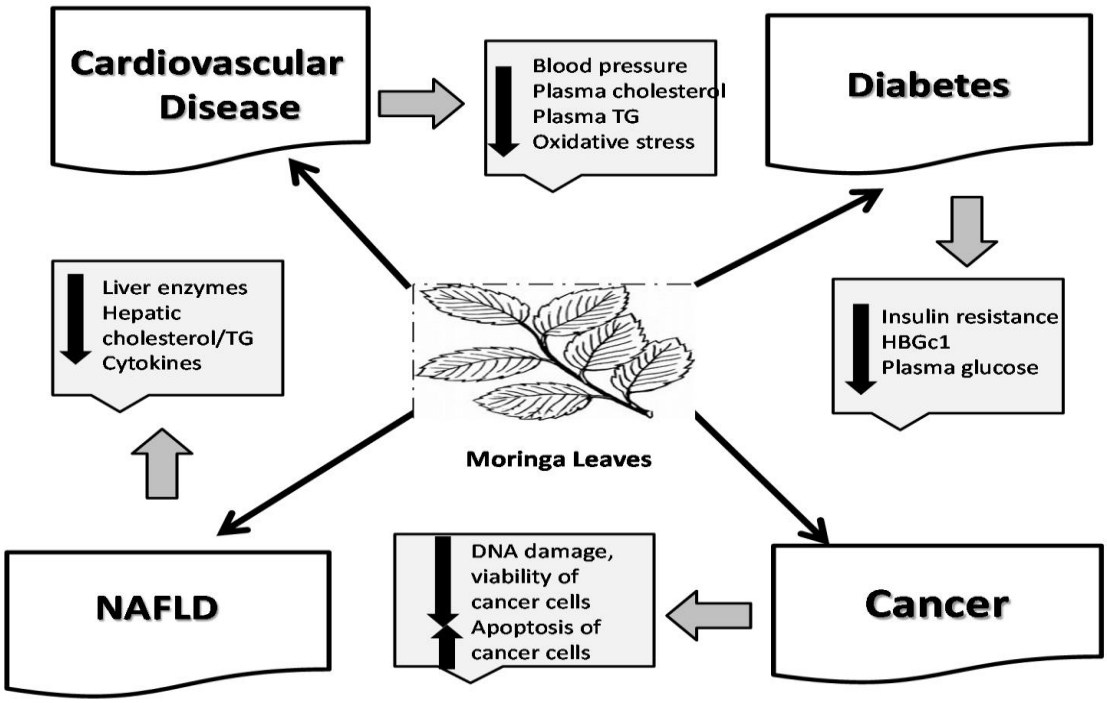

Figure:1

Therapeutic potential of M. oleifera.

The development of generic drugs has, in one way or another, undermined the domestic medical services system; on the other hand, resistance to generic drugs, their high cost, and their unavailability have caused many people to turn back to using natural plants, especially since they are generally considered to be safe.

Any tropical or subtropical nation that has certain natural traits, Moringa oleifera grows best in dry to slightly wet areas with annual precipitation of 760 to 2500 mm (no more than 800 mm), and temperatures between 18 and 28 °C. With a pH between 4.5 and 8, at a height of up to 2000 m, it fills in every type of dirt, no matter how heavy or wet it is. Focusing on the use of Moringa oleifera in neighborhoods and its topographical dispersal throughout Nigeria's major agro-biological district, research clearly established that "despite being widely believed to be a non-native animal species,

Moringa oleifera, is well-known among the diverse ethnic Nigerians who have used it for a variety of uses (e.g., food, medicine, grain, and so on)." At the moment, countries in the Middle East, Africa, and Asia are home to the majority of Moringa oleifera and its progeny, with additional distribution occurring to other regions.

In the 1980s, Ugandan local media played a major role in the development and promotion of Moringa oleifera as a plant purportedly capable of curing a range of ailments, including the aftereffects of Human ImmunodificiencyVirus/Acquired ImmunoDificiency Syndrome. Industrialists bought the seeds and foliage to be used as raw materials, and many families were working on this cutting-edge technology.

As of right moment, ranchers have taken out the plant and are only leaving a few trees surrounding the complex. Even though M. oleifera is native It is now widely dispersed around the world, from the sub-Himalayan regions of Afghanistan, Bangladesh, Pakistan, and India, where it is utilized as medicine. Given its few nourishing, pharmaceutical (Caceres et al., 1991;

1992; Fuglie, 2001), and contemporary uses M. oleifera is referred to as a "wonder tree" or a "supernatural occurrence tree" .It is also claimed that this plant has significant commercial worth. The leaves of this plant have a profile of significant minor components and are an excellent source of minerals, proteins, amino acids, beta-carotine, and other phenolics (Anwar, 2007).Considering all of the advantages that M. oleifera leaves offer, we were perplexed as to why so few people and the media advocate for the use of M. oleifera leaves in a nation where 15% of children under five are sick and 45% are crippled.

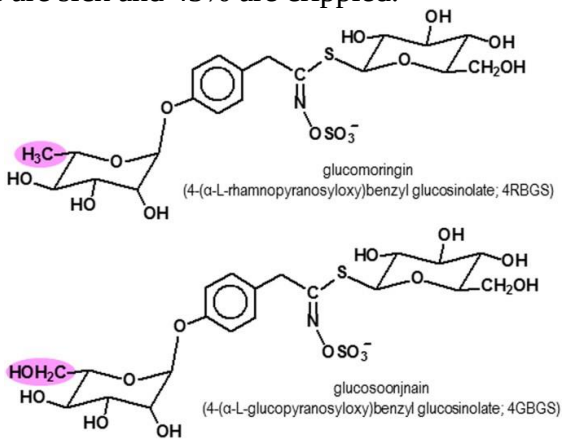

Figure:2 Phytochemical Constituents of moringa olifera.

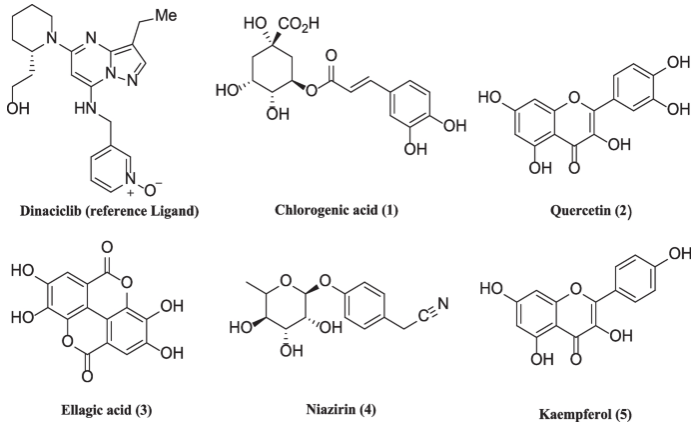

Figure:3 Extracted Phytochemicals chemicals .

Moringa *oleifera* belonging to the family of Moringaceae is an effective remedy for malnutrition. Moringa is rich in nutrition owing to the presence of a variety of essential phytochemicals present in its leaves, pods and seeds. In fact, moringa is said to provide 7 times more vitamin C than oranges, 10 times more vitamin A than carrots, 17 times more calcium than milk, 9 times more protein than yoghurt, 15 times more potassium than bananas and 25 times more iron than spinach [1]. The fact that moringa is easily cultivable makes it a sustainable remedy for malnutrition. Countries like Senegal and Benin treat children with moringa [2]. Children deprived of breast milk tend to show symptoms of malnutrition. Lactogogues are generally prescribed to lactating mothers to augment milk production. The lactogogue, made of phytosterols, acts as a precursor for hormones required for reproductive growth. Moringa is rich in phytosterols like stigmasterol, sitosterol and kampesterol which are precursors for hormones. These compounds increase the estrogen production, which in turn

stimulates the proliferation of the mammary gland ducts to produce milk. It is used to treat malnutrition in children younger than 3 years [3].

About 6 spoonfuls of leaf powder can meet a woman's daily iron and calcium requirements, during pregnancy. This study provides an overview on the cultivation, nutritional values, medicinal properties for commercial use and pharmacological properties of moringa. There are no elaborate reports on treatment of diabetes and cancer using moringa. This study aims to bridge the gap.

Processing of Moringa

Most plants lose their nutritive properties when processed. When compared, the nutritive content of raw, germinated and fermented moringa seed flour, it was found that phytochemicals were higher in raw seed flour and amino acid content was at its peak in fermented and germinated seed flour [17],. This can be a result of the biochemical activities during germination and microbial activity during fermentation. However, a study reviewed the effect of boiling, simmering and blanching to see the retention of nutrient content of moringa leaves. Interestingly, boiling was the most effective of all the techniques as it reduced the cyanide, oxalate and phytate contents, more significantly than the other two methods. The presence of phytate and other anti-nutrients can reduce the bioavailability of certain nutrients and processing can hence be done for maximum utilization of required nutrients from the seeds and leaves [18]. Yang et al. [15] reported that boiling increased the availability of iron and antioxidant content. Hence, the processed moringa seed flour can be used to treat malnutrition problems. However, some studies have shown that children refuse to take in moringa due to its slight bitter taste . designed moringa noodles by three methods of cooking noodles, sautéing, steaming and boiling.

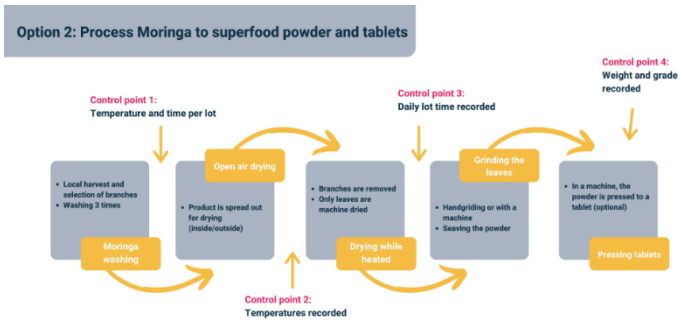

Figure: 4 Processing methods of moringa olifera Linn.

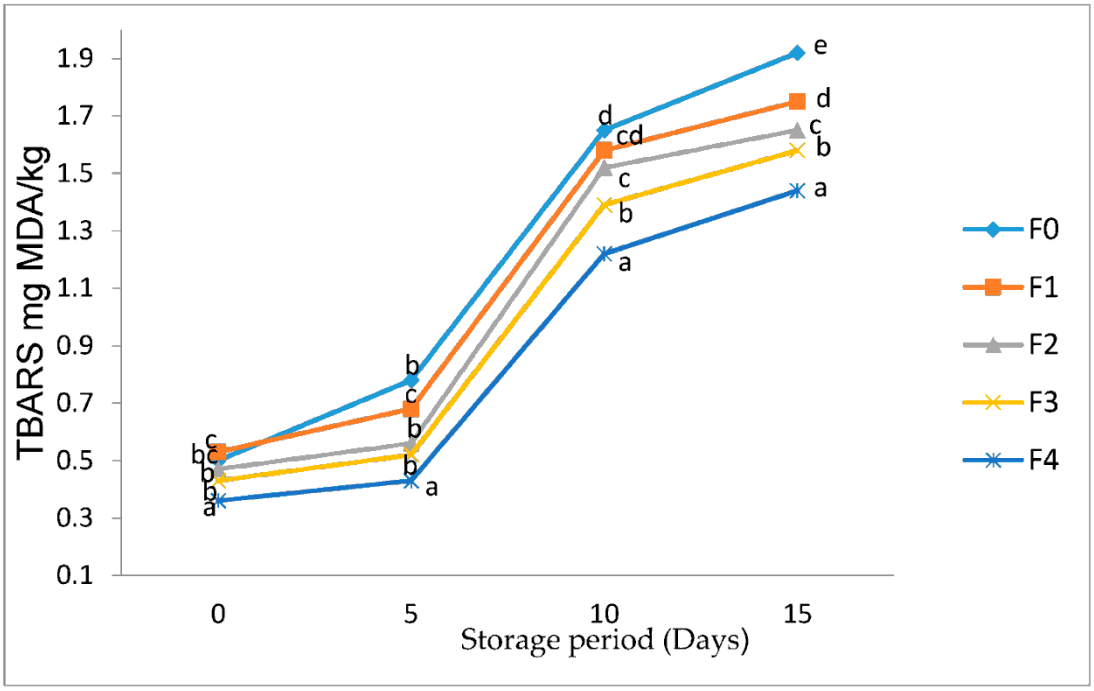

Figure:5 Different absorption of spectra of compounds

Materials and Methods

Reagents and Chemicals

The reagents and chemicals used in this work are methanol and distilled water as solvents for M. oleifera leaf extraction. Folin–Ciocalteu reagents, gallic acid, sodium hydroxide, anhydrous sodium carbonate, quercetin reagents, and anhydrous aluminum chloride are used to analyze the total phenolic and flavonoid contents. Meanwhile, nutrition (Brain Heart Infusion, BD Bacto), artificial seawater (Marine Art SF-1), MTT reagent (Thiazolyl Blue Tetrazolium Bromide), and propan-2-ol are used for assessing the bacterial activity.

Sampling and Identification of Plants

Samples of M. oleifera leaf were obtained from the Botanical Gardan, Noida, Uttar Pradesh.

Preparation of the Extract

The leaves of M. oleifera were carefully cleaned with running water to eliminate soil particles and dust. Then, this M. oleifera leaf was dried under the scorching sun for three days. M. oleifera dried leaf was ground to a size of <60 mesh. 25 g of M. oleifera powdered leaf was added to 150 mL of solvent with ratio concentrations of 100%, 75%, and 50% (v/v) methanol water, respectively. The extraction technique was performed by macerating at room temperature for 3 × 24 hours, and a new solution was replaced every 24 hours. After maceration, filtering is carried out and then concentrated in a rotating evaporator at 50°C. Finally, the M. oleifera leaf extract was refrigerated until further processing. The result of the extraction is determined according to the following formula: mathematical equation (1) where W1 represents the final weight of the M. oleifera leaf extract (concentrated extract) and while W0 represents the initial weight of M. oleifera dried leaf (M. oleifera leaf powder).

Thin Layer Chromatography (TLC) Analysis

The TLC test was observed to analyze the polarity distribution of the active components contained in the M. oleifera leaf extract at various methanol ratios. Identification was performed using silica gel as a stationary phase and various n-hexane to ethyl acetate in a ratio of 1 : 0 to 0 : 1 as a mobile phase. Detection of chromatograms was observed with or without ultraviolet (UV) light. The UV light observations were made at 254 nm and 366 nm wavelengths.

Strains and Cultures of Bacteria

Pseudomonas aeruginosa strain from Indonesian Culture Collection (InaCC) B3 was utilized in the antibacterial research assay. This bacterial strain was obtained from BRIN under the license of InaCC. The oblique bacteria culture was moved to the freshwater brain heart infusion (BHI) broth media and then transferred to the marine BHI broth media as a test material.

Antibacterial Test

The antibacterial activity and minimum inhibitory concentration (MIC) were assessed using the MTT methods [32, 33]. The media was prepared in accordance with the manufacturing instructions. 37 g of BHI were suspended in 1000 mL of distilled water and then mixed, heated, and boiled for 1 minute until fully dissolved. It is then autoclaved at 121°C for about 15 minutes. Furthermore, artificial seawater was prepared with the following manufacturing instructions: 38 g of powders (Marine Art SF-1) were dissolved into 1000 mL of distilled water, followed by heating and mixing. Subsequently, the solution was autoclaved for 15 minutes at 121°C. In summary, an aliquot of 100 µL (BHI-artificial sea water in a ratio of 1 : 1) consisting of different concentrations of the extract was added to each plate of 96 wells. The extract concentration varied from 0 µg/mL and 512 µg/mL up to 6144 µg/mL for this study. Each tube well is filled with a suspension of bacteria cells (2 µL) from a 24-hour culture. Microplates are incubated for 24 hours at room temperature. Furthermore, 10 µL of MTT solution (containing 5 mg/mL) was filled into the tube well and incubated for 1 hour. The well was then filled with 10 µL of MTT solution (5 mg/mL) and incubated for 1 hour. Afterwards, each tube well was filled with 100 µL of propan-2-ol containing 0.04 M of HCl. A microplate reader measured cell suspension absorption at 595 nm (Bio-Rad xMark). All experiments were carried out in three replications. The percentage of inhibition against viable cells was determined using the following equation [30]: mathematical equation (2) where Abst and Absc are cell absorbance treated and cell absorbance control, respectively.

Total Phenolic and Flavonoid Analysis

The total phenolic compounds in the extract were estimated to follow the previously reported [34, 35] using Folin–Ciocalteu methods with gallic acid as reference. The sample solution and the reference solution of gallic acid are each placed into a test tube and then dried into 4 mL by adding aquades, Folin–Ciocalteau 250 µL, and shaken. After 8 minutes, 750 µL of 20% Na_2CO_3 was added and shaken homogeneously. This mixture is then left for 2 hours at room temperature. Absorption was read by using a spectrophotometer at 765 nm. The total flavonoid compounds were analyzed by the colorimetric aluminum chloride method [36] with quercetin as a standard solution. 4 mg of each quercetin was used, and the extracted sample was dissolved in 4 ml of methanol. Pipetting up to 250 µL of sample into the test tube allowed for accurate measurements. 2 ml of aquades and 150 µL of 5% $NaNO_2$ were added to each test tube. 150 µL of 10% $AlCl_3$ was added after 5 minutes. Six minutes later, 2 ml of NaOH 1 M was added, and the volume was calibrated to 5 mL with the addition of aquades. The mixture was homogenized and measured using a UV-Vis spectrophotometer at 510 nm.

Analysis of Structural Compounds

The shape and active compounds in the M. oleifera leaf extract were determined using structural analysis. LC/MS-MS was used to examine the compound structure of the M. oleifera extract (Agilent Technologies 7890). For 17 minutes, structural compounds were measured using LC-MS-MS at 0.3 mL/min of flow rate.

Phytochemical Screening

The extracts of *Moringa oliefera*was subjected to preliminary qualitative phytochemical analysis for the presence of phyto-constituents such as saponin, flavonoids, tannins, alkaloids, glycosides, steroids, carbohydrates and terpenoids using standard laboratory techniques as reported.

Test for Alkaloids: Few drops of Wagner's reagent were added to 2ml of the plant, Formation of orange red precipitate showed the positive test for alkaloid. (Evans, W.C. and Harborne, J.B.).

Test for tannins: about 2ml of the extract will be stirred with 2ml of distilled water and few drops of ferric chloride solution would be added. The formation of a green precipitate would indicate the presence of tannins.

Test for terpenoids: A portion 2ml of organic extract will be dissolved in 2ml chloroform and evaporated to dryness. 2ml of conc. H2SO4will then be added and heated for 2minute. The development of green colour would indicate the presence of terpenoids.

Test for saponins: 5ml of the extract will shake vigorously with 5ml distilled water in a test tube and warmed. The formation of stable foam would indicate the presence of saponins.

Second methods:1ml of pure extract was added to stand for 15minutes and the presence or absence of persistent fronthing indicates the presence of saponins.

Test for flavonoids: 1ml of equeous sodium hydroxide was added to 1ml of the extract. The development of an intense yellow colour indicated the presence of flavonoids.1ml of
hydrochloric acid was added to the solution. reversion to the original colour confirmed the presence of flavonoids.

Test for steroids: salkowski's: 2ml of the extract will be dissolved 2ml chloroform with 2ml of concentratied sulphuric acid. Red colous formed in the lower chlorofoamlarye indicate rhe present of steroid.

Test For Carbohydrates: 0.2 g of the extracts were dissolved each in 5ml of methanol;2ml of the resulting solution were taken into a test tube and 2 drops of molisch`s reagent were added to the solution, followed by addition of 2ml of concentrated sulphuric acid. The mixture was then allowed to stand for 3minutes, formation of a red/dull-violet color at the interface of the two layers indicated the presence of carbohydrate.

Antibacterial assay by agar well diffusion method in Nutrient plate

Agar well diffusion method was used to study the antibacterial activity of phytochemical screening of *moringa oliefera*. The test organisms were inoculated in Nutrient broth and incubated overnight at 37oC to adjust the turbidity to 0.5 McFarland standards giving a final inoculum 1.5×108 CFU/ml. Nutrient plate was lawn cultured with standardized microbial culture broth. Plant extract of 50 mg/ml concentration were prepared in Dimethyl sulfoxide (DMSO). Five wells of 6mm were bored in the inoculated media with the help of sterile cork- borer (6mm). Each of well was filled with 50ul extract. Positive control (ceftriaxone 30mcg) and 300mcs nitrofurantoin for bacteria and 1mg/ml of cyclohexylamine for fungal isolates and negative/solvent control (DMSO), respectively. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37oC.After the incubation, plates were observed for the formation of a clear zone around the well which corresponds to antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm [3,12,19].

1. **Result of the aqueous extract of *Moringa oliefera*leaves**

Table 1: Characteristics of the plant extract

Solvent	**Colour of extracts**	**Weight of extract**	**Percentage yield**
Methanol	Dark green	2.18	10.9%
Distilled water	Dark green	1.53	7.65%

Result of the phytochemical screening of the aqueous extract of *Moringa oliefera*leave

Table 2: Phytochemical results of the plant *Moringa oliefera*Methanol as a solvent

S/N	**Phytochemical**	**Test**	**A.E Result**
1	Alkaloids	Wagner test	+
2	Tannin	Ferric chloride test	+
3	Terpenoid	H2SO4 test	_
4	Saponins	Distilled water test	+
5	Flavonoids	NaOH test	+
6	Steroids	Salkowski's test	_
7	Carbohydrate	Molisch's test	+

Phytochemical results of the plant *Moringa oliefera*distilled water as a solvent

S/N	**Phytochemical**	**Test**	**A.E Result**
1	Alkaloids	Wagner test	+
2	Tannins	Ferric chloride test	+
3	Terpenoids	H2SO4 test	_
4	Saponins	Distilled water test	_
5	Flavonoids	NaOH test	+
6	Steroids	Salkowski's test	_
7	Carbohydrate	Molisch's test	+

Table 3: Phytochemical results of the plant *Moringa oliefera*distilled water as a solvent

Antimicrobial screening of leaf extract of *Moringa oleifera* in terms of average zone of inhibition

Table 4: Result of Antimicrobial screening of *Moringa oliefera*Methanol Extract

Micro-organism		**Zone of inhibition(mm)**
1	Escherichia coli	20mm
2	Salmonella typhi	10mm
3	Pseudomonas aeruginosa	5mm

2. **Result of Antimicrobial screening of Moringa oliefera Distilled Water Extract**

Micro-organism		**Zone of inhibition(mm)**
1	Escherichia coli	10mm
2	Salmonella typhi	15mm

3	Pseudomonas aeruginosa	15mm

Table 5: Result of Antimicrobial screening of *Moringa oliefera*Distilled Water Extract

6: Absorption Frequencies of FT/IR Result Obtained from methanol extract of Moringa oleifera whole leaf

S/No	Absorption frequency (cm-1)	Functional groups	Remark assignment
1	3632.3	O-H group (Alcohol).	OH- Stretching, H- bonded.
2	3337.8	O-H group (Alcohol).	OH- Stretching
3	2929.7	-CH3 (Alkane)	C-H Stretching
4	2830.9	=C-H (Aldehyde)	C=O Stretching
5	2571.9	-CO-OH (Carboxylic acid)	Unknown
6	2432.1	CH3 (Alkyl group)	C-H Stretching
7	1707.1	C=O (Ketone or carboxylic acid)	C=O stretching
8	1541.3	C=C (Aromatic group)	C=C Stretching
9	1507.7	C=C (Aromatic group)	C=C Stretching
10	1448.1	C=C (Aromatic group)	C=C Stretching
11	1420.1	C=C (Aromatic group)	C=C Stretching
12	1364.2	-CH3 (Trimethyl)	C=C Bending
13	1228.2	Ar-OH (Acids)	C-O Stretching
14	1164.8	CH2 (Methylene group)	CH2 Wagging
15	1094.0	C=C-CR^1R^1-OH	C=O Stretching
16	1023.2	C-O (Ester)	Unknown
17	672.8	Cis RCH=CHR^1	Unknown

Table 6: Absorption Frequencies of FT/IR Result Obtained from methanol extract of Moringa oleifera whole leaf

The infrared region is useful in functional group identification of the active components present in extract via the peaks FT/IR values. When the extract was passed into the FT/IR, the functional groups of the components were separated based on its peaks ratio. The FT/IR spectroscopic studies revealed the presences of alcohol, alkanes, aldehydes, carboxylic acid, aromatics and esters were observed from methanol extract of Moringa oleifera whole leaf. The peaks therefore, between 3500-3200 cm-1, 3500- 3100 cm-1, 2750-250 cm-1 and the top at 900 – 675 cm-1 are diagnostic marker for the presence of OH, NH, C=O and C=C functions respectively. The C-H

stretch associated with H-C=O usually differed a little in frequency because of the inductive effect of oxygen attached to the carbon atom and hydrogen atom, thereby making it weaker. The broad band, therefore, in this region of the spectrum is diagnostic of the presence of OH group. The results of IR analysis (1541.3- 420.1 cm-1) also reveal that, the components of Moringa oleifera leaf could be aliphatic or aromatic. It may therefore be inferred that aromatic or aliphatic alcohols or phenols, amine, ketones, esters and some nitrogen containing compounds are some of the constituents of the extract of Moringa oleifera whole leaf. The characterization of Methanoic extract of Moringa oleifera leaf reveals the presence of C=O, C-O, C=C, -CH3 –CO-OH etc. band stretching, suggesting that components of Moringa oleifera leaf may be aromatic or aliphatic. This is also in agreement with the opinion of [25-29] and [30], who conducted a fluorescence spectroscopic study of a coagulating protein extracted from Moringa oleifera seeds.

Gas Chromatography-Mass Spectroscopy analysis

The components present in the methanol extract of Moringa oleifera whole leaf were identified by GC-MS analysis. The GC-MS chromatogram with peak area is given in Figure 3. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and Area (%) are presented in Table 3. Twenty major compounds were detected in methanol extract of Moringa oleifera whole leaf. Compounds 6 and 10 were the same (Pentetetic acid) as predicted by comparing with the standard mass spectral databases. However, they varied in their respective retention time, area and area percentage. The results revealed that, N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid and 2-Myristynoyl pantetheine (100.0%) was found as major component followed by 2- Myristynoyl pantetheine and Deoxyspergualin (92.05%), 5-Octadecenal and 9-Hexadecenoic acid (27.24%) , N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid and Pentetic Acid (26.29%). N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid and 2,6-Bis[2-[2-S thiosulfuroethylamino] ethoxy] pyrazine (8.70%), Acetamide,N-methyl-N-[4-[4-fluoro-1- hexahydropyridyl]-2-butynyl]- and N-Methoxy-1-ribofuranosyl-4-imidazolecarboxylic amide (6.39%), 3-Dodecen-1-ol (6.44%). Glucobrassicin and Pentetic Acid (5.64%), N,N'- Pentamethylenebis[s-3-aminopropyl thiosulfuric acid and 2-Myristynoyl pantetheine (5.37%), 5α-androstane-3,17-dione 17-monooxime and Pyrrolo[3,2k]anthracene-4,6-diol, 3-methoxy- 4b,5,6,7,8,9,10,11,11a,12-decahydro-11-methyl-(0.11%) and Morphinan-3-ol-6-one, 4- methoxy and 5α-androstane-3,17-dione 17-monooxime (0.04%). Most of the compounds identified in the methanol extract of Moringa oleifera whole leaf seem to possess medicinal properties and some of them are commonly present in many medicinal plants. The presence of Pentetic acid, 5-Octadecenal, Glucobrassicin, tetrapentacontane, 2-propenoic acid, pentadecyl ester, 3,4- dihydroxymandelic acid has acted as a various therapeutic and pharmaceutical benefits such as hydroxylation of liver, enzymes antipyretic analgesic, ant rheumatism and antimicrobial activity during phase I metabolism, hair growth promoter,inhibit production of uric acid and arachidonic acid inhibitor in human body respectively.

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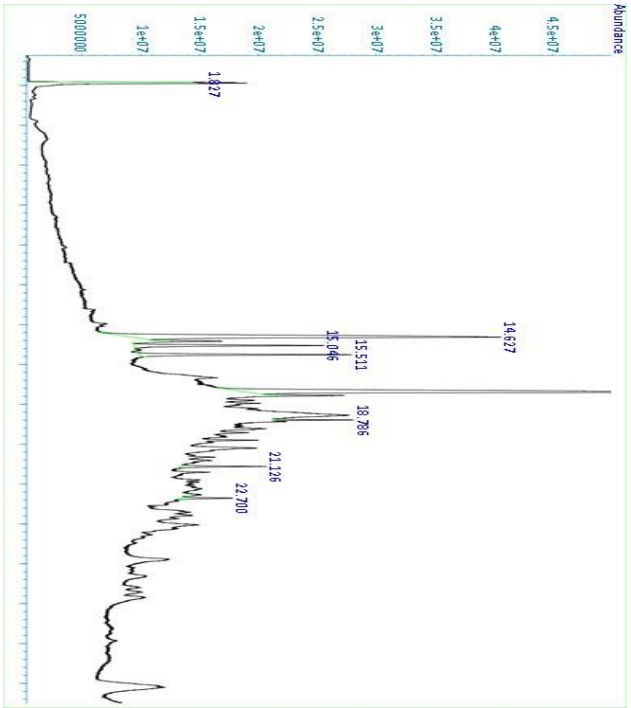
S/No	Retention Time (mins)	Peak Area (%)	Molecular Weight	Name of compound	Molecular Formular
1	0.622	0.11	303	5α-androstane-3,17-dione 17-monooxime	C19H29NO2
			303	Pyrrolo[3,2-k]anthracene- 4,6-diol, 3-methoxy-4b,5,6,7,8,9,10,11,11a,12-decahydro-11-methyl-	C18H25NO3
2	1.210	0.04	287	Morphinan-3-ol-6-one, 4-methoxy-	C17H21NO3
			303	5α-androstane-3,17-dione 17-monooxime	C19H29NO2
3	1.827	6.39	226	Acetamide, N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-2-butynyl]-	C12H19FN2O
			273	N-Methoxy-1-ribofuranosyl-4-imidazolecarboxylic amide	C10H15N3O6
4	14.627	92.05	484	2-Myristynoyl pantetheine	C25H44N2O5S
			387	Deoxyspergualin	C17H37N7O3
5	15.046	27.24	266	5-Octadecenal	C18H34O
			254	9-Hexadecenoic acid	C16H30O2
6	15.511	26.29	410	N,N'-Pentamethylenebis[s-3-aminopropyl thiosulfuric acid	C11H26N2O6S4

Table 7: Phytocomponents identified in the plant Methanolic extracts of Moringa oliefera whole leaf by GC-MS.



Discussion

The yield of methanol and distilled water were 10.9% and 7.65% respectively. Methanol yielded more extract from *Moringa oliefera*, probably due to the chemical component present in the plant. Phytochemical screening results of aqueous extract showed that *Moringa oliefera*contains the presence of alkaloids, tannins, saponins, flavonoid and carbohydrate. but terpenoids and steroid were absent by using methanol as a solvent. And contain the present of alkaloids, tannins and flavonoid, but terpenoids, saponins and steroid were absent by using distilled water as a solvent. Therefore methanol is the best solvent for the extraction [28] of these phytochemical from *Moringa oliefera.* The presence of these diverse secondary metabolites in the plant justifies its use in folklore medicine. Table 4 and 5 showed the antimicrobial sensitivity test of *Moringa oleifera* leaf extract against species of bacteria that the extracts exhibited Antimicrobial activity against Escherichia coli, salmonella typhi (bacteria), and *pseudomonas aeruginosa*, it can be seen that extracts possessed a broad range of activity with the highest zone of 20 mm with Methanol extract, methanol extract showed with E. Coli, 20mm, salmonella typhi 10 mm, and *pseudomonas aeruginosa* 5 mm. The distilled water extract has its zone of inhibition on all the organisms with salmonella tiphy and *pseudomonas aeruginosa* having the highest zone of 15 mm, followed by *Escherichia coli* have the least zone of inhibition 10 mm, This indicates that the activity of the extract is influenced by the solvent used for the extraction [29,30]. Escherichia coli is the pathogenic organism responsible for the intestinal disorder, Salmonella tiphy is the causative organism for typhoid fever, food poisoning, pneumonia, toxic shock syndrome and scalded skin while *Escherichia coli* is responsible for Zygomycosis, allergis, otomycosis and pseudomonas aeruginosa acusescandidasis, vaginitis and thrush. This proves that *Moringa oleifera* leaves could be used in the treatment of infection caused by such pathogens as aureus E. coli, salmonella tiphy,and pseudomonas aeruginosa. The phytochemical screening of Moringa oleifera methanol leaf extract revealed the presence of several bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and phenolics. These compounds are known for their various therapeutic properties. Alkaloids, for instance, are well-documented for their antimicrobial, analgesic, and anti-inflammatory activities. Flavonoids possess strong antioxidant properties, which help in combating oxidative stress and may contribute to the antimicrobial efficacy observed. Tannins are known to inhibit the growth of microorganisms by precipitating microbial proteins, while saponins can enhance immune responses and exhibit antimicrobial properties by interacting with microbial cell membranes. The presence of these phytochemicals suggests that Moringa oleifera has significant potential as a source of natural antimicrobial agents. The combination of these compounds may result in a synergistic effect, enhancing the overall antimicrobial activity of the methanol leaf extract. The antimicrobial activity assay demonstrated that the methanol leaf extract of Moringa oleifera exhibited significant inhibitory effects against a range of bacterial and fungal pathogens. The extract showed varying degrees of inhibition, with the highest activity observed against Gram-positive bacteria, such as Staphylococcus aureus, and moderate activity against Gram-negative bacteria, including Escherichia coli and Pseudomonas aeruginosa. Additionally, the extract was effective against Candida albicans, a common fungal pathogen. The observed antimicrobial activity can be attributed to the high content of bioactive phytochemicals identified in the extract. The effectiveness against both bacterial and fungal pathogens highlights the broad-spectrum antimicrobial potential of Moringa oleifera. The higher susceptibility of Gram-positive bacteria compared to Gram-negative bacteria may be due to the differences in their cell wall structures.

Gram-negative bacteria possess an outer membrane that acts as an additional barrier to antimicrobial agents, which could explain their relatively lower susceptibility

Implications and Future Research

The results of this study suggest that Moringa oleifera methanol leaf extract has potential as a natural antimicrobial agent. This could be particularly beneficial in the development of alternative treatments for infections, especially in the context of rising antibiotic resistance. The use of plant-based antimicrobials offers a promising avenue for new therapeutic agents with potentially fewer side effects compared to synthetic antibiotics.

Future research should focus on the isolation and characterization of individual bioactive compounds within the extract to identify the specific constituents responsible for the antimicrobial activity. Additionally, studies on the mode of action of these compounds will provide deeper insights into how they exert their effects on microbial pathogens. Exploring the synergistic effects of combined phytochemicals could also enhance the efficacy and spectrum of antimicrobial activity.

Conclusion

The phytochemical analysis and antimicrobial activity assessment of Moringa oleifera methanol leaf extract underscore its potential as a source of natural antimicrobial agents. The presence of diverse bioactive compounds and their demonstrated efficacy against various pathogens highlight the importance of further investigation into this plant's medicinal properties. Moringa oleifera holds promise for the development of novel antimicrobial therapies, contributing to the ongoing search for effective and sustainable solutions to combat microbial infections.

REFERENCES:

1. Ramachandran C, Peter KV, Gopalakrishnan PK.Drumstick(*Moringa oleifera*): a multipurpose Indian vegetable. Econ Bot. 1980; 34(3):276-83.

2. Patel,J.P., Bharat, G and Patel, K. (2010). Evaluation of invitroSchizonticidal Properties of Acetone Extract of some Indian Medicinal Plants. *Advances in Biological Research* 4 (5): 253-258, 2010. ISSN 1992-0067. IDOSI Publications.

3. Leone, A.; Spada, A.; Battezzati, A.; Schiraldi, A.; Aristil, J.; Bertoli, S. Cultivation, genetic,ethnopharmacology, phytochemistry and pharmacology of Moringa oleifera Leaves: An overview. Int. J. Mol.Sci. **2015**, 16, 12791–12835.

4. Sreelatha S, Jeyachitra A, Padma PR: Antiproliferation and induction of apoptosis by Moringa oleifera leaf extract on human cancer cells.Food Chem Toxicol 2011, 49:1270–1275.

5. Cajuday LA, Pocsidio GL: Effects of Moringa oleifera Lam. (Moringaceae)on the reproduction of male mice (Mus musculus). J Med Plants Res 2010,4:1115–1121.Siddhuraju P, Becker K: Antioxidant properties of various solvent extractsof total phenolic constituents from three different agroclimatic origins ofdrumstick tree (Moringa oleifera Lam.) leaves. J Agric Food Chem 2003,51:2144–2155.

6. Lam K.S. (2007). New aspects of natural products in drug discovery. *Trends Microbiology*,15: 279–289.

7. Lee C.P. (2006). Who's in the business of saving lives? *Journal of Medicine and Philosophy*, 31: 465–482.
8. Nouman, W.; Basra, S.M.A.; Siddiqui, M.T.; Yasmeen, A.; Gull, T.; Alcayde, M.A.C. Potential of *Moringa oleifera* L. as livestock fodder crop: A review. *Turk. J. Agric. For.* **2014**, *38*, 1–14.
9. Popoola, J.O.; Obembe, O.O. Local knowledge, use pattern and geographical distribution of*Moringa oleifera* Lam. (Moringaceae) in Nigeria. *J. Ethnopharmacol.* **2013**, *150*, 682–691.
10. Fahey J (2005). A review of the medical evidence for its nutritional, therapeutic and prophylactic properties. Trees life .
11. J.L. Rockwood, B.G. Anderson, D. a. Casamatta, Potential uses of Moringa oleifera and an examination of antibiotic efficacy conferred by M. oleifera seed and leaf extracts using crude extraction techniques available to underserved indigenous populations, Int. J. Phytothearpy Res. 3 (2013) 61–71.
12. J.N. Kasolo, G.S. Bimenya, L. Ojok, J. Ochieng, J.W. Ogwal-okeng, Phytochemicals and uses of Moringa oleifera leaves in Ugandan rural communities, J. Med. Plants Res. 4 (2010) 753–757.
13. Mutiara Titi, T. Estiasih, E.S. W, Effect Lactagogue Moringa Leaves (Moringa oleifera Lam) Powder in Rats, J. Basic. Appl. Sci. Res., 3 (2013) 430–434.
14. 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) 1–13.
15. 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) 417–421.
16. W.J. Asante, I.L. Nasare, D. Tom-Dery, K. Ochire-Boadu, K.B. Kentil, Nutrient composition of Moringa oleifera leaves from two agro ecological zones in Ghana, African J. Plant. 8 (2014) 65–71.
17. 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) 67-72.