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Phytochemical Analysis and Antimicrobial Activities of Fresh and Powdered form of Leaves, Stem and Bark of *Moringa oleifera*

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Abstract: *Moringa oleifera*, a plant with medicinal property is analyzed for its phytochemical constituents present in leaves, stem and bark in fresh and powdered form. Different solvents such as ethanol, hot water and cold water were used for making the extract of all three plant parts (leaves, stem and bark). The fresh plant parts like leaves, stem and bark show presence of maximum secondary metabolites in cold and hot water in comparison to extract in ethanol. The powdered form of leaves, stem and bark shows presence of steroids, flavanoids, volatile oils etc. in ethanolic as well as in hot and cold water extract. Antimicrobial activity was observed against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans* and *Candida albicans* using powdered extract in all the three solvents by agar well diffusion method using pure ethanol as control. The ethanolic extract of the bark showed a broad spectrum of antimicrobial activities against all test organisms with highest zone of inhibition (10 mm) to *E. coli*. Hot water extract of leaves showed inhibition against *P. aeruginosa* (10 mm). The ethanolic leaf extracts showed an inhibition of 4 mm and 9 mm *to S. aureus* and *C. albicans*, respectively. In cold water, leaves extract was effective against *P. aeruginosa* and *C. albicans* (6 mm and 3 mm, respectively).The stem extract in ethanol showed antimicrobial activities against *C. albicans* (8 mm) only while in hot water showed antimicrobial activities against *P. aeruginosa* and *S. mutans* (4 mm and 2 mm, respectively) and in cold water effective against *S. aureus* only. *Staphylococcus aureus* was found to be the most sensitive test organism to different extracts of *Moringa oleifera*. It may be concluded that *M. oleifera* may be a potential source for the treatment of different infections caused by the resistant microbes.

Keywords: Phytochemicals, Antimicrobial, Agar well diffusion, Inhibition, *Moringa oleifera*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Candida albicans*

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Introduction

From ancient times, plants have served as medicine for disease treatment. The broad diversity of structural biochemicals of the plant kingdom counts bioactive phytochemicals with medicinal properties antimicrobial resistance has emerged as a global health problem, thus effective and affordable alternative medicines making is especially important in the developing world. Some bacterial species such as *Staphylococcus aureus* and *Streptococcus mutans,* can cause respiratory and skin infections together with *Pseudomonas aeruginosa* and certain elusive bacterial species of Enterobacteriaceae can trigger gastrointestinal, neurological and urogenital infections in addition to wound contamination. These bacteria have been shown to be universally resistant to conventional antibiotics highlighting the urgent need to scientifically evaluate and promote the antimicrobial potential of plant medicines against the multi-drug resistant pathogens and microbes with the potency of contaminating some types of beers like pito such as *Staphylococcus aureus*, *E. coli*, *Shigella spp*, *Salmonella spp*, and lactic acid bacteria (Devi *et al*., 2020; Owusu-Ansah *et al*., 2023). Despite the large number of under-explored plants and plant parts, little effort has been put to use modern scientific tools to validate traditional medicine. Intensive research into therapeutic approaches, as such clinical efficacy, and safety profile of herbal medicines is an important prerequisite for fully exploiting the vast reservoir of plant bioactive compounds as drug targets for antimicrobial agents instead of a failed infection.

Moringa oleifera also known as miracle plant is a native plant species belonging to Moringaceae famiy found in Northern India, Southwest parts of Asia, Southwest and Northwest Africa, and many other parts of the world including the Pacific and Caribbean Island. *Moringa oleifera* is used for its nutritional, phytochemical and pharmacological properties (Rani *et al*., 2018; Arora and Arora, 2021; Hassan *et al*., 2021; Chhikara *et al*., 2021). It is considered a very versatile plant due to its great capacity to provide edible food, which includes different vegetative structures, such as leaves, pod shells, stem, flowers, fruits and seeds which are rich in bioactive compounds providing health benefits (Sagona *et al*., 2020; Okechukwu *et al*., 2021; Giuberti *et al*., 2021). The plant can be used for functional food and other industrial food applications (Oyeyinka and Oyeyinka, 2018; Hodas *et al*., 2021). The nutritional level of *Moringa* has all the valuable amino acids, vitamins, and minerals even in greater amounts compared to other foods and are utilized by both human and animal food, and also has important applications in agriculture, biotechnological industries, and medicine (Anzano *et al*., 2021; Akter *et al*., 2021; Ercan *et al*., 2021).

The main contributions made by *Moringa* in terms of macro and micronutrients are found in the leaves. This is the part of the *Moringa* most used due to its high protein value. They are also rich in antioxidant components, among which the isothiocyanates stand out as one of the main carriers of anti-carcinogenic and antibiotic properties (Fidrianny *et al*., 2021; Islam *et al*., 2021). The leaves are used as a source of vitamins A and C. They are also good sources of vitamin B and are also sources of minerals. More than 12 flavonoids including quercetin, kaempherol-glucoside of thiocarbamate and lass, moringyne, pterygospermin, niaziridin, 4-((α-L- rhamno-pyranosyloxy) benzyl) isothiocyanate, 4-O-(α-L-rhamnopyranosyloxy)-benzyl glucosinolate and glucoside malonates have been reported in this plant (Rani *et al*., 2018). Among these, quercetin and kaempherol are used to treat cancers. The plant also shows high degree of nutritional properties as well as medicinal properties (Sánchez *et al*., 2006; Oduro *et al*., 2008; Penalver

et al., 2022). *M. oleifera* contains significant amount of alkaloids, flavonoids, glycosides, saponins, tannins, steroids in different parts which contributes to both nutritive and medicinal value of this plant. The aim of this study was to evaluate the phytochemical composition of different parts of the *M. oleifera* and carry out an antimicrobial analysis of the crude extracts of different parts using different solvents.

Materials and Methods

Collection of plant material and preparation of plant extracts:

The fresh leaves, stem and the bark of *Moringa oleifera* used in this study were collected from the campus of Maharishi Markandeshwar Deemed to be University, Mullana, India. The collected plant parts were taken to the Department of Biosciences and Technology Microbiology Laboratory. Fresh materials of the plant samples were cleaned washed under running tap water and each sample was split into two halves, one to be used as fresh sample and the other half dried and homogenized into fine powdered form. Each sample was prepared and extracted with ethanol, hot water and cold distilled water. Fresh extracts were prepared by grinding fresh leaves, stem and bark into fine particles by the help of a pestle and mortar. Five gram of each sample was mixed with 100 ml ethanol, hot water and cold distilled water in separate flasks and shaken vigorously for 5 min, filtered using Whatman filter paper No. 1 and then centrifuged at 10,000 rpm for 10 min at room temperature. The supernatant was collected and stored at 4°C for further analysis.

Powdered extract of the leaves, stem and bark was soaked in a flask containing 50 ml of each solvent (ethanol, hot water and cold distilled water) and kept in a rotary shaker set at 200 rpm at 30°C for 48 h. The extract was then filtered and the filtrate was centrifuged at 10,000 rpm for 10 min at room temperature and the supernatant was stored at 4°C for further analysis.

Phytochemical analysis:

A qualitative analysis screening of the phytochemical constituents of *Moringa oleifera* from the prepared extracts was done to assess the presence of Alkaloids, Flavonoids, Saponins, Steroids, Tannins, Glycosides, Reducing sugars and volatile oils (Padayachee and Baijnath, 2020). The following procedure was employed in this study:

(i) *Alkaloids*: Baljet's reagent was prepared by adding 95ml of 1% picric acid to 5ml of 10% NaOH. A drop of Baljet's reagent was added to 0.1 ml of each extract, a yellow-orange colour solution indicates the presence of alkaloids.

(ii) *Flavonoids*: 3 ml of distilled water was mixed with 0.1 ml of the extract and shaken and few drops of 10% NaOH was added to the mixture. Yellow colour is an indicator for the presence of flavonoids.

(iii) *Saponins*: 0.1 ml of extract was diluted with 1 ml of distilled water. The test tube is shaken vigorously for about 30 sec. Honeycomb froth indicates the presence of saponins.

(iv) *Steroids*: 5 drops of conc. H_2SO_4 was added to 1 ml of each extract. A reddish-brown colour confirms the presence of steroids.

(v) *Tannins*: 0.1 ml of extract was boiled gently for 2 min and allowed to cool and 2 drops of Ferric Chloride solution was added. The formation of a blue or green colour indicates the presence of tannins.

(vi) *Glycosides*: 2.5 ml of dilute H_2SO_4 was added to 0.1 ml extracted and boiled for 15 min, cooled and neutralized with 10% NaOH, and then 1 ml Fehling's solution was added. The blue coloured solution indicated the presence of glycosides.

(vii) *Reducing sugars*: 1 ml of distilled water and 5-8 drops of Fehling's solution (A and B) was added to 0.5 ml extract and heated over water bath. A brick red precipitate indicates the presence of reducing sugars.

(viii) *Volatile oils*: 0.1 ml extract was shaken with 0.1 ml dilute NaOH and a small quantity of dilute HCl, a layer of oil shows the presence of volatile oils.

Test microorganisms:

This study utilized two gram-negative bacterial cultures (*Escherichia coli* and *Pseudomonas aeruginosa*), two gram-positive bacterial cultures (*Streptococcus mutans* and *Staphylococcus aureus*) and one yeast culture of *Candida albicans* which were obtained from the Department of Microbiology Laboratory, MM Institute of Medical Sciences. The isolates were cultured on selective media and identified by gram staining procedures (for bacterial cultures), colony examination and biochemical analysis. The cultures were maintained in broth media at 37 °C for the analysis of the antimicrobial activities of the extracts.

Test for Antimicrobial activity:

The agar-well diffusion method was used to determine the antimicrobial activities of *M. oleifera* extracts in nutrient agar media. 25 ml of autoclaved and sterile media was poured in 6 different petri plates and allowed to solidify. 0.1 ml culture of selected microorganisms was inoculated by glass spreader on the surface of the agar and allowed to dry for maximum absorption of the liquid culture onto the agar media, and 6 mm wells were punched in the agar using a sterile borer. 25 µl of plant extracts in different solvents along with positive and negative controls were loaded in the wells. The plates were incubated at 37°C for 24 h and the zone of inhibition was measured.

Results and Discussion

Phytochemical analysis:

The phytochemical profile of ethanolic, hot water and cold distilled water extracts of *Moringa oleifera* from different plant parts (leaves, stem and bark) is shown in Table 1, which reflects the analysis of the presence of eight phytocompo-nents.

The ethanolic extract, fresh plant parts show the presence of alkaloids and glycosides as fresh leaves shows presence of flavonoids and stem and bark shows the presence of volatile oils while saponins are present in ethanolic extract of fresh bark. Similar results were also reported in *M. oleifera* (Shu *et al*., 2020). Meanwhile, in the ethanolic powdered leaves extract alkaloids, flavonoids, saponins, glycosides, reducing sugars and volatile oils were present. In powdered stem, alkaloids, saponins, tannins, glycosides and volatile oils showed their presence whereas in powdered bark, steroids were presence in addition to alkaloids, flavonoids and volatile oils (Fig. 1). Hot water extracts from fresh leaves show the presence of alkaloids, flavonoids, saponins, tannins and reducing sugars. The fresh stem and bark reveal almost similar results with the presence of alkaloids, flavonoids, glycosides and volatile oils with bark showing saponins unlike the stem. The powdered extracts in the hot water reveals the presence of flavonoids and volatile oils in the leaves, stem and bark. Alkaloids was present in stem and bark, saponins in leaves and bark, steroids in leaves and stem whereas glycosides showed their presence in stem and bark (Fig. 2). In cold distilled water, fresh leaves, stem and bark showed the presence of alkaloids, flavonoids, saponins, glycosides and volatile oils with leaves showing the presence of tannins and stem reducing sugars in addition (Imohiosen *et al*., 2014). Powdered extract in cold distilled water reveals the presence of alkaloids and tannins in all plant parts as glycosides and saponins are present in leaves and stem. Flavonoids and steroids are present in powdered leaves and bark, reducing sugars present in leaves and volatile oils is present in both stem and bark.

Antimicrobial activities:

The antimicrobial analysis was performed on five test microorganisms, two gram negative bacterial cultures (*Escherichia coli* and *Pseudomonas aeruginosa*), two gram positive bacterial cultures (*Streptococcus mutans* and *Staphylococcus aureus*) and one yeast culture of *Candida albicans* (Table 2).

In this study, the ethanolic extract of the bark showed a broad spectrum of antimicrobial activities against all test organisms with highest zone of inhibition (10 mm) for *E. coli*. Ethanolic

Table 1: Phytochemical screening of fresh and powdered form of leaves, stem and bark extracts of *M. oleifera* in three selected solvents (ethanolic, hot and cold distilled water)

	Ethanolic Extract						**Hot water Extract**						**Cold Distilled Water Extract**					
	Fresh Extract			**Powdered Extract**			**Fresh Extract**			**Powdered Extract**			**Fresh Extract**			**Powdered Extract**		
Plant Constituent	**Leaves**	**Stem**	**Bark**	**Leaves**	**Stem**	**Bark**	**Leaves**	**Stem**	**Bark**	**Leaves**	**Stem**	**Bark**	**Leaves**	**Stem**	**Bark**	**Leaves**	**Stem**	**Bark**
Alkaloids	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
Flavonoids	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+
Saponins	-	-	+	+	+	-	+	-	+	+	-	+	+	+	+	+	+	-
Steroids	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	+
Tannins	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-	+	+	+
Glycosides	+	+	+	+	+	-	-	+	+	-	+	+	+	+	+	+	+	-
Reducing sugars	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-
Volatile Oils	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+

(+) indicates the presence and (-) indicates the absence

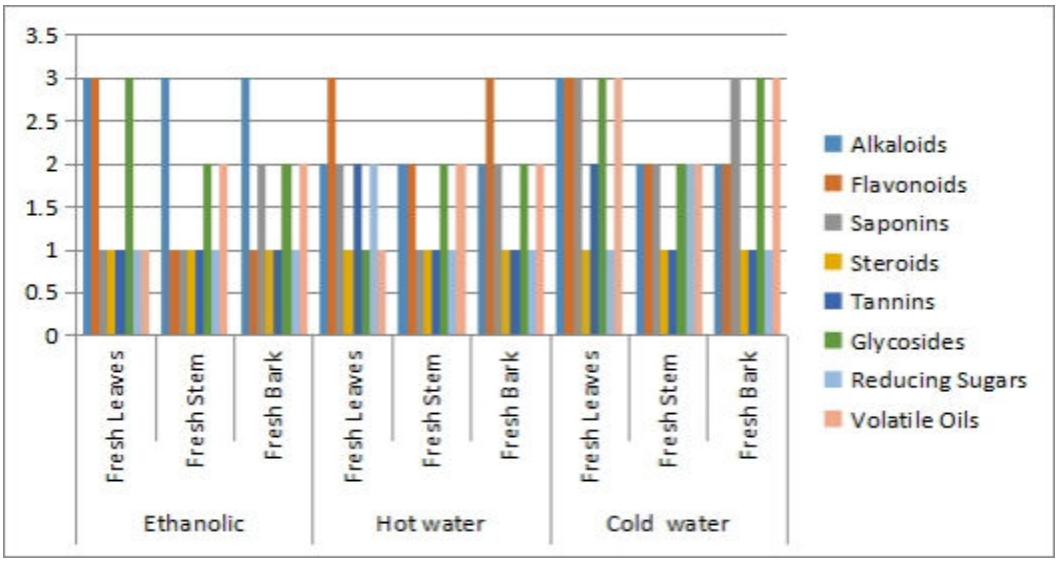

Fig. 1: Phytochemical quantity present in fresh leaves, stem and bark in three different solvents (ethanolic, hot water and cold distilled water).

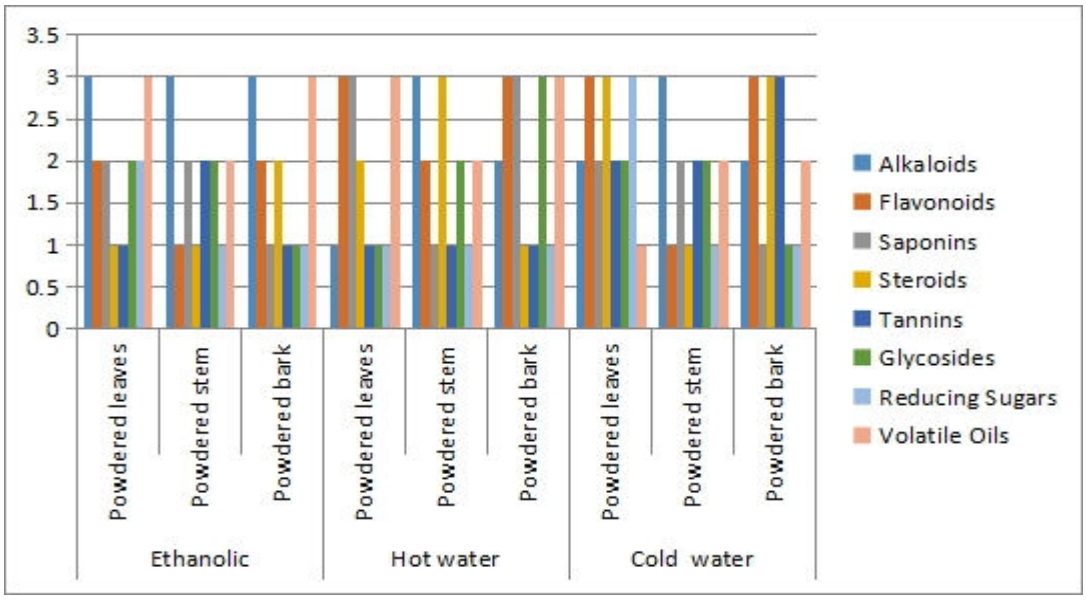

Fig. 2: Phytochemical quantity present in powdered leaves, stem and bark in three different solvents (ethanolic, hot and cold distilled water).

leaf extracts showed an inhibition of 4 mm and 9 mm for *S. aureus* and *C. albicans*, respectively. The stem extract in ethanol showed antimicrobial activities against *C. albicans* (8 mm) only. In hot water, the leaves showed a maximum zone of inhibition (10 mm) against *P. aeruginosa* and it was not effective against the other test organisms (Table 2). The stem extract in hot water showed antimicrobial activities against *P. aeruginosa* and *S. mutans* (4 mm and 2 mm, respectively). Using cold distilled water as a solvent, the bark extract showed maximum antimicrobial activities against all test organisms except *S. mutans*. The stem extract in cold water was effective against *S. aureus* alone and the leaves were effective against *P. aeruginosa* and *C. albicans* (6 mm and 3 mm, respectively).

Many studies have shown that different parts of *M. oleifera* including leaves, bark, stems, seeds, root and flowers are rich in several phytocomponents and they have antimicrobial activities (Fahey, 2005; Bhattacharya *et al*., 2018; Shu *et al*., 2020). Different parts of *M. oleifera* have been established to be rich in alkaloids, flavonoids,

Table 2: Antimicrobial analysis of the leaves, stem and bark of *M. oleifera* against different test microorganisms using different solvents (ethanolic, hot and cold distilled water)

		Zone of inhibition (in mm)					
Plant extract		Volume of the sample	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. mutans*	*C. albicans*
Ethanolic	Leaves	25 µl	0	0	4	0	9
	Stem	25 µl	0	0	0	0	8
	Bark	25 µl	10	7	9	7	6
Hot water	Leaves	25 µl	0	10	0	0	0
	Stem	25 µl	0	4	0	2	0
	Bark	25 µl	4	2	2	0	2
Cold water	Leaves	25 µl	0	6	0	0	3
	Stem	25 µl	0	0	2	0	0
	Bark	25 µl	2	4	2	0	4

glycosides, saponins, tannins, steroids (Amaglo *et al*., 2010; Coppin *et al*., 2013), among other important phytoconstituents and this study agrees with the previous studies on the constituents present in *M. oleifera* leaves, stem and bark. In this study, the highest amount of phytochemicals was present in both fresh and powdered leaves extracted using cold distilled water as the solvent. High concentration of phytochemical in *M. oleifera* leaves compared to other parts was also shown by Napolean *et al.* (2009). Similarly, this study demonstrates that in the bark, the phytocomponents are in high concentration after the leaves and the least concentration was present in the stem.

For over decades *M. oleifera* has been used as a potent medicinal plant for treatment and prevention of a large number of diseases. The antimicrobial testing of leaves, stem and bark in different solvents in this study indicated that this plant has a wide spectrum of antimicrobial activities ranging from gram positive bacteria, gram negative bacteria and fungi. Based on the plant part and the solvent used to make the extract, this study has revealed the potential antimicrobial activity of *M. oleifera* against *E. coli, P. aeruginosa, S. aureus, S. mutans* and Yeast *C. albicans*. This result is similar to previous studies in which different parts of *M. oleifera* including bark, stem, fruits, flowers and roots showed effective antimicrobial activities against *S. albus*, *S. aureus*, *B. subtilis*, *S. pyogenes*, *Salmonella gallinarum*, *P. aeruginosa*, and *E. coli* (Thilza *et al*., 2010; Kumar *et al*., 2012). The high concentration of large number of phytochemicals suggests that this plant is very important and this gives it an enormous health benefits against large number of diseases with possibly less adverse effects compared to chemically synthesized pharmaceutical drugs. For instance, alkaloids present in leaves, stem and bark are naturally occurring compounds, containing nitrogen and these have ability to intercalate between the DNA of the microorganisms making it an effective therapeutic agent targeting to inhibit microbial DNA processes (Kasolo *et al*., 2012). In this study, high amount of saponins were present and this component has been used for body washing as a natural soap due to its moisturizing and antimicrobial nature. Steroids also present together with terpenoids have shown the ability to inhibit *S. aureus* (Cowan,1999), having anti-carcinogenic effects making it effective in preventing cancer (Raju *et al*., 2004; Khalafalla *et al*., 2010). Flavonoids present in *M. oleifera* were effective against inhibiting the membrane bound enzymes of different test microorganisms due to its antioxidant activities (Cowan, 1999). Also, these natural antioxidants are important for health and can be utilized as cosmetics for topical applications to prevent skin infections. Studies also show that *M. oleifera* exhibit higher

antioxidant activity against free radicals, revealing its strong nutritional worth (Gebregiorgis *et al*., 2011; Melesse *et al*., 2012; Nahar *et al*., 2016; Khalid *et al*., 2023). Other studies have shown the potency of *M. oleifera* against chronic and lifestyle illness and showed a significant reduction in body mass index of obese patient after oral treatment with *M. oleifera* leaf powder (Ghimire *et al*., 2021; Bhalla *et al*., 2021; Milla *et al*., 2021). In another study, aqueous extract of leaves showed significant protection against Alzheimer's disease by altering brain monoamine levels and electrical activity and also shows beneficial effects in dentistry too (Ganguly *et al*., 2005; Ganguly and Guha, 2008; Meena *et al*., 2021; Llorent-Martinez *et al*., 2023). This is evident by the fact that *M. oleifera* leaves are rich in Vitamin C and E which plays a role in boosting memory in patients suffering from Alzheimer.

Conclusion

Multidrug resistance is an emerging global conern that pose a great threat to human life, and this creates an avenue for extensive research towards finding a solution to gap the antimicrobial resistance towards the commonly known drugs. This study demonstrates the high value of *M. oleifera* due to its broad spectrum towards a large number of microorganisms as a medicinal plant together with nutritive values. Ongoing scientific research validates the vast medicinal properties of *M. oleifera* that traditional therapeutics has recognized. Existing literature on extract preparations of *M. oleifera* substantiate the beneficial roles highlighting its mechanisms as an antibiotic, antifungal, anti-obesity, anti-ulcer, anti-hypersensitive, anti-inflammatory, and neuroprotective phytoactive agent among others. As modern research continues to highlight more potent properties of this medicinal plant, proper isolation, extraction, identification, screening and analysis of these components should be encouraged. For instance, this study has revealed more phytocomponents were present in leaves and barks and when extracted using cold water. Similarly, the study has shown that flavonoids, alkaloids, saponins glycosides andvolatile oils are in abundant in leaves, stem and bark of *M. oleifera*.

According to antimicrobial screening, the study shows that maximum antimicrobial activities are shown by broad spectra of the ethanolic extracts of the bark. In this result, ethanol is an organic solvent having both polar and non-polar properties and more antimicrobial phyto-compounds are more soluble in ethanol than water and ethanol achieve higher diffusion rates in agar wells than water. In addition, ethanol can also have an effect on the cell membrane of microorganisms allowing more phytochemicals to diffuse inside the cell causing the death of microorganism. Therefore, this study concludes that for better results of *M. oleifera* against microorganisms, the extract should be prepared using ethanol as a solvent whereas for maximum amount of phytochemicals, leaves and bark should be prepared using cold distilled water. Hence, it is evident that this study strengthens existing scientific evidence and further research is recommended to outline more about the dosage and other related pharmacological parameters.

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