Research Article

Antimicrobial effect of polyphenols enriched fractions of *Moringa oleifera* Lam. leaves at the flowering stage against microbial strains causing oral and wound infections

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ABSTRACT

Purpose: Various parts of *Moringa oleifera* Lam. (Moringaceae) are used in traditional systems of medicine. This study investigates the antimicrobial activity of the crude aqueous extract and polyphenol enriched fractions of *M. oleifera* leaves at the flowering stage against microbial strains causing oral and wound infections.

Methods: Polyphenol enriched fractions (hydroalcoholic extract, methanol fresh leaves extract, methanol dry leaves extract and decoction) were prepared using four different methods and total polyphenolic content (TPC) and total flavonoid content (TFC) of the extracts were determined. Crude aqueous extract and the fractions were tested against ATCC reference strains of *Escherichia coli* and *Candida albicans* (oral pathogens) and *Streptococcus aureus* and *Staphylococcus pyogenes* (wound pathogens) using agar well diffusion method. Gentamycin and clotrimazole were used as the positive controls for bacteria and fungi respectively and distilled water was used as the negative control.

Results: Methanol dry leaves extract demonstrated highest values of TPC (11.92 ± 0.39 mg GAE/g) and TFC (41.68 ± 0.82 mg QE/g). Each polyphenol fraction demonstrated antibacterial activity against *E. coli*, *S. aureus* and *S. pyogenes* at concentrations of 500 - 1000 mg/ml. Hydro-alcoholic and methanol (fresh leaves) extracts demonstrated antifungal activity against *C. albicans*. The crude aqueous extract exhibited dose dependent antibacterial activity against *S. aureus*. All extracts demonstrated less antibacterial activity against *S. pyogenes* compared to *S. aureus*.

Conclusion: Polyphenol rich fractions of *M. oleifera* leaves at the flowering stage possessed antimicrobial activity against *E. coli*, *S. aureus*, *S. pyogenes* and *C. albicans*.

Keywords

Antimicrobial activity; *Moringa oleifera*; Polyphenolic content; Flavonoid content; Flowering stage

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INTRODUCTION

Herbal medicines have high demand due to the problems associated with the synthetic medicines such as high cost, side-effects and inefficient access.(1) Therefore, it is essential to develop novel herbal medicines to meet the demand for herbal medicines.(2) Oral infections have become one of the major health problems worldwide in all populations, especially in children and infants.(3) Major bacterial species responsible for oral infections are *Staphylococcus mutans*, *S. oralis*, *S. sanguinis* and *Escherichia coli*. These organisms are known to cause gingivitis and periodontal diseases.(4) Oral infections of fungal origin are mainly caused by *Candida* leading to “oral thrush” and results in white or creamy patches on the moist surfaces of the mouth.(5) Wound infections are a common cause of morbidity and mortality. Between 7% - 15% of hospitalized patients are affected by wound infections globally.(6) Growth and persistence of infected bacteria on a wound is promoted by the polymicrobial biofilm formation.(7) Both Gram-negative bacteria (*E. coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and Gram-positive bacteria (*S. aureus*, *Streptococcus pyogenes* and *Enterococcus faecalis*) can be identified as wound pathogens.(8) Polyphenols are secondary metabolites and exhibit a significant structural variability. These compounds possess a wide range of biological activities including antimicrobial activity.(9) Antimicrobial activity of plant phenols against Gram-positive bacteria is reported in literature.(9) Moreover, *in vitro* experiments have proven the activity of phenolic compounds against *Candida* species.(10)

Moringa oleifera. Lam (Family: Moringaceae), is known as Murunga in Sinhala and Sahinjan in Hindi. Other vernacular names of *M. oleifera* are Horse

radish tree, Drumstick plant and Murungai. *M. oleifera* is a fast-growing soft wood tree which can reach 12 m in height. This plant can grow in tropical and sub-tropical regions in dry to moist climates. Therefore, it has an ability to grow in a range of temperatures between 18 - 28°C.(11) Flowers and fruits of *M. oleifera* originate twice a year.(12) This plant is indigenous to North India and is widely grown in South Eastern Asian countries like Thailand and Philippines.(11)

Dried leaves of *M. oleifera* are rich in polyphenols where flavonoids (quercetin, rutin, myricetin) and phenolic acids (gallic acid, caffeic acid, ellagic acid, ferulic acid, coumaric acid, chlorogenic acid, syringic acid, gentisic acid and sinapic acid) are prominent.(13) Various parts of *M. oleifera* are used in traditional systems of medicine to treat malaria, typhoid fever, parasitic disease, arthritis, hypertension and diabetes. Further, parts of *M. oleifera* is used as an immunomodulator, cardiac stimulant and a contraceptive.(14,15) Reported biological activities of *M. oleifera* leaves include antioxidant, anti-inflammatory, antimicrobial, anticancer, antihypertensive and antiproliferative activities.(16-18) Polyphenols of *M. oleifera* leaves have demonstrated antimicrobial activity against *E. coli*, *K. pneumonia*, *P. aeruginosa* and *Bacillus subtilis*.(19) Total phenolic content (TPC) of leaves of many plants is higher at the flowering stage.(20,22) Despite previously reported data suggesting the abundance of polyphenols in leaves at the flowering stage, polyphenol enriched fractions of *M. oleifera* leaves at the flowering stage were not tested for antimicrobial activity. Therefore, the aim of the present study was to investigate the antimicrobial activity of *M. oleifera* leaves at the flowering stage against microbial strains causing oral and wound infections. Thus, a crude aqueous extract and polyphenol

enriched fractions of *M. oleifera* leaves at the flowering stage were used for this study.

MATERIALS AND METHODS

Collection, identification and authentication of *Moringa oleifera*: Fresh leaves of *M. oleifera* were collected at the flowering stage of the plant from Kanangama area in Sabaragamuwa province (GPS location: <https://maps.app.goo.gl/PuupK1yUSr9kNbn38>), Sri Lanka during the months of May and September, 2020 and authenticated at the National Herbarium, Botanical Garden, Peradeniya, Sri Lanka.

Preparation of the crude aqueous extract of *Moringa oleifera* leaves: Leaves of *M. oleifera* were washed, air dried for three days and dried in a hot air oven (Mammert DIN 40050-IP20, Apeldoorn, Nederland) at 40°C, powdered to a coarse powder and stored in airtight bottles. A sample (50 g) of the dry powder was added to a round bottom flask containing 500 ml of distilled water and refluxed for four hours. Then the extract was filtered and concentrated using a rotary evaporator. The extract was stored at 4°C until use.

Extraction of polyphenol enriched fractions from leaves of *Moringa oleifera* leaves: Four different extraction methods were used to extract polyphenol enriched fractions.

Hydroalcoholic extract: Dried and powdered *M. oleifera* leaves (50 g) were blended (BL440, Kenwood, China) with 400 ml of a hydro alcoholic solution (ethanol: distilled water, 70:30 v/v) and shaken using a mechanical shaker (Shaker RJH5005, Richard James Hilfiger, China) at room temperature for 60 minutes. The extract was filtered and concentrated by rotary evaporation.(23)

Methanol fresh leaves extract: Fresh leaves of *M. oleifera* (200 g) were finely crushed and blended with 500 ml of methanol at room

temperature (28–30°C). The blended material was filtered and the filtrate was left for 48 hours in a refrigerator. The extract was concentrated by rotary evaporation.(24)

Methanol dry leaves extract: Dried leaves of *M. oleifera* (100 g) were crushed and blended with 300 ml of methanol. Blended material was filtered, and the filtrate was left for 48 hours in a refrigerator. The extract was concentrated by a rotary evaporator.(25)

Decoction: Crushed dry leaves of *M. oleifera* (50 g) were blended with 750 ml of distilled water. The extract was stirred at 100°C for 45 minutes under magnetic stirring. The decoction was filtered and concentrated with rotary evaporation.(23)

Quantification of total polyphenol content of the crude aqueous extract and polyphenol enriched fractions of *Moringa oleifera* leaves at the flowering stage: A weight of 0.1 g of each extract was dissolved in its respective solvent (1 ml) and considered as the stock solution. The stock solution was diluted to half of the original stock solution concentration. A volume of 20 µl of each extract were mixed with 110 µl of the Folin-Ciocalteu reagent (diluted 1:10 with deionized water) and was neutralized with 70 µl of a sodium carbonate solution (7.5 %, w/v). The reaction mixtures were incubated at room temperature for 30 minutes with intermittent shaking for colour development, followed by diluting the reaction mixtures with 3 ml of distilled water. The absorbance of the resulting solution was measured at 765 nm using a UV–VIS Spectrophotometer (Spectrophotometer UV-1601, Shimadzu, Japan). TPC of each extract was determined from a linear equation of a standard curve prepared with gallic acid. The content of total phenolic compounds was expressed as mg gallic acid equivalents (GAE)/g of the dry extract.(12)

Quantification of the total flavonoid content of the crude aqueous extract and polyphenol enriched fractions of *Moringa*

***oleifera* leaves at the flowering stage:** A stock solution was prepared by adding 0.1 g of each extract into respective solvent (1 ml). The stock solution was diluted to half of the original stock solution concentration. Then 100 µl aliquots of each extract were mixed with 100 µl of a prepared aluminium chloride solution (2% w/w of aluminium chloride in methanol). The reaction mixtures were incubated at room temperature for 10 minutes with intermittent shaking for colour development. The absorbance of the resultant solution was measured at 415 nm using a UV–VIS Spectrophotometer. The total flavonoid content (TFC) of each extract was determined from a linear equation of a standard curve prepared with quercetin. The content of total flavonoid compounds was expressed as mg quercetin equivalents (QE)/g of dry extract. (26)

Determination of the antimicrobial activity of crude aqueous extract and polyphenol enriched fractions of *Moringa oleifera* leaves at the flowering stage against microbial strains causing oral infections: The antimicrobial activity of the crude aqueous extract of *M. oleifera* leaves and each polyphenol enriched fractions against microbial strains causing oral infections, was determined by the agar well diffusion method.(27) Isolates of ATCC reference strains of *E. coli* (25922) and ATCC reference strains of *C. albicans* (10231) were obtained from the Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka. Subcultured organisms were used to prepare microbial suspensions. The turbidity of the suspensions was adjusted to 0.5 McFarland standard (1×10^8 CFU/ml). A weight of 38.0 g of Mueller Hinton agar was dissolved in 1L of distilled water. Media were sterilized by autoclaving at 121°C for 15 minutes. Liquid agar was poured to agar plates and was kept aside for the medium to

solidify.(28) Prepared inocula of *E. coli* and *C. albicans* were spread on the surface of Mueller Hinton agar plates using a sterile cotton swab. Plates were allowed to dry for 5 minutes. Wells were cut in the agar plate by using a sterile dropper with 6 mm diameter. A drop of molten agar was added to each well to seal the bottom of the well. The extract and fractions were diluted by half to construct a concentration series (62.5 mg/ml, 125 mg/ml, 250 mg/ml, 500 mg/ml and 1000 mg/ml). A volume of 40 µl of each concentration was added to the wells. Bacterial cultures were incubated at 37°C for 24 hours and fungal cultures for 48 hours. Gentamycin (0.3% w/v) and clotrimazole (1% w/v) were used as positive control for bacteria and fungi respectively. Antimicrobial activity was determined by measuring the zone of inhibition around the well.

Determination of the antimicrobial activity of the crude aqueous extract and polyphenol enriched fractions of *Moringa oleifera* leaves at the flowering stage against microbial strains causing wound infections: The antimicrobial activity of the crude aqueous extract of *M. oleifera* leaves and each polyphenol enriched fractions against microbial strains causing wound infections, was determined by the agar well diffusion method.(29) ATCC reference strains of *S. aureus* (25923) and *S. pyogenes* (19615) were obtained from the Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka. Subcultured organisms were used to prepare microbial suspensions. The turbidity of the suspensions was adjusted to 0.5 McFarland standard (1×10^8 CFU/ml). Prepared inocula of *S. aureus* and *S. pyogenes* were spread on the surface of Mueller Hinton agar and blood agar respectively, using sterile cotton swabs. Blood (7 % v/v sterile, defibrinated blood) was added aseptically to the medium during

the preparation of blood agar plates.(30) Plates were allowed to dry for 5 minutes. Wells were cut by using a sterile dropper with 6 mm diameter. A drop of molten agar was added to each well to seal the bottom of the well. The extract and the fractions were diluted by half to construct a concentration series (62.5 mg/ml, 125 mg/ml, 250 mg/ml, 500 mg/ml and 1000 mg/ml). A volume of 40 μ l of each concentration was added to the wells. Cultures were incubated at 37°C for 24 hours and gentamycin (0.3% w/v) was used as the positive control. Antimicrobial activity was determined by measuring the zone of inhibition around the well.

Statistical analysis: The data were presented as mean $\pm$ SD (Standard Deviation) of three replicates. One-way analysis of variance (ANOVA) was performed by using IBM SPSS statistics 25 Software. P values < 0.05 was considered significant.

RESULTS

Extraction yields of the crude aqueous extract and its fractions of *Moringa oleifera* leaves at the flowering stage: The yields (w/w, dry weight basis) of crude aqueous extract, hydroalcoholic extract, methanol fresh leaves extract, methanol dry leaves extract and decoction were 20.6%, 17.5%, 10.4%, 15.2% and 11.6% respectively.

Quantification of the total polyphenol content and the total flavonoid content of crude aqueous extract and polyphenol enriched fractions of *Moringa oleifera* leaves at the flowering stage: The TPC and TFC of the crude aqueous extract and polyphenol enriched fractions of *M. oleifera* leaves at the flowering stage are represented in Table 1 and Table 2, respectively. According to the results, methanol dry leaves extract of *M. oleifera* contained the highest TPC and TFC.

Table 1: Total polyphenolic content of crude aqueous extract of *Moringa oleifera* leaves and polyphenol enriched fractions at the flowering stage

	Crude aqueous extract	Hydroalcoholic extract	Methanol fresh leaves extract	Decoction	Methanol dry leaves extract
Total polyphenol content (mg GAE /g)	0.14 $\pm$ 0.11	3.10 $\pm$ 0.16	10.76 $\pm$ 0.41	2.56 $\pm$ 0.63	11.92 $\pm$ 0.39

Data presented as Mean $\pm$ SD

Table 2: Total flavonoid content of crude aqueous extract of *Moringa oleifera* leaves and polyphenol enriched fractions at the flowering stage

	Crude aqueous extract	Hydroalcoholic extract	Methanol fresh leaves extract	Decoction	Methanol dry leaves extract
Total flavonoid content (mg QE/g)	11.27 $\pm$ 0.87	27.72 $\pm$ 0.96	21.89 $\pm$ 0.13	8.48 $\pm$ 0.045	41.68 $\pm$ 0.82

Data presented as Mean $\pm$ SD

Determination of the antimicrobial activity of the crude aqueous extract and polyphenol enriched fractions of *Moringa oleifera* leaves at the flowering stage against microbial strains causing oral infections: Varying antimicrobial activity was observed as different zones of inhibition of bacteria and fungi among the crude aqueous extract of *M. oleifera* leaves and the polyphenol enriched fractions. The results obtained are reported in Figure 1.

The results indicate that the highest concentration of the crude aqueous extract of *M. oleifera* leaves exerted antibacterial activity against *E. coli*. The crude aqueous extract of *M. oleifera* leaves did not show antifungal activity against *C. albicans* at the concentrations tested. The diameter of the zone of inhibition (mm) of gentamycin against *E. coli* was 25.66 ± 0.57 mm and that of clotrimazole against *C. albicans* was 37.0 ± 0.29 mm.

Figure 1: Diameter of the zones of inhibition of different concentrations of crude aqueous extract of *Moringa oleifera* leaves and polyphenol enriched fractions at the flowering stage against microbial strains causing oral infections.

A: The diameter of zone of inhibition (mm) of different extracts against *Escherichia coli*
B: The diameter of zone of inhibition (mm) of different extracts against *Candida albicans*.

Determination of the antimicrobial activity of the crude aqueous extract and the polyphenols enriched fractions of *Moringa oleifera* leaves at the flowering stage against microbial strains causing wound infections: Antimicrobial activity of the crude aqueous extract and each

polyphenol rich fractions of leaves of *M. oleifera* at the flowering stage was tested against *S. aureus* and *S. pyogenes* that cause wound infections (Figure 2). The diameter of zone of inhibition (mm) of gentamycin against *S. aureus* and *S. pyogenes* was 31.0 ± 0.2 mm.

Figure 2: Diameter of the zones of inhibition of different concentrations of crude aqueous extract and polyphenol enriched fractions of *Moringa oleifera* leaves at the flowering stage against microbial strains causing wound infections.

A: The diameter of zone of inhibition (mm) of different extracts against *Staphylococcus aureus*

B: The diameter of zone of inhibition (mm) of different extracts against *Streptococcus pyogenes*.

DISCUSSION

Moringa oleifera is a plant rich in polyphenols. The principal polyphenolic compounds reported in *M. oleifera* leaves are flavonoids and phenolic acids.(15) Some of the isolated and characterized polyphenols from *M. oleifera* are quercetin-3-*o*-glucoside, kaempferol-3-*o*-malonylglucoside and quercetin-3-*o*-(6''-malonyl)glucoside.(31)

There are various extraction methods adapted for isolation of polyphenols including Soxhlet extraction, maceration, reflux extraction, distillation and low-pressure solvent extraction. Microwave and ultrasonic assisted extractions, supercritical fluid extraction, and accelerated solvent extraction are considered as novel extraction methods of polyphenols.(32)

According to literature, TPC of many plants leave is higher at the flowering stage.(20-22) In the present study, crude aqueous extract and polyphenolic rich fractions (methanol dry leaves extract, methanol fresh leaves extract, hydroalcoholic extract, and decoction) of *M. oleifera* leaves at the flowering stage were tested for microbes that cause oral infections (*E. coli* and *C. albicans*) and wound infections (*S. aureus* and *S. pyogenes*). There is a paucity of studies, reporting on extraction of polyphenols of *M. oleifera* leaves at the flowering stage. Hence, this would be the first report of a study exploring the antimicrobial activity of polyphenol enriched fractions of *M. oleifera* leaves at the flowering stage against microorganisms causing oral and wound infections.

In the present study, the highest amounts of TPC and TFC were observed in the polyphenol enriched fractions extracted using methanol. A similar phenomenon has been discovered by other scientists as well. According to Rocchetti *et al.* different

solvent extractions were used to quantify the TPC and TFC amounts in *M. oleifera* leaves. Moreover, they found that highest amounts of TPC and TFC were present in methanol extract.(13) Reported values of TPC and TFC of *M. oleifera* leaves were at a range of 12.2 -20.9 mg GAE/g of dry weight and 5.059 - 12.16 mg QE /g of dry weight respectively.(13) In the present study, the TFC in methanol dry leaves extract (41.68±0.82 mg QE/g) of *M. oleifera* was higher than the reported value. Zhu *et al.* have reported a significant correlation between the polyphenol profile and the collection area of plant material.(33) Due to the differences in cultivation conditions, climate, or soil environment, accumulation of different polyphenols was reported.(33) Moreover, such wide range of reported values would also be due to different environmental conditions in the various countries of origin, the harvesting season, the genetic of the plant, the drying method, the leaf maturity stage and the extraction method used.(33)

Microorganisms were selected for testing on the basis of causing oral infections. *E. coli* is responsible for the many types of bacterial oral infections in humans. Common oral infections such as gingivitis, periodontitis, mucositis and stomatitis occur due to *E. coli* infection.(34) *C. albicans* is the most common disease causing *Candida* species in the oral cavity. Candidiasis frequently occurs in the very young, elderly and immune deficient persons. It can result in prolonging the use of antibiotics and resulting in the decrease of normal bacterial flora in the mouth. (5)

The results of the present study indicated that the highest concentration of the crude aqueous extract of *M. oleifera* leaves exerted antibacterial activity against *E. coli*. Further, *E. coli* is reported to be resistant to Moringa

extracts.(35-36) Antimicrobial activity of the crude aqueous extract of *M. oleifera* leaves was greater against Gram-positive bacteria than against Gram-negative bacterial strains.(36) Moreover, according to the results of the present study, the crude aqueous extract of *M. oleifera* leaves did not show antifungal activity against *C. albicans* at the tested concentrations. Similar observations were reported in several studies where the water extract of *M. oleifera* leaves was resistant to *C. albicans*.(27,37). However, the *C. albicans* was sensitive to the water and alcohol extracts of *M. oleifera* seeds.(37) In addition, essential oil of *M. oleifera* seeds demonstrated antifungal activity against *C. albicans*.(38)

All four polyphenol enriched fractions of *M. oleifera* leaves at the flowering stage, at concentrations of 1000 mg/ml and 500 mg/ml demonstrated antibacterial activity against *E. coli* and was sensitive to the methanol fresh leaves extract even at 250 mg/ml (Figure 1A). TPC was highest in the methanol dry leaves extract, as evident by the results of the present study. *M. oleifera* dry leaves extract in methanol at a concentration of 1000 mg/ml was more active against growth inhibition of *E. coli*. This is probably attributable to the high TPC in the methanol dry leaves extract. A similar finding is reported in literature where it is shown that the TPC is directly proportional to the antibacterial activity.(18) According to the findings by Rocchetti *et al.* the methanol extract was characterized by the highest TPC.(13) These findings emphasize the choice of extraction solvent in isolation of polyphenols as a critical factor for obtaining the desired antimicrobial activity. The phenolic class is highly affected by the solvent used to extract. However, it is noteworthy that the existing literature does not provide supporting information on

extraction of polyphenols of *M. oleifera* leaves at the flowering stage.

Conversely, *C. albicans* was only sensitive to the methanol fresh leaves extract (250-1000 mg/ml) and the hydroalcoholic extract (500-1000 mg/ml). Among these two extracts, the methanol fresh leaves extract has shown the highest antifungal activity against *C. albicans* (Figure 1B). A similar finding was observed in the study conducted by Maqsood *et al.* where the methanol extract exerted higher antifungal activity against *C. albicans* than the hydroalcoholic extract. (39)

Microorganisms causing wound infections (*S. aureus* and *S. pyogenes*) were selected in this study. As evident by the results, the tested concentrations of the crude aqueous extract of *M. oleifera* leaves exhibited dose dependent antimicrobial activity against *S. aureus*. Similar type of dose dependent antibacterial activity of the crude extract of *M. oleifera* seeds against *S. aureus* was reported by Jabeen *et al.*(40) However, as depicted in Figure 2, *S. pyogenes* was resistant to the crude aqueous extract of *M. oleifera* leaves at concentrations ranging from 62.5-250 mg/ml. Similarly, *S. pyogenes* was resistant to the water extract of *M. oleifera* leaves at the concentration range 15.6 – 500 mg/ml.(37) Different polyphenol enriched fractions exerted varying degrees of inhibition zones against wound pathogens (*S. aureus* and *S. pyogenes*). Virtually all the fractions showed appreciable inhibition of *S. aureus*. All four polyphenol enriched fractions of *M. oleifera* leaves at the flowering stage, at concentrations of 1000 mg/ml and 500 mg/ml demonstrated antibacterial activity against *S. aureus* and *S. pyogenes* (Figure 2). *S. aureus* was sensitive to the methanol fresh leaves extract at concentrations ranging 62.5-1000 mg/ml. However, the methanol dry leaves extract demonstrated antibacterial activity at

concentrations ranging from 250-1000 mg/ml against *S. aureus* (Figure 2). In the present study comparing the antibacterial activity of the fresh and dried *M. oleifera* leaves in methanol extract against *S. aureus*, exhibited that the fresh leaves extract was more effective than the dried one. This observation is in line with reported data.(41) All polyphenol enriched fractions have demonstrated less antibacterial activity against *S. pyogenes* when compared to *S. aureus* (Figure 2). Similar results were reported in previous studies that proved *S. aureus* is more sensitive to plant antibacterial agents than *S. pyogenes*.(42) The decoction of *M. oleifera* leaves has demonstrated least antibacterial activity against *S. aureus* among all the tested extracts (Figure 2). A similar finding was observed in a study conducted by Ibrahim and Kebede where the methanol extracts of Moringa leaves had the highest antibacterial activity against *S. aureus*.(43) This study and literature demonstrate that, the methanolic extracts of the leaves of *M. oleifera* were more effective than the aqueous extracts, indicating that methanol is a better solvent than water.(44) Among the four polyphenol enriched fractions obtained from *M. oleifera* leaves, the fractions extracted to methanol showed the highest TPC. Methanol extracts were more active against the growth of *S. aureus* (Figure 2). This is probably attributable to the high TPC in polyphenol enriched fractions extracted to methanol.

Polyphenol enriched fractions extracted to methanol exhibited antibacterial activity against *E. coli*, *S. aureus*, *S. pyogenes* and antifungal activity against *C. albicans*. However, detailed studies on the specific bioactive molecules in each polyphenolic enriched fraction responsible for the antimicrobial attributes and their mechanism of action is required. Therefore, it is highly recommended to conduct bioactivity guided

fractionation of the methanol fractions in order to isolate antimicrobial compounds. Mechanism of action of polyphenols which are thought to be responsible for antimicrobial activity includes enzyme inhibition by the oxidized compounds, irreversible binding with proteins, inactivating enzymes, binding to adhesins on the microbial cell surface, binding to cell wall proteins, and interacting with substrates, rendering them unavailable to the microorganism, complexing with metal ions, and others.(16) However, the mechanism of action of antimicrobial activity of the polyphenol enriched fractions was not evaluated under this study.

Despite the common belief that medicinal plants are safe, it is necessary to evaluate any possible adverse-effects which associate with them. Similarly, many *in vitro* and *in vivo* studies were conducted to evaluate the toxicity of *M. oleifera* leaves. The hot water extract of *M. oleifera* leaves was safe at ≤ 1000 mg/kg for Sprague-Dawley rats *in vivo* and cytotoxicity was at 20 mg/ml *in vitro*. Moreover, hot water extract of *M. oleifera* leaves was genotoxic at a dose of 3000 mg/kg *in vivo*. (45) According to Awodele *et al.* cold water extract of *M. oleifera* leaves did not exhibit any significant toxicity in terms of haematological and biochemical parameters, sperm quality or weight gain in male Wistar albino mice and the LD₅₀ value was 1585 mg/kg.(46)

CONCLUSION

In conclusion, crude aqueous extract and polyphenols enriched fractions of *M. oleifera* leaves at the flowering stage have potential antimicrobial effects on oral (*E. coli* and *C. albicans*) and wound (*S. aureus* and *S. pyogenes*) pathogens. The study warrants the necessity of bioactivity guided fractionation

in order to isolate and characterize antimicrobial compounds from polyphenol enriched fractions of leaves of *M. oleifera* at the flowering stage.

Author's Declaration: The authors declare that all persons listed as authors have read and given approval for the submission of this manuscript.

Competing Interests: The authors declare that they have no competing interests to disclose.

REFERENCES:

1. Konaté K, Hilou A, Mavoungou JF, Lepengué AN, Souza A, Barro N, Datté JY, M'batchi B, Nacoulma OG. Antimicrobial activity of polyphenol-rich fractions from *Sida alba* L.(Malvaceae) against co-trimoxazol-resistant bacteria strains. *Annals of Clinical Microbiology and Antimicrobials*. 2012 Jan;11(1):1-6.
2. Akinyeye AJ, Solanke EO, Adebisi IO. Phytochemical and antimicrobial evaluation of leaf and seed of *Moringa oleifera* extracts. *Int J Res Med Health Sci*. 2014; 4(6):1-10.
3. Naser SSA, El Haddad IA. An expert system for genital problems in infants. *World Wide J Multidiscip Res Dev*. 2016; 2:83–6.
4. Rostinawati T, Aryani H, Iskandar Y. Identification of bacteria causing dental caries through genetic testing and activity assay of toothpastes. *J Pharm Sci Res*. 2018; 10(3):511–3.
5. Vila T, Sultan AS, Montelongo-jauregui D. Oral candidiasis: A disease of opportunity. *J fungi* 2020; 6(1):15.
6. World Health Organization. Global guidelines for the prevention of surgical site infection (2nd ed). Geneva; 2018.
7. Hurlow J, Couch K, Laforet K, Bolton L, Metcalf D, Bowler P. Clinical biofilms: A challenging frontier in wound care. *AdvWound Care*. 2015;4(5):295–301.
8. Sisay M, Worku T, Edessa D. Microbial epidemiology and antimicrobial resistance patterns of wound infection in Ethiopia: a meta-analysis of laboratory-based cross-sectional studies. *BMCPharmacolToxicol*. 2019;20(1):35.
9. Álvarez-Martínez FJ, Barrajón-Catalán E, Encinar JA, Rodríguez-Díaz JC, Micó V. Antimicrobial capacity of plant polyphenols against Gram-positive bacteria: A comprehensive review. *Curr Med Chem*. 2018;27(15):2576–606.
10. Martins N, Barros L, Henriques M, Silva S, Ferreira ICFR. Activity of phenolic compounds from plant origin against *Candida* species. *Ind Crops Prod*. 2015;74:648–70.
11. Nkya JW, Erasto P, Kilambo D, Chacha M. *In vitro* evaluation of antifungal activity of *Moringa oleifera* Lam extracts against coffee wilt pathogen, *Gibberella xylarioides* Heim and Saccas. *Am J Res Commun*. 2014; 2(12): 53–62.
12. Alhakmani F, Kumar S, Khan SA. Estimation of total phenolic content, *in-vitro* antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*. *Asian Pac J Trop Biomed*. 2013; 3(8): 623–7.
13. Rocchetti G, Pagnossa JP, Blasi F, Cossignani L, Piccoli RH, Zengin G, Montesano D, Cocconcelli PS, Lucini L. Phenolic profiling and *in vitro* bioactivity of *Moringa oleifera* leaves as affected by different extraction solvents. *Food Research International*. 2020 Jan 1;127:108712..
14. Department of Ayurveda. Compendium of medicinal plants, a Sri Lankan study. Vol. IV. Maharagama, Sri Lanka, 2004.

15. 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(6):12791–835.
16. Othman L, Sleiman A, Abdel-Massih RM. Antimicrobial activity of polyphenols and alkaloids in middle eastern plants. *Front Microbiol.* 2019;10:911.
17. Othman AI, Amer MA, Basos AS, El-Missiry MA. *Moringa oleifera* leaf extract ameliorated high-fat diet-induced obesity, oxidative stress and disrupted metabolic hormones. *Clin Phytoscience.* 2019; 5: 48.
18. Tekle EW, Sahu N, Makesh M. Antioxidative and antimicrobial activities of different solvent extracts of *Moringa oleifera*: an *in vitro* evaluation. *Int J Sci Res Publ.* 2015; 5(5): 255–66.
19. Torondel B, Opare D, Brandberg B, Cobb E, Cairncross S. Efficacy of *Moringa oleifera* leaf powder as a hand- washing product: A crossover controlled study among healthy volunteers. *BMC Complement Altern Med.* 2014; 14(1):1–7.
20. Feduraev P, Chupakhina G, Maslennikov P, Tacenko N, Skrypnik L. Variation in phenolic compounds content and antioxidant activity of different plant organs from *Rumex crispus* L. and *Rumex obtusifolius* L. at different growth stages. *Antioxidants.* 2019; 8: 237.
21. Saffaryazdi A, Ganjeali A, Farhoosh R, Cheniany M. Variation in phenolic compounds, α -linolenic acid and linoleic acid contents and antioxidant activity of purslane (*Portulaca oleracea* L.) during phenological growth stages. *Physiol Mol Biol Plants.* 2020; 26: 1519-29.
22. Adegbaaju OD, Otunola GA, Afolayan AJ. Effects of growth stage and seasons on the phytochemical content and antioxidant activities of crude extracts of *Celosia argentea* L. *Heliyon.* 2020; 06: 04086.
23. Baldisserotto A, Buso P, Radice M, Dissette V, Lampronti I, Gambari R, Manfredini S, Vertuani S. *Moringa oleifera* leaf extracts as multifunctional ingredients for “natural and organic” sunscreens and photoprotective preparations. *Molecules.* 2018 Mar;23(3):664.
24. Tumer TB, Rojas-silva P, Poulev A, Raskin I, Waterman C. Direct and indirect antioxidant activity of polyphenol- and isothiocyanate-enriched fractions from *Moringa oleifera*. *J Agric Food Chem.* 2016; 63(5):1505–13.
25. Dzutam JK, Touani FK, Kuete V. Antibacterial and antibiotic-modifying activities of three food plants (*Xanthosoma mafaffa* Lam., *Moringa oleifera* (L.) Schott and *Passiflora edulis* Sims) against multidrug-resistant (MDR) Gram-negative bacteria. *BMC Complement Altern Med* 2016;16:9.
26. Shirazi OU, Khattak MMAK, Shukri NAM, Nasyriq MN. Determination of total phenolic, flavonoid content and free radical scavenging activities of common herbs and spices. *J Pharmacogn Phytochem.* 2014; 3(3): 104–8.
27. Elgamily H, Moussa A, Elboraey A, El-sayed H, Al-moghazy M, Abdalla A. Microbiological assessment of *Moringa oleifera* extracts and its incorporation in novel dental remedies against some oral pathogens. *Open Access Maced J Med Sci.* 2016; 4(4):585–90. doi: 10.3889/oamjms.2016.132
28. Perilla M, Ajello G, Bopp C, Elliott J, Facklam R, Knapp J, et al. Manual for the

- laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health importance in the developing world. World Health Organization (WHO), Geneva, 2003 :163–217
29. Priya V, Abiramasundari PP, Devi SG, Jeyanthi GP. Antibacterial activity of the leaves, bark, seed and flesh of *Moringaoleifera*. *Int J Pharm Sci Res.* 2011; 2: 2045–49. doi:10.13040/IJPSR.0975-8232.2(8).2045-49
 30. Hardcover M. *Merk Microbiology Manual*. 12th ed. Darmstadt; Germany; 2010.
 31. Leone A, Fiorillo G, Criscuoli F, Ravasenghi S, Santagostini L, Fico G, Spadafranca A, Battezzati A, Schiraldi A, Pozzi F, Di Lello S. Nutritional characterization and phenolic profiling of *Moringa oleifera* leaves grown in Chad, Sahrawi Refugee Camps, and Haiti. *International journal of molecular sciences.* 2015 Aug;16(8):18923-37.
 32. Tzima K, Brunton NP, Rai DK. Qualitative and quantitative analysis of polyphenols in Lamiaceae plants: A review. *Plants.* 2018;7:1–30.
 33. Zhu Y, Yin Q, Yang Y. Comprehensive Investigation of *Moringa oleifera* from different regions by simultaneous determination of 11 polyphenols using UPLC-ESI-MS/MS. *Molecules.* 2020; 25(3):676.
 34. Roberts FA, Richardson GJ, Michalek SM. Effects of *Porphyromonasgingivalis* and *Escherichia coli* lipopolysaccharides on mononuclear phagocytes. *Infect Immun.* 1997; 65(8):3248–54.
 35. Fouad EA, Abu Elnaga ASM, Kandil MM. Antibacterial efficacy of *Moringa oleifera* leaf extract against pyogenic bacteria isolated from a dromedary camel (*Camelus dromedarius*) abscess. *Vet World* 2019; 12(6):802–8.
 36. Peixoto JR, Silva GC, Costa RA, Vieira GH, Fonteles Filho AA, dos Fernandes Vieira RH. *In vitro* antibacterial effect of aqueous and ethanolic *Moringa* leaf extracts. *Asian Pacific journal of tropical medicine.* 2011 Mar 1;4(3):201-4.
 37. Kheir SM, Kafi SK, Elbir H. Evaluation of the antifungal activity of *Moringa oleifera* seeds, leaves and flowers. *World J Pharm Res.* 2015; 4(2); 258 -1.
 38. Girish K, Fathima SK. Antifungal activity of essential oils on *Phomopsis azadirachtae* causing die-back of neem. *J Bioprocess* 2019; 12(1):45–50.
 39. Maqsood M, Qureshi R, Arshad M, Ahmed MS, Ikram M. Preliminary phytochemical screening, antifungal and cytotoxic activities of leaves extract of *Moringa oleifera* lam. From salt range, Pakistan. *Pakistan J Bot.* 2017; 49(1): 353–9.
 40. Jabeen R, Shahid M, Jamil A, Ashraf M. Microscopic evaluation of the antimicrobial activity of seed extracts of *Moringa oleifera*. *Pak. J. Bot.* 2008;40:1349–1358.
 41. Dike-Ndudim JN, Anyanwu GO, Egbuobi RC, Okorie HM, Udujih HI, Nwosu DC, Okolie NJ. Anti-bacterial and phytochemical potential of *Moringa oleifera* leaf extracts on some wound and enteric pathogenic bacteria. *Eur J Bot Plant Sci Phytol.* 2016;3(1):50-60.
 42. Sahraei S, Mohkami Z, Golshani F, Javadian F, Saeidi S, Baigi GS. Antibacterial activities of five medicinal plant extracts against some human bacteria. *Eur J Exp Biol.* 2014;4:194–196.
 43. Ibrahim N, Kebede A. *In vitro* antibacterial activities of methanol and aqueous leaf extracts of selected medicinal plants

- gainst human pathogenic bacteria. Saudi J Biol Sci. 2020; 27: 2261–68.
44. Truong DH, Nguyen DH, Ta NTA, Bui AV, Do TH, Nguyen HC. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and *in vitro* anti-inflammatory activities of *Severiniabuxifolia*. J Food Qual. 2019
 45. Asare GA, Gyan B, Bugyei K, Adjei S, Mahama R, Addo P, Out-Nyarko A. Toxicity potentials of the nutraceutical *Moringa oleifera* at supra supplementation levels. J Ethnopharmacol. 2012; 139: 265 – 272.
 46. Awodele O, Oreagba IA, Odoma S, De Silva JAT, Osunkalu VO. Toxicological evaluation of the aqueous leaf extract of *Moringa oleifera* Lam (Moringaceae). J Ethnopharmacol. 2012; 139 (2): 330-336.