



Antibacterial screening of different part of Drumstick tree (*Moringa oleifera* Lam)

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ABSTRACT

Antimicrobial resistance remains global public health problems. Several pathogenic diseases were found to resist a number of drugs e.g. *S. serotypes*, *S. pneumonia*, *Enterococcus spp*, *S. typhimurium*, and *S. pyogenes* among others. However many other strains of bacterial pathogens were found to develop resistivity against more than one antibiotics, hence this phenomenon is refer to as multidrug resistance. Thus approaches to recover the present condition are needed. This study was aimed to assess antibacterial effect of different part of *Moringa oleifera* Lam. The results showed that bark and combination of stem with bark extracts had inhibitory effect against all gram positive bacteria studied. And the most susceptible bacteria was found to be *S. aureus* (ATTC 33591) while the most resistant one was *P. aerogenosa* (ATTC 12453). Hence it proves the claimed that many part of *Moringa oleifera* contains phytochemicals compounds capable of acting as either bacteriostatic or bactericidal activity.

Keywords: Antimicrobial, resistance, *Moringa oleifera* Lam., MIC.

INTRODUCTION

Antimicrobial resistance remains global public health problems. Several diseases were found to develop resistance against many drugs e.g. *S. serotypes*, *S. pneumonia*, *Enterococcus spp*, *S. typhimurium*, and *S. pyogenes* among others. Approaches to recover the present Condition may consist of research to discover new and Novel antimicrobials [1]. Infectious diseases continue to be the major causes of death everywhere in the world. Despite many interventions were made by different organisation in managing antimicrobial resistance, yet there is need of further interventions so as to reduce the influence of antimicrobial resistance on mortality, morbidity and even treatment cost [2]. Recently the challenges of bacterial resistance is becoming worse, where many more strains of bacterial pathogens were revealed to have resistance against more than one antibiotics, hence this phenomenon is refer to as multidrug resistance [3]. Examples of some bacteria and the related resisted drug are *S. typhimurium* resist a number of drugs such as Ampicillin, Chloramphenicol, Streptomycin, Tetracycline, and sulphonamides [4]. [3]Point out the need of many researchers to continuous in defining the molecular facts about the mechanism of microbial resistance. That may result to new and suitable to multidrug inhibitors.

Moringa oleifera Lam. It is a multipurpose plant, popularly called drumstick, Horseradish or Ben oil tree [5, 6].It is also called multipurpose plant due to its various functions almost every parts of the plant is important to many. Some of the valuable effect of *Moringa oleifera* are medicine, nutrition, water management, livestock feed, and landscaping among others [7]. Many part of the plant such as leaves, fruits, roots were used as either vegetables [8], source of vitamins, iron, calcium, beta-carotene, riboflavin and phenolic acid among others [9, 10].Different studies have showed that various parts of *Moringa oleifera* such as stem, flowers, bark, roots as well as seeds have antimicrobial activities [6, 11, 12]. *Moringa oleifera* have been considered as antimicrobial agent after the discovery of several Antimicrobial components with inhibitory activity against many microorganisms [13].*Moringa oleifera*

leaves have been reported to be outstanding source of phytochemicals compounds such as saponins, flavonoids, tannins and other phenolic compounds that have antimicrobial properties [14, 15, 16]. Nevertheless, [17] stated that the antioxidant activities of Moringa plant extracts have been related to the presence of polyphenolic compounds, which have great potential as antimicrobial agents. It was known that *Moringa oleifera* is a rich source of compound popularly called pterygospermin, which serve as potential antibacterial and also have fungicidal effects [18]. Furthermore, *Moringa oleifera* leaves have been used to isolate Compounds such as benzyl isothiocyanate, benzyl glucosinolate, and pterygospermin, however the antimicrobial activities of these compounds have been tested against many bacteria, which showed bactericidal and bacteriostatic activity [6]. Additionally polypeptide was also reported to have bactericidal property against many pathogenic bacteria some of which include Streptococcus, Legionella, and Staphylococcus species [19].

This study was carried out with the sole aims of studying antibacterial effect of different part of *Moringa oleifera* Lam.

EXPERIMENTAL SECTION

Preparation of inoculum

A sterile inoculating loop was used to touch four or five isolated colonies of each bacteria use, the bacterial colonies were suspended in 2 ml of sterile Mueller Hinton broth. It was homogenised which create a smooth suspension. The turbidity of this suspension was adjusted to a 0.5 McFarland standard by adding more organism especially when the suspension was found to be too light or even diluted with sterile saline if it appeared too heavy. The prepared suspension was then Use 15 minutes after preparation [20].

Inoculation of the MH plate

A sterile swab was use and dipped into the inoculum suspension tube, it was rotated against the tube sides, the dried surface of the MH agar plate used was inoculated by streaking the swab for about three times throughout the entire agar surface, the plate was rotated approximately 60 degrees each round so as to be well distributed. The rim plate was then swabbed leading to removal of excess liquid, and the plate was placed at room temperature at least 15 minute, so as to dry the surface of the agar plate [20].

Placement of the antibiotic disks (control)

Antimicrobial impregnated disks were place on the surface of the agar medium, with the help of forceps, each antimicrobial disk was dispense one at a time, at least 5 disc were used in each petri dish with at least 24 mm distance between one another, each disk was pressed down gently using forceps to confirm complete contact with the medium surface, the lid was then replace so as to minimise exposure of the medium surface to air and the petri dishes were subsequently inverted, follow by immediate incubation at 37°C for 24 hours [20].

Screening and standardization of the test organisms

Screening and standardisation of the test organism was achieved using methods of [21] with a slight modification. The pure culture of the test organisms used *Bacillus cereus* (ATCC 10876), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 33591) and *Pseudomonas aeruginosa* (ATCC 12453) were collected from microbiology laboratory of Universiti Sultan Zainal Abidin, (UNISZA). Tembila campus Besut, Terengganu, Malaysia. The isolates were subcultured on agar slant and stored at 4°C until further use. A loopful of the test organisms was inoculated into 5.0 ml of nutrient broth and incubated at 37°C for 24 hours. 0.2 ml from the 24 hours culture of the organism was dispensed into 20 ml sterile nutrient broth and incubated for 3-5 hours to standardize the culture to 10⁶ cfu/ml.

Screening of extracts for antibacterial activity

Potential of Extract as antimicrobial compounds was tested through adopting the method followed by [21]. With little modification, where 10 mg of each crude extract was dissolved in 10 % DMSO. The agar well diffusion method was used. Sterile nutrient agar plates were prepared. A cotton swab was used to inoculate the test organisms used, each plate was properly labelled. A sterile cork borer (6 mm) was used to make ditches in each plate for the extract. 50 µL of the reconstituted extract with the concentration of 10 mg/ml was dispensed into each ditch. The plates were left to allow for diffusion of extract before incubation at 37°C for 24 hours. The zones of clearance produced around the ditches after incubation were observed, measured and recorded. Gentamycin impregnated disc was used as the standard (125 mg/ml).

Determination of Minimum Inhibitory Concentration (MIC)

MIC determination was achieved through adopting the method followed by [21], with some modification, where test tubes was replaced with 96 wells. The broth dilution method was used, the first test tubes contains 100 µL of MHB,

while the rest 7 wells contains 50 μ L of MHB each. 50 μ L of reconstituted extract at 10 mg/ml was dispensed into the first well. After one fold serial dilution, the wells were inoculated with a 50 μ L solution of the test organisms. Three extra wells, the first containing MHB only, the second containing MHB plus extract and the third containing MHB plus the test organisms were incubated along with the batch of the test tubes at 37°C for 24 hours. MTT (dimethylthiazol-2-yl]-2, 5 diphenyl tetrazolium bromide) was added on the overnight incubated 96 well plate, 20 μ L of MTT (1 mg/ml) was added to each well, followed by subsequent incubation of the plates for 30 minutes. After which the concentration that shows no visible growth was considered the minimum bactericidal concentration. The changes of natural colour of MTT from yellow to purple colour Formazan was observed and recorded along the dilution gradient.

RESULTS

Screening of extracts for antibacterial activity

The results showed that bark and combination of stem with it bark extracts had inhibitory effect against all gram positive bacteria studied. Seed extract have no any antibacterial activity against all the bacteria tested. All the extract used were susceptible against *S.aureus*. Except fruit and seed extract. However Bark extracts found to be more potent against *S.aureus* 33591 at the concentration used with zone of inhibition 14.0 ± 0.50 mm. Followed by Stem with bark extract having zone of inhibition 13.5 ± 1.50 mm. While for *B.Cereus* only bark and stem with bark were susceptible having zone of inhibition 10.67 ± 1.37 mm and 10.0 ± 0.50 mm respectively. While *E.coli* 25922 only fruit and leaf were able to show antibacterial activity with 10.0 ± 1.00 mm each. From this experiment the most susceptible one was found to be *S. aureus*, and the most resistant bacteria was *P. aerogenosa* (table 1)

Table 1: Antibacterial screening from Methanolic extract of different part of *Moringa oleifera* Lam. on gram positive and gram negative bacteria (mm)

| plant extract | <i>S.aureus</i> 33591 | <i>B.Cereus</i> 10876 | <i>E.coli</i> 25922 | <i>P.aerogenosa</i> 12453 |
|----------------|-----------------------|-----------------------|---------------------|---------------------------|
| Bark | 14.0 \pm 0.50 | 10.67 \pm 1.37 | - | - |
| Stem | 12.0 \pm 1.00 | - | - | - |
| Stem with bark | 13.5 \pm 1.50 | 10.0 \pm 0.50 | - | - |
| Fruit | - | - | 10.0 \pm 1.00 | - |
| Leaf | 12 \pm 0.50 | - | 10.0 \pm 1.00 | - |
| Seeds | - | - | - | - |
| <i>n=3</i> | | | | |

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was tested using MTT (dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide), where by yellow tetrazole, is reduced to purple formazan in the presence of living cell. The intense of the purple colour formation was categorised into three as follows deep purple (+++), moderately purple (++) and bright (+) (table 2). The deep purple colour indicate the inability of the extract to eliminate the bacterial cell, while bright colour shows presence of low number of bacterial colony. The susceptibility strength of the bacterial used was found to be increasing in this order *S. aureus* > *E. coli* > *P. aeruginosa* > *B. cereus*. The activities of the extract were found to be low at the concentration used. In terms of *S. aureus* the extract from leaf, stem with bark and bark were found to show appreciable bactericidal activity at various concentration. Bark and stem with bark were found to have high activity against *B. cereus*. For *E. coli* the best activity was found from leaf, followed by stem with bark and bark. Where're stem with bark and leaf revealed high bactericidal effect on *P. aeruginosa*. Followed by bark extract.

Table 2: Minimum inhibitory concentration (MIC) of different extract from *Moringa oleifera* Lam.

| Samples | Concentrations (mg/ml) | <i>S. aureus</i> | <i>B. cereus</i> | <i>E. coli</i> | <i>P.aeruginosa</i> |
|---------|------------------------|------------------|------------------|----------------|---------------------|
| | | colour change | colour change | colour change | colour change |
| Leaf | 10 | + | + | + | + |
| | 0.7 | + | + | + | + |
| | 0.46 | + | ++ | + | + |
| | 0.31 | ++ | ++ | ++ | + |
| | 0.21 | ++ | +++ | ++ | ++ |
| | 0.14 | ++ | +++ | +++ | ++ |
| | 0.09 | ++ | +++ | +++ | +++ |
| Stem | 10 | ++ | +++ | + | +++ |
| | 0.7 | ++ | +++ | ++ | +++ |
| | 0.46 | +++ | +++ | +++ | +++ |
| | 0.31 | +++ | +++ | +++ | +++ |
| | 0.21 | +++ | +++ | +++ | +++ |
| | 0.14 | +++ | +++ | +++ | +++ |
| Bark | 10 | + | + | + | + |
| | 0.7 | + | + | + | + |

| | | | | | |
|--|------|-----|-----|-----|-----|
| | 0.46 | ++ | + | + | ++ |
| | 0.31 | ++ | ++ | ++ | ++ |
| | 0.21 | ++ | +++ | ++ | +++ |
| | 0.14 | +++ | +++ | +++ | +++ |
| | 0.09 | +++ | +++ | +++ | +++ |
| Seed | 10 | ++ | +++ | ++ | +++ |
| | 0.7 | ++ | +++ | +++ | +++ |
| | 0.46 | +++ | +++ | +++ | +++ |
| | 0.31 | +++ | +++ | +++ | +++ |
| | 0.21 | +++ | +++ | +++ | +++ |
| | 0.14 | +++ | +++ | +++ | +++ |
| Fruit pods | 0.09 | +++ | +++ | +++ | +++ |
| | 10 | ++ | +++ | ++ | +++ |
| | 0.7 | +++ | +++ | +++ | +++ |
| | 0.46 | +++ | +++ | +++ | +++ |
| | 0.31 | +++ | +++ | +++ | +++ |
| | 0.21 | +++ | +++ | +++ | +++ |
| Stem with bark | 0.14 | +++ | +++ | +++ | +++ |
| | 0.09 | +++ | +++ | +++ | +++ |
| | 10 | + | + | + | + |
| | 0.7 | + | + | + | + |
| | 0.46 | ++ | + | + | + |
| | 0.31 | ++ | ++ | ++ | + |
| | 0.21 | ++ | +++ | +++ | + |
| | 0.14 | ++ | +++ | +++ | ++ |
| | 0.09 | +++ | +++ | +++ | ++ |
| <i>deep purple (+++), Moderately purple (++)</i> , bright (+). | | | | | |

DISCUSSION

Screening of extracts for antibacterial activity

Many researches have proven that various parts of *Moringa oleifera* such as bark, stem, flowers, roots as well as seeds have antimicrobial activities [6, 11, 12]. *Moringa oleifera* have been considered as bactericidal agent after the discovery of several antibacterial constituents with inhibitory activity against many bacteria [13]. From the result obtained bactericidal effect of different part of *Moringa oleifera* extract have shown weak activity against tested organisms at the concentration used, as the approximate growth inhibition zones found to be almost half of that observed from control (Gentamycine), this agree with the result obtained by [22] who discovered the weakness of antimicrobial effect of *Moringa oleifera* leaves extract, where the growth inhibition zones did not reach 1.5 mm. Nevertheless, the result contradict with the findings of [23] whom obtained a significant result from ethyl acetate fraction of fruit and bark as well as Chloroform fraction of fruit and leaf of *Moringa oleifera*, and the result revealed inhibition zone ranging from 9-38 mm from different organisms. This variation may be source from solvent, organisms and concentrations used. the current study shows that out of all the bacterial species used gram positive bacteria was found to be more susceptible, especially *S. aureus* this correspond to the findings of [24]. But the most resistant bacteria was found to be *P. aeruginosa*. Seed was found to have no any antibacterial activity from the concentration used, this contradict the result obtained by [25, 26], although the source of variation may be due the differences in concentration and the solvent used for the extraction.

Minimum inhibitory concentration (MIC)

MTT assay was used to measure cell viability and proliferation, this help to determine the minimum inhibitory concentration (MIC). MTT is taken up by cells via endocytosis and eventually reduces MTT formazan, which gathers especially in endosomal or lysosomal part of the cell, and subsequently conveyed to the cell surface via exocytosis [27]. Hence the colour of MTT, is either reduced to purple formazan in the presence of cells or remains when the cells are absent. In the present study the MTT was slightly reduced along the concentration gradient. This indicate that the concentration used has little bactericidal activity. This agree with the finding of [28]who revealed that methanolic crude extract of *Moringa oleifera* have no activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Nevertheless, [29] obtained 50 mg/ml being effective minimum bactericidal concentration of Moringa seed extract which inhibited the growth of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* compare to the greater concentration used in our study (10 mg/ml). Different part of *Moringa oleifera* have been reported to contain phytochemicals compounds that have either bacteriostatic or bactericidal activity.[14, 15, 16] reported the presence of phytochemicals compounds such as flavonoids, tannins saponins, and other phenolic compounds that have antimicrobial properties from Moringa leaf. Nevertheless, [17] reported the presence of polyphenolic compounds which can be related to antimicrobial agents. [18]Added that *Moringa oleifera* is an excellent source of compound known as pterygospermin, which believe to inhibit the growth of both bacteria

and fungi. Furthermore, compounds such as benzyl isothiocyanate, benzyl glucosinolate, and pterygospermin have been tested against many bacteria, which revealed bactericidal and bacteriostatic activity [6].

CONCLUSION

Infectious diseases remains global public health problems. Hence there is need of researchers to engage in finding way out, to manage their consequences and also reduce the influence of antimicrobial resistivity against many drugs.

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