

Determination of Antibacterial Activity of Aqueous Extract of *Moringa oleifera* Leaves against *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa***Manga SS¹, Isah M¹, Kalgo MZ², Bazata AY²**¹Department of Biological Sciences, Kebbi State University of Science and Technology Aliero, Kebbi State, Nigeria²Department of Microbiology, Federal University Birnin Kebbi, Kebbi State, Nigeria***Corresponding author**

Manga SS

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Abstract: Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking for remedies and health approaches from side effects caused by synthetic chemicals. This has aggravated the search for antimicrobials from plants sources. Therefore, this study was aimed at investigating the efficacy of aqueous extract of *Moringa oleifera* leaves against some clinical isolates (*Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*). The study was carried out between January and June, 2014. The *Moringa oleifera* leaves sample were collected from the Kebbi State University of Science and Technology Aliero Orchard and screened for antimicrobial activity against the isolates using agar well diffusion assay, and preliminary phytochemical analysis were also conducted using standard procedures. Concentrations of the extract used were 30mg, 60mg, 90mg and 120mg. The extract demonstrated the highest activity at 120mg, with the zone of inhibition of 28.5mm, 25.5mm and 24.0mm on *Salmonella typhi*, *Escherichia coli* and *Pseudomonas aeruginosa* respectively. The results of preliminary phytochemical screening revealed the presence of Saponins, Tannins, Flavonoids, Glycosides and Alkaloids. The antibacterial assay results portrayed broad spectrum activity of *Moringa oleifera* leaf extract against the test organisms with the comparable inhibitory zones by standard antibiotic (Ciprofloxacin). In conclusion the results from these findings have shown the antibacterial potentials of *Moringa oleifera* leaf extract implying that the extract could help as a chemotherapeutic agent or might be a lead compound for the development of new potent antibacterial agents.

Keywords: *Moringa oleifera*, Aaqueous, Extract, Clinical, Isolates

INTRODUCTION

Moringa oleifera is a small to medium-sized deciduous tree up to 10m-12m in height that develops a swollen underground rootstock. It produces large elongated capsules each containing numerous seeds, the tree also has an open crown dropping fragile branches with flowers, feathery foliage of trip innate leaves and thick corky whitish bark. It is often cut back annually to 1 - 2 meters and allowed to re grow. The pods and leaves remain within arm's reach [1]. The moringa plant has been the object of much research due to its multiple uses and well-known bactericidal potential [2-5]. *Moringa oleifera* is a well-documented world renowned plant herb for its extraordinary nutritional and medicinal properties. It is a natural antihelmintic, antibiotic, detoxifier, outstanding immune builder and is used in many countries to treat malnutrition and malaria. It is also used in water purification and therefore helps in reducing the incidence of water borne diseases [6]. The Moringa plant which is a native of north-eastern India has been refers to by many names in Nigeria, Zogale and Bagaruwar makka (Hausa), Ewe igbale and idagbo Monoye (Yoruba), Ikwa oyibo (Igbo) and Kabi (Kilba) [7].

The indiscriminate use of antibiotics and several other factors has led to the emergence of multidrug-resistance pathogens [8]. The plants of medicinal value are said to have minor side effects compared to the chemical agents [9]. In addition, certain antibiotics present undesirable side effects such as nausea, depression of bone marrow, thrombocytopenic purpura and agranulocytosis leading to the emergence of previously uncommon diseases [10,11]. This has given scientist the impetus to search for newer and alternative microbial compounds from medicinal plants [12]. Besides, the high cost of conventional drugs, particularly in resource limited communities. This has increased the use of plants as an alternative for treatment of infectious diseases.

Plant extracts and phytochemicals with antimicrobial properties are of great significance in therapeutic treatments. Their antimicrobial properties are due to compounds synthesized in the secondary metabolism of the plant. The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential

source of novel antibiotic prototype [13]. The plant extract of *Moringa oleifera* have been recently found to contain the following phytochemical constituents; alkaloids, saponins, tannins and phenols [14]. The presence of of these phytochemical constituents has been reported to account for the exertion of antimicrobial activity by the plants [15]. Moringa tree is rich in nutrients and apart from a range of industrial and medicinal applications, is used to purify water for human consumption [16]. Not surprisingly, Moringa oleifera is of economic importance in the production of several commodities such as; oils, foods, condiments and medicines [17]. Many studies had demonstrated the potential activity of different parts of moringa plant in vitro, against medically important pathogens, using various extract [7,18–21]. The susceptibility of microorganisms to antimicrobials differs between countries or even regions in the same country, this variation may be attributed to differences in geographical locations and community saturation of these antimicrobials. Therefore the objective of this study was to evaluate the efficacy of aqueous extract of *Moringa oleifera* leaves against some clinical isolates (*Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*) in the study area.

MATERIAL AND METHODS

Sample collection

Leaves of *Moringa oleifera* were collected from the Kebbi State University of Science and Technology Aliero orchard. It was ensured that the plant was healthy and uninfected. The botanical identification was carried out (with voucher No. 121) at the Biological Sciences Department of Kebbi State University of Science and Technology, Aliero.

Sample Processing

The leaves were washed under running tap water to eliminate dust and other foreign particles and air-dried for two weeks in an area protected from light to prevent loss of vitamins and protected from dust and pest to avoid contamination. The dried leaves were then made into powder using a sterile mortar and pestle, the powder was sieved to remove large particles later collected in a sterile container and stored for future use.

EXTRACTION OF PLANT MATERIALS

Aqueous extract

Plant material (50g) was crushed in sterile water (150 ml) for preparation of aqueous extract as described by Himal *et al.*, (2008) [22]. The extract was separated using sterile muslin cloth and filtered through sterile Whatman filter paper (no. 02).

Phytochemical analysis

The extracts were analyzed by the following procedures as described by Parekh and Chanda [23] to test for the presence of the alkaloids, saponins, tannins, Terpenoids, flavonoids, volatile oils and glycosides

Test Microorganisms

The stock culture of the test organisms used (*Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*), were isolated from clinical cases and provided by Microbiology laboratory of Usmanu Danfodiyo University Teaching Hospital, Sokoto. The isolates were sub cultured on agar slants and stored at 4°C until prior to being used.

Preparation of concentration of the plant leaves extract

Concentration of extract 30mg, 60mg, 90mg and 120mg were measured using weighing balance and dissolved in 5ml of distilled water in sterile test tubes and allowed to soak for 24 hours before [24].

Antibacterial activity

The antibacterial activity of the leaf extracts were identified using agar well diffusion method. Mueller Hinton agar was prepared according to the manufacturer's instruction. The test organisms were inoculated by spread plate method. Wells were punched using punch borer (6 mm) in the agar and loaded with plant extracts. Control wells containing neat solvent and ciprofloxacin were also run parallel in different plate. The plates were allowed to stand for 15 minutes on the bench to allow pre-diffusion of the extract to take place and then incubated at 37°C for 24 hours and the antibacterial activity was assessed by measuring the diameter of the zone of inhibition.

RESULT

The result of the phytochemical screening is presented in Table and revealed that the leaves aqueous extract of *M. oleifera* contains some secondary metabolites such as Tannins, Alkaloids, Saponins, Flavonoids, and Glycosides. The antibacterial activity of the extract is presented in Table 2. The extract had the largest diameter zone of inhibition on *S. typhi* and *E. coli* at 120 mg/ml. Also, it was observed that the concentration of extract resulted in diminished activity on *P. aeruginosa*.

Table-1: Phytochemical components of *M. oleifera* Aqueous Leaves Extract

S/NO	Phytochemicals	<i>Moringa oleifera</i> leaves
1	Alkaloid	+
2	Flavonoid	++
3	Tannins	+++
4	Saponins	+
5	Volatile oils	-
6	Glycosides	++

KEY:

- = absent

+ = slightly present

++ = moderately present

+++ = highly present

Table-2: Antibacterial Activity of *M. oleifera* aqueous leaves extract

Test Organism	Zone of Inhibition (mm)					
	Concentration of Plant extract (mg/ml)					
	30	60	90	120	Cfl	Water
<i>S. typhi</i>	15.0±0.4	22.0±0.0	25.5±0.7	28.5±0.4	37.5	-
<i>E. coli</i>	15.5±0.7	20.5±0.7	22.5±0.4	25.5±0.6	32.5	-
<i>P. aeruginosa</i>	13.0±0.4	20.0±0.6	22.5±0.7	24.0±0.4	29.5	-

KEY:

Cfl = Ciprofloxacin

DISCUSSION

The result of the phytochemical screening from Table 1 shows that the leaves aqueous extract of *M. oleifera* contains some secondary metabolites such as Tannins, Alkaloids, Saponins, Flavonoids, and Glycosides. No other group of natural product has contributed to medicine and pharmaceuticals preparation than alkaloids. As a group, it displays an exceptionally wide array of biological activity [25]. Flavonoids are strong antioxidants and are effective antibacterial substances *in vitro* against a large number of microorganisms by inhibition of the membrane-bound enzymes [26]. They also showed substantial anticarcinogenic and antimutagenic activities due to their antioxidant and anti-inflammatory properties [27]. Tannins are a group of polymeric phenolic compounds and cause local tumours. They are able to inactivate and kill microorganisms. Glycosides are also present in the leaves extract of the plant, this shows that, the leaves of *M. oleifera* can be used in the treatment of heart related diseases as reported by [28]. Saponins possess antioxidant, anti-inflammatory, antiapoptosis and immunostimulant properties [29]. The presence of Saponins in *M. oleifera* leaves extract was also confirmed.

The development of microbial resistance to the available antibiotics has informed the need to explore natural disease control options which has led to further investigation of antimicrobial activity of some medicinal plants [30]. *M. oleifera* leaf extract inhibited the growth of almost all the test organisms with varying effectiveness. This study is in line with the study conducted by [31] that indicates antibacterial activity of leaf extract increased with increase in extract concentration. This is an indication of the presence of antibacterial properties in the plants extract of *M. oleifera*. The potential for developing antimicrobial from higher plants appears rewarding, as it will lead to the development of a phytomedicine to act against microbes. Plant based antimicrobial represents the vast untapped source for medicine.

The plant extract under study had varied extents of antibacterial activities which were concentration-dependent. The extract had the largest diameter zone of inhibition on *S. typhi* and *E. coli* at 120 mg/ml as seen in table 2 above. Also, it was also observed that the concentration of extract resulted in diminished activity on *P. aeruginosa*. The antibacterial activities of the extract suggest that the plant could be of relevance in the treatment of infections caused by these organisms. Similar research conducted by [24] showed reasonable zone of inhibition against the test organisms and stop at (90mg/ml). Ciprofloxacin, the control antibiotic had greater diameter zone of inhibition on the test microbes than that of plant extract. Studies have shown that the antimicrobial potential of *M. oleifera* leaves extract may be attributable to the presence of an array of phytochemicals. At concentration of 30mg, 60mg, 90mg and 120mg, the extract was active against the test organisms, but had the largest diameter zone of inhibition at 120mg concentration. The most susceptible organisms to the antibacterial activity of the extract at (120mg/ml) were *S. typhi* and *E. coli* while the least susceptible was *P. aeruginosa*.

CONCLUSION

The present study verified the antibacterial activity of aqueous extract of *M. oleifera* leaves for some human pathogens (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) and partly explained its use in herbal medicine as rich source of phytochemicals with the presence of tannins, alkaloids, saponins, flavonoids and glycosides. Thus this plant can be utilized as an alternative source of useful drugs.

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