

PHYTOCHEMICAL AND ANTIBACTERIAL PROPERTIES OF *MORINGAOLEIFERA* EXTRACTS AGAINST SELECTED BACTERIA ISOLATES.

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Abstract

The phytochemical screening and antibacterial activity of *Moringaoleifera* plant were carried out to determine the efficacy of its parts against some selected bacterial isolates of medical importance. The aqueous and methanolic extracts of *Moringaoleifera* tested positive for alkaloids, tannins, flavonoids, steroids and saponins in the three plant parts of leaves, stem bark and root. Glycosides tested positive in the three plant parts of the extracts. The acetone extracts of *Moringaoleifera* tested positive for steroids, tannins, flavonoids, alkaloids, saponins and glycosides in the three plant parts. The acetone extracts of the leaf, stem bark and root of *Moringaoleifera* shows the highest antibacterial activity against *Pseudomonas aeruginosa*, *Klebsiellasp.*, *Escherichia coli*, and *Staphylococcus aureus* isolates, followed by the methanolic extracts of the three plant parts. The aqueous extracts showed the least antibacterial activity against all the bacterial isolates. Acetone and methanolic extracts from the stem bark showed antibacterial activity against *Salmonella typhi*. The zones of inhibition for all the antibacterial activity in this study revealed 2.0mm as the minimum value and 22.0mm as the maximum value against all the bacterial isolates. The phytochemical constituents of the extracts from *Moringaoleifera* plant could be used to combat infections that might arise from the tested bacterial isolates.

Keywords: *Moringaoleifera*, phytochemical, antibacterial activity, bacterial isolates.

INTRODUCTION

Phytochemical screening is the determination of the active chemical constituents (curative compounds) present in plants. These active chemical constituents include steroids, alkaloids, flavonoids, saponins, tannins, etc. These active compounds could delay or prevent the on-set of degenerative diseases, because of their redox properties which allow them to act as hydrogen donors, reducing agents and super-oxide radicals (Govindaraj et al., 2005).

Moringaoleifera is the most widely cultivated species of a monogeneric family, the Moringaceae, that is native to western and sub-himalayan region of India, Pakistan, Asia minor, Africa and Arabia (Mughaiet al., 1999). The plant is now distributed in the Philippines, Cambodia, central, north and south America and the Caribbean Island (Broinet al., 2002). The tree is rather slender with drooping branches that grow to approximately 10m in height (Mughaiet al., 1999) its bark, roots, flowers, leaves, seeds, and gum are used medicinally as antiseptics for treating rheumatism, venomous bites, and other conditions (Dillard and Dillard, 2000).

The different parts of the plant contain a profile of important minerals and good sources of proteins, vitamins, B-carotene, amino acids, and various phenolics (Farooq et al., 2007). A number of medicinal properties have been ascribed to various parts of this highly esteemed tree. Almost all parts of this plant (roots, stem bark, gum, leaves, fruits, flowers and seeds) have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastro-intestinal, hematological and hepatorenal disorders (Singh and Ksumar, 1999).

Moringaoleifera root have antibacterial activity (Rao et al., 1996), and are reported to be rich in antimicrobial agents. The roots contain pterygosperrum, which has powerful antibacterial and fungicidal effects (Ruckmani and Kavimani, 1998). The aglycone of deoxy-

niazimicine (N-benzyl, 5-ethyl thioformate) isolated from the chloroform fraction of an ethanol extract of the root bark was found to be responsible for antibacterial and antifungal activity (Nikon et al., 2003). The juice from the stem bark showed antibacterial effect against *Staphylococcus aureus* (Gayatri et al., 2010).

The fresh leaf juice was found to inhibit the growth of microorganisms such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* that are pathogenic to man (Cacere et al., 1991). The seeds also possess antimicrobial properties (Olsen, 1987; Madsen et al., 1987). Broinet al., (2002), reported that a recombinant protein in seed is able to flocculate gram positive and gram negative bacterial cells. The seeds may also act directly upon microorganisms and thus results in growth inhibition. Antimicrobial peptides are thought to act by disrupting the cells membrane or inhibiting essential enzyme. (Silvestro et al., 2003; Suarez et al., 2003)

This research work is set to know the phytochemical constituents and antibacterial activity of *Moringaoleifera* in order to elucidate their potentials in the treatment of diseases.

MATERIALS AND METHODS

Collection of Plant materials

The leaves, stem bark and roots of *Moringaoleifera* were collected around the staff quarters of Ibrahim Badamasi Babangida University, Lapai, Niger state. The plant parts were taken to the herbarium of the Department of Biological Sciences, Ibrahim Badamasi Babangida University Lapai for proper identification.

Drying of the Plant Parts

The plant parts (leaves, stem bark, and roots) were air dried for a month in a well ventilated room in the laboratory of the Biological Sciences Department, Ibrahim Badamasi Babangida University, Lapai. The plant parts were constantly turned to prevent them from getting

rotten. The purpose of air drying was to prevent ultraviolet rays of the sun from destroying the active ingredients in the plant parts. The dried plant parts were later pound into powder using wooden mortar and pestle.

Extraction

Extraction was done by filtration method; three solvents were used for the extraction, which are acetone, water and methanol. Powdered plant parts (12.5g) of *Moringaoleifera* were dissolved in 100ml acetone, water and methanol separately. The mixtures were kept for two days in tightly sealed containers at room temperature, stirred several times daily with a sterile glass rod. The mixtures were each filtered through filter paper containing cotton wool. To eliminate the solvent, the supernatant was evaporated using a 'Buuchi – R – 200' evaporator at 60°C under vacuum. The extracts were stored in refrigerator (4°C) and used within 24hrs. The dried extracts were exposed to ultra violet rays for 24hrs and check for sterility by plating on NA. The extracts were weighed, and a portion of each used for phytochemicals screening, while the rest were used for antibacterial activity determination (Okigbo, and Igwe, 2007).

Phytochemical Screening

The phytochemical screening were carried out on the acetone, aqueous and methanolic extracts for the qualitative determination of phytochemical constituents as described by Sofowora,(1993) and Trease and Evans, (1999.).

Determination of Antibacterial activity

Clinical strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhii*, *Escherichia coli* and *Klebsiella* spp were obtained from the Department of Microbiology, Federal University of Technology, Minna, Niger state, Nigeria. The well method (Kirby-Bauer) as described by Oluma et al.,(2004) was employed to assay the plant extracts for antibacterial activity. Mueller Hinton agar was prepared according to the manufacturer's instruction and dispensed into the Petri-dishes, and was allowed to solidify. The test bacteria isolates were inoculated using a sterile cotton swab on the solidified agar medium. Six millimeter diameter wells were punched using cork borer in agar and filled with the desired concentrations of extracts using sterile syringe and needle. The inoculated plates were allowed to stand for 4 hours at room temperature for extract to diffuse into the agar, after which the plates were incubated at 37°C for 24 hours. Zones of inhibition were measured using a meter rule and the mean recorded in millimeters as described by Mukherjee et al. (1995).

RESULTS

The phytochemicals screening carried out on the extracts from the three different solvent (Table 1), revealed that alkaloids, tannins, flavonoids, steroids, saponins and glycosides were present as active chemical constituents in the three different parts of *Moringaoleifera* plant.

Table (2) shows the result of the antibacterial activity of *Moringaoleifera* leaf extracts against the listed bacteria. Acetone and methanolic extracts showed the high efficacy against *Klebsiella* sp. and *E.coli*. *Salmonella typhii* was resistant to the extracts regardless of the extractant. However, all the tested bacteria except *Klebsiella* sp. were resistant to the aqueous extracts.

Table(1) The phytochemicals screening of the Aqueous extracts of *Moringaoleifera* plant.

TEST	Mr ₁	Mr ₂	Mr ₃
Alkaloids	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Steroids	+	+	+
Saponins	+	+	+
Glycosides	+	+	+

KEY + = present; - = absent; Mr₁= extract from the leaves; Mr₂ = extract from the stem bark .Mr₃ = extract from the root.

Table(2) Antibacterial activity of *Moringaoleifera* leaf extract against some bacterial isolates

Isolates	Acetone extract	Aqueous extract	Methanolic extract
Diameter of zone of growth inhibition in mm(5mg/ml)			
<i>Klebsiella</i> sp	22.0	12.0	17.0
<i>Salmonella typhii</i>	0.0	0.0	0.0
<i>Escherichia coli</i>	22.0	4.0	12.0
<i>Staphylococcus aureus</i>	7.0	0.0	2.0
<i>Pseudomonas aeruginosa</i>	4.0	0.0	2.0

Table(3) Antibacterial activity of *Moringaoleifera* stem bark extract against some bacterial isolates.

Isolates	Acetone extract	Aqueous extract	Methanolic extract
Diameter of zone of growth inhibition in mm(5mg/ml)			
<i>Klebsiella</i> sp	8.0	0.0	9.0
<i>Salmonella typhii</i>	9.0	0.0	4.0
<i>Escherichia coli</i>	20.0	0.0	2.0
<i>Staphylococcus aureus</i>	8.0	0.0	7.0
<i>Pseudomonas aeruginosa</i>	7.0	0.0	2.0

Acetone extract showed highest efficacy against *Escherichia coli*, and was also moderately active against *Klebsiella* spp (Table.3). Aqueous extract could not inhibit any of the bacterial isolates. All the bacterial isolates showed either moderate or total resistance to the extract from the roots of *moringaoleifera* plant (Table 4) Aqueous extracts could not inhibit any of the bacterial isolates.

Table(4)Antibacterial activity of *Moringaoleifera* root extract against some bacterial isolates.

Isolates	Acetone extract	Aqueous extract	Methanolic extract
Diameter of zone of growth inhibition in mm(5mg/ml)			
<i>Klebsiellasp</i>	8.0	0.0	7.0
<i>Salmonella typhii</i>	0.0	0.0	0.0
<i>Escherichia coli</i>	4.0	0.0	2.0
<i>Staphylococcus aureus</i>	8.0	0.0	4.0
<i>Pseudomonas aeruginosa</i>	7.0	0.0	2.0

DISCUSSION

The result shows the presence of alkaloids, tannins, steroids, saponins, and flavonoids in all the three parts of the plants. Farombi (2003) and Okigbo *et al.*, (2009) earlier reported the presence of steroid, flavonoid, saponin, tannin, alkaloid and glycoside in many plants. These secondary metabolites present in the leaf, stem bark and root extracts of the plant might be responsible for the medicinal properties of the plant, and hence their efficiency in the treatment of numerous diseases. According to Francis *et al.*, (2002) the medicinal values of plants lie in their phytochemical components such as, alkaloid, tannin, flavonoid, and phenolic compounds which produce a definite physiological action on human body. To justify the findings in this study, Salah *et al.*, 1995; Del-Rio *et al.*, 1997 and Okwu, 2004 reported that, medicinal plants with health promotion effects have been documented to contain phytochemical constituents such as alkaloids, terpenoid, steroids and glycosides.

The results of antibacterial activity of the leaf of *Moringaoleifera* obtained in this study confirmed the earlier report that *Moringaoleifera* leaf extract inhibited the activities of *Escherichia coli*, *Salmonella typhii*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Gayatri *et al.* (2010). However, the report that the juice from the stem bark showed antibacterial effect against *Staphylococcus aureus* by Gayatri *et al.*, (2010) was not agreed with the findings in this study, because *Staphylococcus aureus* showed resistance to the stem bark extracts. But in agreement with Bolin and Satyabrat (2011), report that methanolic extract of the stem bark of *Moringaoleifera* has no significant antibacterial activity against *S. aureus* and *P. aeruginosa*, but showed potency against *E. coli*. The result of antibacterial activity of *Moringaoleifera* root extract revealed that all the bacterial isolates showed very low sensitivity towards the acetone and methanolic extracts. The aqueous extract was totally resisted by the bacterial isolates. This result conformed to the findings of Gayatri *et al.* (2010), where the methanolic, acetone and aqueous extracts of *M.oleifera* root bark were not active towards *E. coli*, *S.aureus* and *P.aeruginosa*.

CONCLUSION

Moringaoleifera have medicinal values due to the presence of some or all listed phytochemical constituents in some or all the parts. Thus, this plant could be used in the treatment of various diseases, depending on which phytochemical constituents it contains, and what function the constituent perform in the treatment of a particular disease.

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