Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2015; 3(3):320-324 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

Studies on the Phytochemical and Antibacterial Activities of Aqueous and Ethanol Extracts of *Psidium guajava* and *Moringa oleifera*

***Okechukwu RI[1], Ujowundu CO[2], Okika WO[1], Ukaoma AA[1], Anuforo HU[1], Ezea CO[1]**
[*1]Department of Biology, Federal University Technology Owerri.
[2]Department of Biochemistry, Federal University Technology Owerri, Nigeria.

***Corresponding author**
Okechukwu RI
E mail: rositaokechukwu@yahoo.com

Abstract: Extracts of the leaves, bark and root of *Psidium guajava* as well as the leaves *of Moringa oleifera* were investigated for antibacterial activity against *Staphylococcus aureus, Streptococcus spp, Klebsiella spp, Proteus spp* and *Psuedomonas spp*. The disc diffusion method was adopted using extract concentrations of 10-280 mg/ml. The qualitative phytochemical compositions of both plants were studied. Phytochemical test on the extracts revealed the presence of saponins, alkaliods, tannins, flavoniods, steroids, phenol and glycosides. Minimum Inhibition concentration (MIC) was obtained by plotting logarithm of the concentrations of the extract against the squares of the zone of inhibition. The antilogarithm of the intercept gave the MIC values. Leaves extract of *Moringa oleifera* at 280 mg/ml showed good inhibitory effect against *Streptococcus spp*, comparable to synthetic antibiotics such as Ciproflox and Reflacine. Similarly, the inhibitory effect of *P. guajava* bark at 280 mg/ml against *Klebsiella spp* competed favourably with Reflacine. Extracts of *P. guajava* leaves, root and the synthetic drug Reflacine gave equal antimicrobial effect against *Pseudomonas spp.* These results indicate that extracts of *P. guajava* and *M. oleifera* can be used for the treatment of pathogenic infections caused by some bacteria.
Keywords: *Psidium guajava, Moringa oleifera,* pathogenic bacteria, phytochemicals, inhibition.

INTRODUCTION

The use of plants for medicinal purposes lies on the ability of its chemical constituents to illicit biochemical and physiological actions in living systems. These chemical known as secondary metabolites or phytochemicals have made inroads in pharmacology, biochemistry, microbiology and medical sciences [1]. Plants have limitless ability to synthesize these important chemicals of which about 10 % have been isolated and characterized [2].

Psidium guajava (Guava), is a widely cultivated ornamental fruit tree. Guava leaf extracts are used in various herbal formulations for a myriad of purposes ranging from herbal formula against diarrhea, dysentery, abdominal distention, flatulence to inhibition of prostatic reflex, gastroenteritis, spasmolytic activity [3] as well as anti-inflammatory and haemostatic agent [4]. Toxicity studies with rat, mice as well as controlled human studies show both leaves and fruits to be safe without side effects [5]. The leaves are used to produce tea [6]. Edible guava is rich in protein, carbohydrates, good levels of dietary fibres and minerals. It is also a good source of riboflavin, naicin as well as vitamin A [7].

Moringa oleifera belong to the family Moringaceae and it is commonly referred to as horseradish tree [8] as the taste of the root is similar to the horseradish oil or oil bean tree. In developing countries, Moringa has potentials to improve nutrition, boost food security, foster rural development, and support sustainable land care [9]. Moringa seed pods are particularly high in vitamin C and also serve as a good source of minerals such as potassium, magnesium, manganese and dietary fibres [10]. Besides the nutritive values of *M. Olifera*, diverse research has been carried out to identify the chemical constituents and pharmacological properties [11]. *M. Oleifera* is used as a natural anthelminthic and possible adjuvant [12].

Both *P. guajava* and *M. oleifera* are endowed with nutritive, health and pharmacological benefits, the interests in these plants led to our exploiting other potential properties embedded in them such as its antibacterial potentials against commonly encountered pathogenic microorganisms.

MATERIALS AND METHODS

Collection and Processing of the Plant Materials

The leaves, stem, bark and root of *P. guajava* and leaves of *M. oleifera* was collected from a farm in

the Federal University of Technology (FUTO) in Owerri West L.G.A of Imo State, Nigeria. The plants were identified by Dr. M.C. Duru of Federal University of Technology Owerri (FUTO).

The samples were air dried at room temperature (21-24 ^{0}C) and milled into powder using a sterile manual grinder. These were stored in airtight glass containers protected from light and heat until required for analysis.

Ethanol Extraction of samples

The processed leaves, stem, bark and root of *P. guajava* and leaves of *M. oleifera* were used. Ethanol extractions of the samples were achieved using Soxhlet extraction with ethanol (99%). Twenty grams (20 g) of each of the samples were separately filled into different Thimbles and 200 ml of ethanol was placed in the flat bottom flask and extraction done exhaustively.

Test Organisms

Gram-positive (*Staphylococcus aureus, streptococcus* specie) and Gram-negative (*Klebsiella* spp, *Proteus* spp, *Pse*udominas spp) bacterial were obtained from and confirmed at the Microbiology Laboratory in the Federal Medical Centre Owerri, Imo State, Nigeria. A 24 hours fresh culture was prepared in Nutrient Broth and used for the antibacterial activity test. The media used in all the tests were prepared as described by Wolfgang and Hilda [13].

Phytochemical Screening of the Extract

The phytochemical screening of *P. guajava* and *M. oliefera* for alkaloids, saponins, tannins, flavanoids, steroid, phenol and glycosides were analyzed according to the method described by Harborne [14].

Antibacterial Activity Test

The disc diffusion method was adopted for this study. Filter paper discs of 7 mm were soaked with diluted extract of each plant sample. Using a sterile cotton swab, each labeled medium plate was uniformly inoculated with a test organism and streaked on the plate surface in a form that lawn growth can be observed. The filter paper discs soaked with different extract were placed in the labeled Petri dishes and incubated at 37^{0}C for 24 hours and zones of inhibition measured in mm.

Minimum Inhibition Concentration (MIC) was obtained by logarithm of the concentration of extracts against the square of zones of inhibition. The MIC values equal the antilogarithm of the interception on the concentration axis. Sensitivity of extract was compared to different standard antibiotics drugs such as Tarivid, Reflacine and Ciproflox. These three antibiotics showed the highest level of inhibition amongst ten commonly used antibiotic drug in an earlier sensitivity test.

RESULTS

The result of the phytochemical study of the leaves, bark and root of *P. guajava* and leaves *M. Oliefera* is presented in Table 1. *P. guajava* leaves, bark and root and *M. Oliefera* leaves showed the presence of alkaloids, saponins, tannins, flavanoids and steroid. *P. guajava* bark and *M. oleifera* leaves tested positive for phenol and glycosides. *P. guajava* root tested negative for phenol, and glycoside was absent in *P. guajava* leaves.

Table 1: The phytochemical contents of leaves, bark and root of *P. guajava* and leaves *M. oliefera*

Plant part	Phytochemicals						
	Alkaloids	Saponins	Tannins	Flavonoids	Steroids	Phenol	Glycosides
A	+	+	+	+	+	+	−
B	+	+	+	+	+	+	+
C	+	+	+	+	+	−	+
D	+	+	+	+	+	+	+

A= leaf, B= bark and C =root of *P. guajava* and D=leaf of *M. oliefera*. + = present, − = absent

Table 2 shows the results of sensitivity test on common antibiotics. The result showed that Tarivid (OFX), Reflacine (PEF) and Ciproflox (CPX) are relatively more potent to the selected gram-positive and gram-negative bacteria.

The result of the antibacterial potentials of extracts of the leaves, bark and root of *P. guajava* and leaves *M. Oliefera* is shown in Table 3. At an extraction concentration of 280 mg/ml, *P. guajava* leaves, bark and root showed inhibition of bacterial activities specifically against *Staphylococcus aereus, klebsiella* specie and proteus specie. *P. guajava* leaves and roots did not inhibit streptococcus specie. Also, *P. guajava* bark did not inhibit the activities of pseudomonas specie. At an extract concentration of 140 mg/ml, *P. guajava* bark inhibited klebsiella and proteus species. Its root also inhibited proteus specie at 140 and 70 mg/ml extract concentrations. *M. oliefera* leaves inhibited streptococcus and klebsiella species at extract concentration 280 mg/ml. *M. oliefera* leaves also inhibited streptococcus specie at 140 mg/ml extract concentrations.

Our results indicates that plant extract of *P. guajava* showed a level of inhibition of both gram-

positive and gram-negative bacteria, inhibition occurred more in gram-negative bacteria. It is obvious that at low concentration (140 mg/ml), *P. guajava* bark showed inhibition against klebsiella and proteus specie. Its root extract inhibited the activities of proteus specie at concentration of 140 and 70 mg/ml. *M. oliefera* inhibited gram-positive streptococcus specie at an extract concentration of 140 mg/ml.

Table 2: Microbial sensitivity test on common (synthetic) antibiotics

Antibiotics	Staphylococuss aureus	Streptococcus spp	Klebsiella spp	Proteus spp	Pseudomonas spp
OFX	++	+	+++	+++	++
PEF	+++	+	+	+++	+
CPX	+++	++	+++	+++	++
AU	−	−	−	+	−
CN	++	++	+	++	−
S	++	+	++	++	++
CEP	++	−	−	+++	−
NA	+	−	−	++	−
SXT	++	−	−	+++	−
PN	−	−	+	+++	−

+ level of sensitivity; − not sensitive

OFX=Tarivid, PEF=Refacine, CPX=Ciproflox, AU= Augmentin, CN= Gentamycin, S=Streptomycin, CEP= Ceporex, NA=Nalidixic acid, SXT= Septrin, PN= Ampicilin

Table 3: Result of antibacterial activities of extracts of the leaves, bark and root of *P. guajava* and leaves *M. Oliefera*

samples	Conc. of test substance (mg/ml)	Diameter of zone of inbition (mm)				
		Staphylococuss	*Streptococcus* spp	*Klebsiella* spp	*Proteus* spp	*Pseudomonas* spp
A	280	2	−	2	4	2
	140	−	−	−	−	−
	70	−	−	−	−	−
	35	−	−	−	−	−
	10	−	−	−	−	−
B	280	2	2	4	6	−
	140	−	−	2	4	−
	70	−	−	−	−	−
	35	−	−	−	−	−
	10	−	−	−	−	−
C	280	2	−	4	8	2
	140	−	−	−	4	−
	70	−	−	−	2	−
	35	−	−	−	−	−
	10	−	−	−	−	−
D	280	−	4	2	−	−
	140	−	2	−	−	−
	70	−	−	−	−	−
	35	−	−	−	−	−
	10	−	−	−	−	−
OFX	0.01	8	4	8	24	6
PEF	0.01	12	4	4	24	2
CPX	0.01	8	8	10	24	6

A = leaf, B = bark and C = root of *P. guajava* and D = leaf of *M. oliefera*

DISCUSSION

Phytochemicals are plant secondary metabolites occurring in relatively high amount in several kinds of fruits, grains and vegetables harvested for human consumption [15]. Significant reduction of the risk factors, in significant human pathologies such as diabetes, cancer, neurodegenerative and cardiovascular diseases have been recently associated

with the prevalence of such food in the human diet [16,17,18].

These phytochemical contents indicate that well processed plant parts of *P. guajava* and *M. oliefera* may offer medicinal and chemoprotective benefits to its users [19]. Phytochemicals have diverse functions which include attraction of insects for pollination and feeding, provision of strength to plants, defense against predators, provision of colour, while some are simply waste products [20]. The presence of these phytochemicals demonstrated in this study, could be the source of strength and resistance exhibited by the *M. oleifera* even in harsh tropic conditions, when the plant maintains its lush green appearance. The presence of alkaloid may be the reason for its use in the treatment of alimentary tract diseases and gastro intestinal disorders. Alkaloids are beneficial chemicals to plants with parasitic and predator repelling effects. *M. oliefera* have been used for the treatment of urinary tract infection [21]. This indicates antimicrobial ability, as shown in the results of the antibacterial study.

Flavanoids have aroused considerable interest recently because of their potential beneficial effects on human health. They have been reported to have antiviral, anti-allergic, antiplatelet, anti-tumour and anti-inflammatory effect [22,23], while tannins are used as astringent.

Phytochemical analysis showed the presence of glycosides in *M. oleifera* leaves. A study identified a compound called Pterygosperm, which dissociates into two molecules of benzyl isothiocyanate [24]. Benzyl isothiocyanate has been reported to have antimicrobial properties against a variety of microbes [24]. This can be attributed to antimicrobial effects observed in the present study. The observed values in this study supports earlier studies which identified a number of glycosylated derivatives of benzyl isothiocyanate [25,26,27]. There was also a report on the antibiotic activity of the primary rhamnosylated compound [28] against a wide range of bacteria and fungi. Cultures of *Helicobacter pylori*, a major risk factor for gastric cancer [29] also turned out to be extraordinarily susceptible to 4-(L-rhamnopyranosyloxy) benzyl isothiocyanate and to a number of other isothiocyanates [30].

Our result (Table 3) showed the presence of flavonoids in the plants. This corroborates the report of [31] which showed the presence of quercetin-a flavonoid in the leaves of *P. guajava*. Much of guavas therapeutic activity can be attributed to flavonoids. Flavonoids have demonstrated antibacterial activities. Quercetin is thought to contribute to the anti diahorrea effect of *P. guajava*; it is able to relax intestinal smooth muscles and inhibit bowel contractions [32]. This can be attributed to its rich content of morphine. Ethanolic leave extract shows morphine-like effect by inhibiting the gastrointestinal release of chemicals in acute diarrhea disease [32].

In addition, chemicals in *Psidium guajava* were shown to bind to *E. coli* (a common diarrhea-causing organism) preventing its adhesion to the intestinal wall and thus preventing infection [32]. This action may be a result of the presence of lectin [32] or tannins. Tannins are astringents; they are also known to form complexes with macromolecules such as proteins. Complexes formed with peripheral or integral proteins embedded in the cell membrane of bacterial could disrupt the integrity of bacterial cell membrane, which is bacteriostatic or bacteriocidal to microbes. In several studies, bark and leaves extracts of *Psidium guajava* have been reported to show significant antibacterial activity against both gram-positive and gram-negative bacteria including fungi [31,11,3,4]. Although standard drugs such as Ciproflox, Reflacine and Tarivid appeared to be more sensitive, compared to crude extracts of *M. Oleifera* and *P. guajava*, isolation of the active ingredient in both plant extracts may provide a more sensitive antibacterial activity.

REFERENCES

1. Ujowundu CO, Okafor OE, Agha NC, Nwaogu LA, Igwe KO, Igwe CU; Phytochemical and Chemical Composition of Combretum zenkeri Leaves. Journal of Medicinal Plants Research, 2010; 4(10): 965-968.
2. Mallikharjuna PB, Rajanna LN, Seertharm YN, Sharanabasappa GK; Phytochemical studiesof Strychnos potatorium L.F. – A medicinal plant. E. J. Chem, 2007; 4(4): 510-518.
3. Manosroi J, Dhumtanom P, Manosroi A; Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines . Cancer Lett, 2005; 235(1):114-120.
4. Gutiérrez RM, Mitchell S, Solis RV; Psidium guajava: A review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol, 2008; 117:1–27.
5. Lutterodt GD, Ismail E, Basheer RH, Bahamdin HM; Antimicrobial effects of Psidium guajava extract as one mechanism of its anti-diarrhoea action. Malayan Journal of Medical Science, 1999; 6(2):17-20.
6. Manhathanatawec K, Manthey JA, Luzio G, Talcotl ST, Goodner K, Baldwin EA; Total antioxidant activity and fiber content of select Florida-grown tropical fruits. Journal of Agricultural and forel Chemistry, 2006; 54(19): 7355-7365).
7. Begum S, Hassan SI, Saddiqui BS; Two new triterpenoids from the fresh leaves of Psidium guajava. Plant Med, 2002;68(12) : 1149-1152.
8. Rolof A, Weisgerber H, Lang UM; Moringa Oleifera, encyclopaedia der holzgewachse. 2009; pp. 978–3.

9. Fahey JW; Moringa Oleifera: A review of the medicinal evidence for its nutritional, therapeutic and prophylactic properties. Trees for Life Journal, 2005; 1:5. http://www.tfljournal.org/article.php/20051201124931586.
10. Schneider E; Vegetables from Amaranth to Zucchini: The Essential Reference: 500 Recipes, 275 Photographs. New York, USA, Harper Collins Publishers. 2001; 777.
11. Nundkumar N, Ojewole JA; Studies on antiplasmodial properties of some South African Medicinal plants used as antimalaria remedies in Zulu Folk Medicine. Methods Find Exp. Chn. Pharmacol, 2002; 24(7): 397-401.
12. Mahajan SG; Comparative evaluation of sensitivity of human pathogenic bacteria to tea, coffee and antibiotics. Ph.D Thesis MD University, Rohtak. India, 1992.
13. Wolfgang KJ, Hilda PW; Zinsser Microbiology,16th. Edition; Appleton Century Crofts, New York, 1976.
14. Harborne JB; Phytochemical Methods. A Guide to Modern Technique of plant Analysis. London. Chapman and Hall. 1984; 54-60.
15. Manach C, Scalbert A, Morand C, Remsey C, Jimenez L; Polyphenols: food sources and bioavailability. American Journal of Clinical Nutrition, 2004;79(5): 727-747.
16. Luchsinger J, Mayeux R; Dietary factors and Alzheimer's disease. Lancet Neurology, 2004; 3(10): 36-143.
17. Morris BJ; A forkhead in the road to longevity: the molecular basis of life span becomes clearer. Journal of Hypertenionsionq, 2005; 23(7): 1285-1309.
18. Vita JA; Polyphenols and cardiovascular disease. Effects on endothelia and platelet function. American Journal of Clinical Nutrition, 2005; 81(1): 36-37.
19. Trease GE, Evans WC; Phenols and phenolic glycosides. In: Textbooks of pharmacology (12th edition). Balliese, Tindall and Co publishers, London. 1983; 343-383.
20. Ibegbulam CO, Ayalogu EO, Uzohu MN; Phytochemical, antinutritional contents and hepatoxicity of zobo (Hibiscus Sabclariffa) drink. Journal of Agriculture and Food Science, 2003; 1(1): 335-339.
21. Sen T, Nasralla HSH, Chaudhuri AKN; Studies on the anti-inflammatory and related pharmacological activities of Psidium guajava: A Preliminary Report. Phytotherapy Research, 1995;9(2): 118-122.
22. Middleton JE, Kanda swamin C; Effects of flavonoids on immune and inflammatory cell function. Biochemical pharmacology, 1992; 43: 1167-1179.
23. Read MA; Flavonoids: naturally occurring anti-inflammatory agents. American Journal of pathology, 1995; 147: 235-237.
24. Kurup PA, Narasimha PL; Antibiotic principle from Moringa Pterygosperma. Part II. Chemical nature of Pterygospermin. Indian Journal of Medical Research, 1954; 42:85-95.
25. Badgett BL; Part I. The mustard oil glucoside from Moringa oleifera seed. Rice University PhD Thesis (student of Martin G. Ettlinger), Houston, TX, USA. 1964.
26. Fahey JW, Zalcmann AT, Talalay P; The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry, 2001; 56(1): 5-51.
27. Bennett RN, Mellon FA, Foidl N, Pratt JH, DuPont MS, Perkins L, Kroon PA; Profiling glucosinolates and phenolics in vegetative and reproductive tissues of the multi-purpose trees Moringa oleifera L. (Horseradish tree) and Moringa stenopetala L. Journal of Agricultural and Food Chemistry, 2003; 51: 3546-3553
28. Eilert U; Antibiotic principles of seeds of Moringa oleifera. Indian Medical Journal, 1978; 38(235): 1013-1016.
29. Stephan M, Andreas H, Alexander M, Ulrike VA, Petra ML, Thomas OH, Norbert L, Peter M, Manfred S, Ekkehard BR; Histological Diagnosis of Helicobacter Pylori Gastritis is Predictive if a High Risk of Gastric Carcinoma. Int. J. Cancer, 1997; 73:837–839
30. Haristoy X, Fahey JW, Schottus I, Lozniewski A; Evaluation of antimicrobial effect of several Isothiocyanates on Helicobacter.pylori. Planta Medica, 2005; 71: 326-330.
31. Tona L, Kambu K, Mesia K, Cimanga K, Apers S, de Bruyne T, Pieters L, Totte J, Vlietinck AJ; Biological Screening of traditional preparations from some medicinal plants used as antidiarrhoea in Kinshasa, Congo. Phytomedicine, 1999; 6(1): 59-66.
32. Lin J, Puckree T, Mvelace TP; Anti-diahorrhea evaluation of some medicinal plants used by Zulu traditional healers. Journal of Ethnopharmacology, 2002;79(1): 53-56.