

## Antibacterial Activity of *Azadirachita indica* and *Psidium guajava* Extracts against Three Bacterial Strains.

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### Abstract

Ethanol and aqueous extracts of two Nigerian plant species used in folk medicine were investigated for their antibacterial activities against three bacteria strains: *staphylococcus aureus*, *pseudomonas aeruginosa* and *proteus mirabilis*. Phytochemical screening of the extracts revealed the presence of some bioactive components like alkaloids, saponin, tannin, glycoside and flavonoids. The plants exhibited antibacterial activity with significant difference between the two plants. The most active plant was *psidium guajava*. Most of the tested plant extracts were active against *staphylococcus aureus*. Of all extracts, the hot water extract of *psidium guajava* was the most active (diameter ranges between 3.09±0.14 – 12.14±0.09 mm) followed by its ethanol extract with diameter ranges between 3.07±0.07 – 9.25±0.03mm. Most of the studied plants are potentially a good source of antibacterial agent and have been demonstrated to be important in medicine and in assisting primary health care in many parts of the world.

**Keywords:** Antibacterial activity, bacterial strains, Phytochemical components.

### 1. Introduction

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contribution to human health and well-being (Case, 2005). In Nigeria, many indigenous plants are used in herbal medicine to cure disease and heal injuries (Okwu and Josiah, 2006). Herbal therapies have been used successfully in treating various disorders for 1000's of years in many parts of the world (Krim *et al.*, 2002).

The learning about herbal remedies and their uses in treatment of disease is now challenging. People who use traditional remedies may not understand the scientific rationale behind their medicines but they know from personal experience that some medicinal plants can be highly effective if used at therapeutic doses (Van Wyk and Ilvan Oultshrin, 2000).

In terms of World Health, traditional medicinal plants continue to play a central role in the health care system of large proportions of the world's population (Akerle, 1993). The World Health Organization (WHO) reported that 25.50% of modern medicines are made from plant first used traditionally (WHO, 2003). Indeed, increasingly, more pharmacognostic investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agents (Beringar, 1997). The major part of the traditional therapy involves the use of plant extract (Bishnus *et al.*, 2009). Following the advent of modern medicine, herbal medicine suffered a set back, but during last two to three decades advances in phytochemistry and in identification of plant compounds effective against certain disease has renewed the interest in herbal medicine (FAO, 1993).

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs. This situation caused to search for new antimicrobials. Given the alarming incidence of antibiotic resistant in bacterial, there is the need for new and more effective therapeutic agents (Agarwal *et al.*, 1996).

***Azadirachita indica*:** *A. indica* whose common name is the Neem tree belongs to the family of plant called Meliaceae. The Neem tree is one of the most important trees of eastern India, where it occurs both wild and cultivated. In Nigeria, it is one of the most widespread introduced tree species (Gill, 1992). *A. indica* contains bitter principles, Nimbin, Nimbilim derived from the seed oil (Iwelewa *et al.*, 1990). Other active constituents of the plant include tannin and gum potash and phosphates are also found in the leaf (Iwelewa *et al.*, 1990). In Nigeria, *A. indica* is used traditionally for the treatment of malaria. The extracts from the leaves have antimicrobial activity (Oliver, 1986). Apart from its antimicrobial activity, *A. indica* also has antipyretic, analgesic and anti-inflammatory properties (Soforowa, 1986).

***Psidium guajava*:** Which has the common name guava, has been popularly noted for its use as an anti-malaria herb (Ilonzon, 1996). The fresh tender leaves are also used in case of stomach ache. The leaves when boiled bring relief for diarrhoea. The stem bark when boiled is used in case of irregular menstruation (Adodo, 2002). Guava is commonly found all over Nigeria but it is a native of Central America. The seeds are edible and rich in tannin A and C, iron, calcium and phosphate (Ezeanuzie, 1991). The leaves contain alkaloids and some potent anti-inflammatory, antimicrobial and antimalarial activities in their extracts (Bever, 1986).

## 2. Materials and Methods

### 2.1 Sample Collection and Extraction Procedure:

Fresh bark of *A. indica* and fresh leaves of *P. guajava* were collected locally from the plant. The fresh bark of *A. indica* was dried under sunlight. Extraction was done by first washing the plant materials thoroughly, air dried and then cut into smaller sizes. 50g of the plant materials were soaked with 200ml of 96% ethanol (BDH Chemical Ltd. England) and then left for about 36 hours at room temperature with occasional shaking. The same amount of plant materials were also boiled with 100ml of water and then allowed to cool. The preparations were filtered with No. 1 Whatmann filter paper, evaporated to dryness in a steady air current and then the residue was exposed to U.V rays for 18hours after which it was checked for sterility by streaking on nutrient agar plate. The residues were stored in clean sterile labeled container until they were needed.

### 2.2 Collection, Confirmation and Standardization of Test Organism

The clinical test isolates (*staphylococcus aureus*, *pseudomonas aeruginosa* and *proteus mirabilis*) were received from the Microbiology section of the medical laboratory of federal medical centre, Owerri. These organisms were reconfirmed by some biochemical tests and preserved as pure culture on nutrient agar plant at 4<sup>0</sup>c. Standard culture inoculums of test organisms were prepared using MacFarland Nephelometry as recommended by the National Committee for Clinical Laboratory Standard (NCCLS, 1998).

### 2.3 Antibacterial Screening

The agar well diffusion method of Okoli *et al.*, (1989) was employed to determine the antibacterial activities of both ethanolic and aqueous extracts of the plants. The inoculum of each of the test organisms was seeded onto sterile Muller-Hinton agar plates. Subsequently, 100 $\mu$ l of 200mg/ml concentration of the extracts was separately introduced in duplicate well of the agar culture. The plates were allowed to stand for 1 hour to allow diffusion of the extracts to take place and then incubated for 37<sup>0</sup>c for 24 hours. The zones of inhibitions were recorded to the nearest millimeter (mm).

The determination of Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC) were done following the specification by NCCLS (1998). The MIC was determined by macro-broth dilution techniques. A twofold serial dilution of the reconstituted extract was prepared in a Muller-Hinton broth. Each dilution was seeded with 100 $\mu$ l of the standardized suspension of the test organisms and the culture, incubated for 24 hours at 37<sup>0</sup>c. MIC was determined as lowest concentration of the test samples that showed visible growth. For MBC, Nine 0.1ml volume of broth from each macro-broth MIC testing showing no bacterial growth was taken and incubated in a sterile Muller-Hinton agar at 37<sup>0</sup>c for 24 hours. The MBC was determined as the least concentration showing no growth on subculture.

### 2.4 Statistical Analysis

Diameter of the zone of inhibition was analyzed using paired sample T-test. Values are reported as means of duplicate determination  $\pm$  standard derivation.

## 3. Results

The pH of both plant extracts ranged from 4.4-7.4. The hot water extract of *A. indica* has the highest percentage yield (39.40) followed by the ethanol extract of *P. guajava* with the percentage yield of 31.70. The extracts contained alkaloid, flavonoid, tannin, saponin and glycoside. Anthraquinone was not detected from any of the extracts.

*Staphylococcus aureus* was mostly inhibited by both the ethanol and hot water extracts of the two plants with inhibition zone diameter range of 7.08 $\pm$ 0.02 to 12.14 $\pm$ 0.09 followed by *Proteus mirabilis* with inhibition zone diameter range of 2.07 $\pm$ 0.01 to 4.69 $\pm$ 0.13. The hot water extracts showed no significant activity against *Pseudomonas aeruginosa* while the ethanol extracts slightly inhibited the organism (2.17 $\pm$ 0.01-2.07 $\pm$ 0.07). In general, the hot water extract of *P. guajava* produces the highest zone of inhibition (12.14 $\pm$ 0.09).

The result of the MIC and MBC reveals that the hot water extracts of *A. indica* and *P. guajava* exhibited a bactericidal effect against *Staphylococcus aureus* with an index of 1.00. Ethanol extract of *P. guajava* showed a bacteriostatic effect against *Staphylococcus aureus* and *Proteus mirabilis* with activity index of less than 1.00 while that of *A. indica* was bacteriostatic against *Staphylococcus aureus* only. The MIC values of these extracts ranged from 100->200 mg/ml.

## 4. Discussion

The phytochemical metabolites detected in this study, namely alkaloid, tannin, saponin, flavonoid and glycoside have been associated with the antimicrobial activities of several herbs (Okwu and Josiah, 2006). The highest yield of the ethanol extract of the plants compared to hot water extracts may be explained by the fact that higher proportions of the plants constitutes are alcohol soluble, lower yield of hot water extraction of the plants may probably result from the getting of the plants constituents in hot water with a slime that may reduce yield because it made filtration through the Whatmann No. 1 filter paper some what difficult and slower.

The confounding observations made were that while the plants may contain similar secondary metabolites, one may show more antibacterial activity (in spectrum and/or degree) than the other. However, higher intensity of the secondary metabolites may not always indicate higher antibacterial activity. For example the ethanol extract of the plants contain relatively higher amounts of the secondary metabolites than the hot water extract but did show lower antibacterial activity against *Staphylococcus aureus*.

Although Microbial growth and survival are influenced by pH of the medium, the pH of the extracts used in this study varied widely and fell within the pH range that favours the growth and survival of Microorganisms used in this study. Therefore, most zones of inhibition obtained with the extracts may not be due to the influence of pH of the extracts, hence the extracts may contain some bioactive antibacterial properties. However, the result obtained with plant extracts such as phenolic compounds had been found to be modified by the pH of the compounds in dilutions for example anise oil had higher antifungal activity at pH 4.8 than at 6.8. Also in the work done by Shittu *et al*, (2007), ethanolic extracts (less acidic) of sesam radiatum was more effective against *Candida albican* than the methanolic extract which had no inhibitory effect. In addition, aqueous extract had antifungal activity at a higher pH but less potency compared to the ethanol extracts.

The result of antibacterial activities of the plant extracts on selected human pathogens indicated that the plant samples were active against a wide variety of human pathogenic Microorganisms. In general, the predominance of ethanol extracts as against hot water in exhibiting antibacterial activities against the test isolates might not be unconnected with the solubility of the plant in the different extracting solvents (Oloke and Kolawole, 1988), more inhibiting effect observed with ethanol extracts than the hot water extract could be explained by the fact that ethanol is acidic and in solution donates a proton which makes the medium acidic. Any organism in the solution will accept the proton as a base. The increase in concentration of the hydrogen ions inhibit the activities of the Microorganism thereby resulting to their death (Uruquiaga and Leighton, 2000). The apparent reduction in spectrum of activity in hot water extract may signal a possible loss of potency in the event of extraction and further purification of the plant components.

The antibacterial property of plants extracts may not always be demonstrated as zone of inhibition to commensurate its efficacy due to the fact that the bioactive constituents diffuses at different rate in the agar medium. Therefore MBC values have also been computed in this study. This is evident by the fact that small zone of inhibition diameter or no inhibition were observed with some extracts yet they exhibited bacteriostatic or bactericidal effect against the test isolates.

Although the ethanol and hot water extracts of *A. indica* and *P. guajava* showed no significant/slight antibacterial activity against *Proteus mirabilis* and *Pseudomonas aeruginosa*, studies have recorded their high activity against other organisms and this explains the high traditional use of *A. indica* and *P. guajava* extracts for traditional management of infection (Iroha *et al*, 2008)

It is noteworthy that the spectrum of antibacterial activity of the extracts of *A. indica* and *P. guajava* in this study are comparable to that of the antibiotics (Gentamycin, penicillin and trimethoprin), the superiority of the latter in terms of larger inhibition zone diameter notwithstanding. Whether active components of higher purity from the plant extracts would show activity approaching those of the antibiotics or lower than presently observed in this study are yet to be determined. Even though crude plant preparations have generally been reported to exhibit lower antimicrobial activity than pure antibiotic substances such as gentamycin, penicillin and trimethoprin (Ebi and Ofoefule, 1997, Ibrahim *et al*, 2001), the higher bactericidal activity and low MBC/MIC ratio (0.50-1.00) observed from this study indicated strong antibacterial properties.

## 5. Conclusion

Results obtained from this study depict the fact that the left and bark crude extracts of *P. guajava* and *A. indica* respectively inhibited some medically important bacterial pathogens. This proved that these plants possess some potential as alternative sources of antimicrobial substances.

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**Table 1:** Yield (mg) and pH of the plant extracts

Plant type	Solvent of extraction	Yield (mg)	Percentage yield	pH
<i>A. indica</i>	Ethanol	13.54	27.08	4.4
	Hot water	19.70	39.40	7.4
<i>P. guajava</i>	Ethanol	15.60	31.70	4.8
	Hot water	5.64	11.28	5.2

**Table 2:** Result of Phytochemical analysis of the crude ethanol and aqueous extracts

Solvent of extraction	Plant type	Components					
		Alkaloids	Flavonoids	Tannin	Saponin	Glycosido	Anthraquinone
Ethanol	<i>A. Indica</i>	+	+	+	+	+	-
	<i>P. guajava</i>	+	+	+	+	+	-
Hot water	<i>A. Indica</i>	+	+	-	+	-	-
	<i>P. guajava</i>	+	-	+	+	+	-

**Table 3:** Inhibition zone diameter of extracts of *A. indica* and *P. guajava* against test bacterial strains.

Test isolate	Ethanol extracts		Hot water extract		Control Antibiotics		
	1	2	1	2	Genta.	Pen.	Tri.
<i>Staphylococcus aureus</i>	7.08±0.02	9.25±0.03	10.17±0.08	12.14±0.09	26.50±0.06	13.17±0.09	7.29±0.09
<i>Pseudomonas aeruginosa</i>	2.17±0.01	3.07±0.07	1.62±0.01	0.00±0.00	14.75±0.21	4.49±0.01	2.61±0.06
<i>Proteus mirabilis</i>	4.15±0.02	4.69±0.13	2.07±0.01	3.09±0.14	25.85±0.01	9.07±0.02	5.07±0.01

**Key:** 1= *A. indica*, 2= *P. guajava*, Genta= Gentamycin, Pen=Penicillin, Tri = Trimethoprin.

**Table 4:** Minimum Inhibiting Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the plant extract (mg/ml)

Plant type	Test isolates	Extracts					
		Ethanol			Hot water		
		MIC	MBC	MIC/MBC	MIC	MBC	MIC/MBC
<i>A.indica</i>	<i>Staphylococcus aureus</i>	100	200	0.50	100	100	1.00
	<i>Pseudomonas aeruginosa</i>	>200	>200	<0.50	100	>200	<0.50
	<i>Proteus mirabilis</i>	100	>200	<0.50	>200	>200	<0.50
<i>P.guajava</i>	<i>Staphylococcus aureus</i>	100	200	0.50	100	100	1.00
	<i>Pseudomonas aeruginosa</i>	200	>200	<0.50	>200	>200	<0.50
	<i>Proteus mirabilis</i>	100	200	0.50	200	>200	<0.50

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