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Comparison of Bioactive Compounds in Medicinal Plants Used as Folklore Remedies in Southwestern Nigeria

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Abstract
In southwestern part of Nigeria *Psidium guajava* and *Mangifera indica* are commonly used for herbal preparations in the treatment of toothache, gastrointestinal disorders, dynsentery, diarrhoea, sore gums, and sore throats. This has therefore led to the investigation of the antimicrobial activities of methanolic extracts of *Psidium guajava* and *Mangifera indica*. Fifteen different bacterial isolates comprising of both Gram negative and Gram positive organisms were used. The results show that *P. guajava* and *M. indica* extracts exhibited autimicrobial activities at a concentration of 20 mg/ml. The zones of inhibition exhibited by *P. guajava* extract ranged between 12 mm and 30 mm while that of *M. indica* varied between 11 mm and 28 mm. The Minimum Inhibitory Concentration (MIC) exhibited by *P. guajava* extract against the tested organisms ranged between 0.313 mg/ml and 0.625 mg/ml. On the other hand MIC exhibited by M. indica extract varied between 1.25 mg/ml and 10.0 mg/ml. Overall, *P. guajava* extract show more antimicrobial activity than *M. indica* extract against tested organisms.

Keywords: *Psidium guajava, Mangifera indica,* antimicrobial activity.

Introduction

Many works have been done which aim at knowing the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections (both topical and systemic applications) as possible alternatives to the chemically synthetic drugs to which many infectious microorganisms have become resistant.

During the last ten years the pace of development of new antimicrobial drugs has slowed down while the prevalence of resistance (especially multiple) has increased astronomically (1). The increase in number of antibiotic resistant bacteria is no longer matched by expansion in the arsenal of agents available to treat infections. Literature reports and ethnobotanical records suggest that plants are the sleeping giants of pharmaceutical industry (2). They may provide natural source of antimicrobial drugs that will/or provide novel or lead compounds that may be employed in controlling some infections globally.

Psidium guajava is a shrub known as "guofa" in Yoruba and "goba' in Hausa tribes in Nigeria. *P. guajava* typically has very thin skins, the leaves are evergreen, opposite, short petioled, oval or oblong. The plant is used in folk medicine to treat fevers, diarrhoea, and as tonic in psychiatry (3,4). The hydroalcoholic extract was shown to decrease motor activity in mice (4). Leaf extract from *P.guajava* was also shown to possess anti-inflammatory effect in rats (5) as well as exhibiting antidiabetic effects in mice (6). The methanolic extract of *P. guajava* was also shown to possess antibacterial effect on *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (7). Clinical studies on phytodrugs from leaves of *P. guajava* on some volunteers with gastrointestinal ailments was found to be effective (8). Bark extract of *P. guajava* also is used for diarrhoea, stomach ache and diabetes (9). Phytochemical constituents isolated from the leaves of *P. guajava* includes tripenoids, approximately 10% tannin as well as quercetin (10, 11). Other phytochemical compounds obtained from *P. guajava* are guavins A, C and D (12). Ester of hexahydroxydiphenic acid with L-arabinose, leucocyanidin and oxalates were obtained from unripe fruits of *P. guajava* while ripe fruits contained only ellagic acid (13). A bio-antimutagenic compound (+)-galoocatechin was identified in *P. guajava* leaves (14). In Guatemala, *P. guajava* is used for the tratment of gastro-intestinal disorders (15,16).

Mangifera indica belongs to the family Anacardiaceae which consists of about sixty genera and six hundred species, which are mainly tropical trees and shrubs (17). Stem bark of *M. indica* was found to possess antihelminthic and antiallergic properties (18). It was also observed that *M. indica* aqueous leaf extract produce a reduction of blood glucose level and glucose-induced diabetic mice (19). Among the compounds isolated from *M. indica* extract are two new terpenoidal saponins (20). Other phytochemical compounds obtained from *Mangifera indica* are polygalacturonase I, II, III from mango pulp (21) and a new triterpenoid, 29-hyroxymangiferonic acid (22). Tetracyclic triterpenoid (23) and pentacyclic triterpenoid (24) were isolated from stem bark of *M. indica*. Another phytochemical compound isolated from unripe mango fruit was fructose-1,6-diphosphatase (25).

Materials and Methods

Plant materials

Fresh bark of *P. guajava* and *M. indica* were collected from Abeokuta, Nigeria in the month of July, 2004 and were identified by Botany Department, Obafemi Awolowo University, Ile-Ife, Nigeria. Voucher samples were prepared and deposited in the Herbarium of the Botany Department, Obafemi Awolowo University, Ile-Ife, for reference. The bark were later air-dried, powdered and stored in an air-tight container for further use.

Preparation of extracts:

Exactly 150 g each of the powdered bark of the two plants were separately extracted in cold using 60% methanol for 4 days. The mixture was then filtered and the filtrate was dried in vacuo using a rotatory evaporator. The yield collected from *P. guajava* was 17% w/w while that of *M. indica* was 13.93% w.w.

Preparation of microorganisms for experiment:

The following microorganisms were used *Bacillus polymyxa* (LIO)*, *Bacillus anthracis* (LIO), *Bacillus cereus* (NCIB 6349), *Bacillus stearothermophilus* (NCIB 8222) *Bacillus subtilis* (NCIB 3610), *Clostridium sporogenes* (NCIB 532), *Corynebacterium pyogenes* (LIO), *Escherichia coli* (NCIB 86), *Klebsiella pneumoniae* (NCIB 418), *Pseudomonas aeruginosa* (NCIB 950), *Pseudomonas fluorescens* (NCIB 3756), *Serratia marcescens* (NCIB 1377), *Shigella dysenteriae* (LIO), *Staphylococcus aureus* (NCIB 8588) *and Strepotcoccus faecalis* (LIO)*. For use in experiments, the organisms were sub-cultured in nutrient broth and nutrient agar (Oxoid Ltd.) while diagnostic sensitivity test agar (DST) (Oxoid Ltd.) was used in antibiotic sensitivity testing.

*LIO = Locally Isolated Organism (Isolated from pus and wound infection).

Phytochemical analysis of the extract:

A small portion of the dry extract was used for phytochemcial screening test (17, 26). Dragendorffs reagents was used to test for alkaloids, ferric chloride for tannins while Benedict's solution was used to test for saponins.

Sensitivity testing:

The sensitivity testing of the extracts were determined using agar-well diffusion method (27, 28). The MIC of the extracts were also determined using a two-fold dilutions method (27). The bacterial isolates were first grown in nutrient broth for 18h before use. The inoculum suspensions were standardized and then tested against the effect of the two plant extracts at a concentration of 20 mg/ml each in DST medium. The plates were later incubated at $37^0C \pm 0.5^0C$ for 24 h after which they were observed for zones of inhibition (See Table 1). The effects were compared with that of the standard antibiotic streptomycin at a concentration of 1 mg/ml (29).

Results and Discussion

In this study the results of the investigations show that the two extracts from the bark of *P. guajava* and *M. indica* possess antimicrobial activities against some of the tested organisms at a concentration of 20 mg/ml (Table 1). The two extracts compared favourably with the standard antibiotic streptomycin. On the other hand, *P. guajava* extract showed more activity than *M. indica* extract as shown in Table 1. Both the plant extracts exhibited a broad spectrum of activity.

Table 1: Antimicrobial activities of P. guajava and M. indica bark extracts

MICROORGANISMS	ZONES OF INHIBITION (mm)*			
	P. guajava (20 mg/ml)	*M. indica* (20 mg/ml)	Streptomycin (1 mg/ml)	Ampicillin (10 µg/ml)
Bacillus anthracis	20	0	18	16
Bacillus cereus	15	14	28	25
Bacillus polymyxa	0	0	15	12
Bacillus suitilis	18	17	20	0
Clostridum sporogenes	18	0	25	20
Corynbacterium pyogenes	15	12	20	36
Escherichia coli	16	12	0	0
Klebsiella pneumoniae	12	14	0	0
Pseudomonas aeruginosa	13	12	21	0
Pseudomonas fluorescens	15	12	30	22
Serratia marcescens	0	0	19	16
Shigella dysenteriae	23	28	22	23
Staphylococcus aureus	25	12	21	0
Streptococcus faecalis	30	11	23	16

Key: *mm* = Mean of three replicates*
LIO = Locally Isolated Organism
NCIB = National Collection of Industrial Bacteria
O = Resistant

Table 2: The MIC of the extracts and streptomycin against the bacterial isolates:

Microorganisms	MIC (mg/ml)		
	P. guajava	*M. indica*	Streptomycin
Bacillus cereus	0.313	1.25	0.0313
Bacillus anthracis	1.25	-	0.0313
Bacillus subitilis	1.25	5.00	0.0625
Bacillus stearothermophilus	1.25	1.25	0.0625
Corynbacterium pyogenes	0.625	2.50	0.0313
Escherichia coli	-	10.00	-
Klebsiella pneumoniae	0.313	-	-
Pseudomonas fluorescens	0.313	5.00	0.25
Shigella dysenteriae	0.625	5.00	0.25
Staphylococcus aureus	0.625	2.50	0.50
Streptococcus faecalis	2.50	2.50	0.0625

The MIC of *P. guajava* extract against the tested organisms varied between 0.313 mg/ml and 0.625 mg/ml while that of *M. indica* ranged between 1.25 mg/ml and 10.0 mg/ml. The standard streptomycin had MIC values varying between 0.0313 mg/ml and 0.500 mg/ml. The results indicated that standard antibiotic streptomycin has stronger activity than the two plant extracts as shown in Table 2.

The phytochemical analysis of the *P. guajava* extract revealed the presence of tannins while that of *M. indica* showed the presence of alkaloids, saponins and tannins. These compounds are known to be biologically active. Tannins have been found to form irreversible complexes with proline-rich proteins (30) resulting in the inhibition of the cell protein synthesis. This activity was exhibited on test organisms against the two plant extracts, thus, this property can be used to explain the mechanism of action of the *P guajava* and *M. indica* bark extracts.

Apart from antimicrobial activity exhibited by tannins, they also react with proteins to provide the typical tanning effect, medicinally, this is important for the treatment of inflamed or ulcerated tissues (31). Tannins have important roles such as stable and potent antioxidants (17). Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery (32), thus, exhibiting antimicrobial activity. One of the largest groups of chemical produced by plants are the alkaloids and their amazing effect on humans has led to the development of powerful pain killer medication (33).

P. guajava and *M. indica* are used among the Yorubas of the southwestern part of Nigeria in preparing decoction, thus, showing them to be non-toxic, hence different formulations could be prepared for clinical trials. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin. Studies are in progress to further evaluate the mechanisms of action of *Psidium guajava* and *Mangifera indica* extracts on some organisms associated with human diseases.

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