THE ANTIMICROBIAL ACTIVITIES OF EXTRACTS OF SIDIUM GUAJAVA AND CITRUS AURANTIFOLIA

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ABSTRACT

The antibacterial effects of extracts from *P. guajava* (guava) and *C. aurantifolia* (lime) leaves were demonstrated *in vitro* against some Gram positive and Gram negative bacteria. An ethanolic extract from *C. aurantifolia* exhibited mean zones of inhibition of 14.2mm, 9.2mm, 8.5mm, 8.1mm and 7.2mm on *Bacillus subtilis. Salmonella* sp, *Escherichia coli, Streptococcus faecalis,* and *Staphylococcus aureus* respectively. The water extracts exhibited mean zones of inhibition of 13.7m and 9.7mm on *E. coli* and *P. guajava* were bactericidal against *Salmonella* sp and *E. Coli* with mean zones of inhibition of 15.2mm and 13, 9mm respectively. Results from this study provide evidence for the medicinal values of the tested plants and thus their therapeutic utilization singly or jointly by patients with fevers and gastrointestinal disorders.

INTRODUCTION

Plant parts have been reported to have various uses in folklore medical practice. From ancient Assyria to the Paranoic Egypt records describe how medicinal plants used to cure illnesses. Ikenebomeh and Metitire (1988) described the antimicrobial properties of *Cassia alata* on some fungi and bacteria. Habtemariam *et al.* (1990) also reported that *Premna schimperi* a verbenaceaec is useful in the treatment of inflammation and secondary infections associated with superficial wounds. According to these workers *P. schimperi* is also employed by farmers to treat common eye infections in cattle.

Ali *et al.* (1988) had earlier demonstrated that *Citrus* wastes are a potential sources of biologically active principles, they tested *C. reticulate* seed extracts against some furgal species, namely *Fusarium solani, Helminthosporium sativum, Alternaria solani, Aspergillus flavus* and *A. niger.* All except *flavus* and *H. sativum* were strongly inhibited by extracts from *C. reticulate* seed. Oboh *et al.* (1992) tested *Citrus aurantifolia* (lime) juice against some Gram positive and negative bacteria, of the nine bacteria species studied by these workers, *Yersinia enterocolitica, Staphylococccus aureus, E. Coli* K12 and 2030 and *Bacillus subtilis* were strongly inhibited.

It is therefor obvious that over the years the African people have developed a store of empirical information concerning the therapeutic values of spices and local plants. Following this, several published reports described the effects of certain bioactive principles such as essential oils tannins, glycosides, pyrazole, saponnins, and sesquiterpens on microorganisms as reviewed by Kokwaro (1976); Sayed *et al.,* (1979),

Marwan and Nagel (1986), Iwu, (1984) and Okwute and Mitscher (1994). It is a common experience to see patients with fevers and infections relying on extracts from *P. guajava* and *C. aurantifolia* leaves for possible cure.

In this paper the antibacterial effects of *P. guajava* and *aurantifolia* leaves against some gram positive and gram negative bacteria were investigated. This is in pursuance of our efforts to search for drugs from higher plants and also provide scientific justification for the use of these plants by person suffering from fevers.

MATERIALS AND METHODS

Plants Materials

Psidium guajava (guava) and *Citrus aurantifolia* (lime) leaves were collected at Ekpoma in Edo State. They were identified botanically in the Department of Botany, Edo State University, Ekpoma.

Microorganisms

The nine bacterial species: *Bacillus subtilis, Streptococcus faecalis, Staphylococcus aureaus, Escherichia, coli, Salmonella* sp. *Klebsiella* sp. *Proteus* sp, *Pesudomans aeruginsa* and *Aeromonas hydrophila* were the stock cultures collection of the Department of Microbiology, Edo State University, Ekpoma. They were identified according to the standard methods of Cowan and Steel (1974) and Cruickshank *et al.* (1975). Stock cultures were maintained on nutrient agar slants at 4°C and subclutured in nutrient broth at 37°C prior to each antibacterial testing. Standardization of cultures were done according to the methods of Baker *et al.* (1983) and NCCLS (1990) by suspending 5 colonies of an overnight culture in 5 ml. of nutrient broth and comparing the turbidity to that of 0.5 MacFarl and standard, after incubating at 35°C for 2 - 5 hrs.

Preparation of Extracts

Fresh leaves were obtained from *P. guajava* and *C. aurantifolia.* These samples were marcerted with pestle in a mortar and treated separately with 70% ethanol and water at a ration of 1:2 (W/V). The contents of each flask were shaken for 3 - 5 h after which they were filtered. The ethanolic filterate was evaporated to dryness in a rotary evaporator. The extracts was reconstituted in known volume of water and stored at 4°C when not in use. The unevaporated water extract was however used as crude.

Susceptibility Testing

Agar plates (Mueller-Hinton) were inoculated with 0.1ml broth culture of test organisms and spread with an L - bent glass rod to ensure confluent growth. Sterile disks from Whatman No 1 filter paper, 5mm in diameter were impregnated with 0.5ml of the extracts. The disks were applied to the plates and tapped to positions using the smooth end of an inoculating needle. For each organism and plant extract the experiment was performed in triplicate. Ethanol and water, 0.5ml of each were used as negative controls. Commercial multidisks containing Gentamicin, Streptomycin, Tetracycline and Ampicillin

were also applied to separate plates was done at 37°C for 18 - 24h. and the resulting zones of inhibition measured.

RESULTS

Impregnated disk diffusion assay indicated that both ethanol and water extracts exhibited significant antibacterial activity towards six of the nine strains of the test organism (Table 1).

Table 1: Diameter (mm) of Zones of inhibition produced by extracts from the leaves of *P. guajava* and *C. aurantifolia* in Millimeters.

Organism	*C. aurantifolia*		*P. guajava*	
	Ethanol	Water	Ethanol	Water
A. hydorophial	-	-	10	-
B. Subtilis	14.2	7.0	8.4	7.2
E. coli	8.5	13.7	13.0	13.98
Klebsiella spp	-	-	-	-
Proteus spp	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-
Salmonella spp	9.2	9.7	-	15.2
Staphylococcus aureus	7.2	-	-	7.2
Streptococcus faecalis	8.1	6.8	-	-

Note that figures are means of four measurement at different points of the Zones on agar plates.

(-) means no zone of inhibition.

Distilled water and ethanol used as negative control did not show any discernible effect towards the test organism.

Results from doubling dilutions of both ethanol and water extracts showed decrease antibacterial activity due to increase in dilution. Also results from commercial multidisks antibiotics (Table 2) showed that eight of the nine organism were generally susceptible to both Streptomycin and Gentamicin. None of the nine organisms was susceptible to Ampicillin.

Table 2: Diameter (mm) of Zones of inhibition produced by multidisks in Millimeter

Organisms	Antibiotics		
	Gentamicin	Streptomycin	Ampicillin
A. hydrophila	-	14	-
B. subtilis	13.6	19.6	5.7
E. coli	19.7	17.3	-
Klebsiella sp	18.8	16.8	-
Pseudomonas spp	17.3	19.5	-
Salmonella sp	11	14.5	-
Staph. aureus	15.4	14.8	-
Strep. faecalis	17.4	9.8	-
Proteus sp	16.2	9.1	-

(-) No zone of inhibition.

DISCUSSION

The antibacterial potential of both *P. guajava* and *C. aurantifolia* leaves extracts has been elucidated by the results from this study. This finding agrees with that of Obasi *et al.,*(1993) who had earlier reported that extract from the roots of *P. guajava* was medicinal. They reported that preparations from *P. guajava* roots along side with extracts from *Phyllanthus amarus* are used locally in Nigeria to treat diarrhoea and other common gastrointestinal disorders.

That the ethanolic extracts of both *P. guajava* and *C. aurantifolia* were bactericidal towards *B. subtilis, E. coli, Salmonella, Streptococcus faecalis* and *A. hydrophila* showed that these extracts could be useful chemotherapeutic agents against infections and fevers arising from the activities of these pathogens. The water extracts of both plants were completely inhibitory to *Salmonella* spp and *E. coli.* This also underscored the use of water extracts from these plants locally by Nigerians during gastrointestinal disorders.

Further studies are being carried out on the isolation and characterization of the bioactive principles of both *P. guajava* and *aurantifolia* leaf extracts.

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