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Inhibitory activity of *Psidium guajava* extracts on some confirmed Extended-Spectrum β-Lactamases producing *Escherichia coli, Klebsiella pneumoniae and Proteus vulgaris* isolates

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ABSTRACT: *Psidium guajava (L.)* leaves powder was extracted with ethanol and methanol using percolation method. The extracts were tested for antimicrobial activity against clinical isolates of confirmed extended spectrum β-lactamase producing *Escherichia coli, Klebsiella pneumoniae* and *Proteus vulgaris* isolates using disc diffusion method. The extracts were further subjected to phytochemical tests using standard procedures. Sensitivity test results showed that water fraction of the plant was active on. Sensitivity test results showed that ethanol extract of the plant was active against *K. pneumoniae* and *Proteus vulgaris* (8mm) but inactive against *E. coli* at 30µg/disc concentration while methanol extract of the plant was active against *Proteus vulgaris* isolates (8mm) but inactive against the remaining isolates at 30µg/disc concentration. Both extracts were active against ESBLs producing *Proteus* isolates with similar zone of inhibition in response to equal concentration of the extracts. The results of phytochemical screening indicated the presence of alkaloids, reducing sugars, saponins, steroids and tannins in either or both extracts.

Keywords: Sensitivity, ESBLs, Enterobacteriaceae*, Psidium guajava.*

Introduction

Medicinal plants are cheap and renewable source of pharmacologically active substances and are known to produce certain chemicals that are naturally toxic to bacteria (Basile *et al.*, 1999).
Psidium guajava is a small tree about 33ft (10in) high with spreading branches found throughout the rain forest and in the tropical countries like Latin America, central and west Africa, pacific tropical regions, Amazon basic and south East Asia (www. tropical plant database). The bark is smooth thin, copper showing a greenish layer beneath the leaves aromatic when crushed are ever green opposite, short petiole oval or oblong – elliptic 7- 15cm long and 3 – 5cm wide with conspicuous our parallel veins and more or less downy on the underside. Faintly fragrant the white leaves are borne single or in small clusters in the leaf axils are 2 – 5cmwide. The fruit exuding a strong sweet musky odor when ripe may be round ovoid or pear gaped, with light yellow skin frequently blushed with pink. The seed counts found in the fruit ranged from 112 to 535 but some are nearly seedless (Aliyu, 2006).

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Biomedical studies by Lozoya *et al.* on the use of *Psidium guajava* plant resource in the treatment of gastrointestinal ailments has shown from the results obtained that the used guava product decreased the duration of abdominal pain in these patients. Anti-cough activity of *P. guajava* leaf extract evaluated in rats and guava pigs' results showed that water extract of the plant decreased the frequency of cough. Moreover growth of *S. aureus* and β Streptococcus group A as determined by disc diffusion method was inhibited by water, methanol and chloroform extract of dry leaves (Jaiary *et al.* 1999).

Extended spectrum β-lactamases (ESBLs) are enzymes that confer variable level of resistance to oxyiminocephalosporins such as cephotaxime, ceftazidime and monobactams. They occur predorminantly in the family enterobacteriaceae with *Klebsiella pneumoniae* been the most commonly reported worldwide and it is responsible for 5-20% of outbreaks of nosocomial infections in intensive care units, burn, oncology and neonatal units (Kotra, *et al*, 2002). At present there exist more than 200 different natural variants worldwide which constitute serious threat to current β-lactam therapies and represent major therapeutic challenges for clinicians (Lin *et al.,* 2005).

Patients at risk of infection with ESBLs-producing organisms are seriously ill patients with prolonged hospital stays and those in whom invasive medical devices (such as catheters) are present for prolonged duration with the length of hospital stay prior to isolation of ESBL producer ranging from 11-67 days (Lautenbach *et al.*, 2001). Other risk factors for infection include presence of nasogastric tubes (Asensio *et al.*, 2000), recent surgery and poor nutritional status (Paterson and Bonomo, 2005), haemodialysis (D'Agata *et al*, 1998) as well as selective pressure on the use and overuse of antibiotics (Cosgrove *et al.*, 2002).

The objective of this research was to determine sensitivity of ESBls producers to *Psidium guajava* extracts with the aim of finding alternative treatment(s) to infections caused by such organisms.

Materials and Methods

Collection of plant materials

Psidium guajava leaves were collected from Biological sciencesngarden at Bayero University Kano, washed and air dried at room temperature. Dried leaves were ground into fine powder using mortar and pestle in the laboratory as described by Mukhtar and Tukur (1999).

Extraction

Fifty grams each of the powdered plant was soaked in 500ml of ethanol and methanol in separate conical flasks and kept for two weeks in a shaker after which the mixture was filtered. The filtrate was evaporated at room temperature (Fatope *et al.*, 1993).

Phytochemical screening

Test for alkaloids

To 0.1ml of each extract in two separate test tubes 2 – 3 drops of Dragendoff's reagent were added. An orange red precipitate/turbidity denoted the presence of alkaloids (Ciulci 1994).

Test for flavonoids

To 4mg/ml of each of the fractions a piece of magnesium ribbon was added this was followed by concentrated HCl drop wise. A colour change ranging from orange to red indicates flavones; red to crimson indicates flavonoids (Sofowora 1993).

Test for glycosides

Ten mls of 50% H_2SO_4 was added to $1cm^3$ of the filtrates in separate test tubes. The mixtures were heated for 15mins. $10cm^3$ of Fehling's solution was added and the mixture boiled. A brick red precipitate indicated presence of glycosides (Sofowora 1993).

Test for reducing sugars

1ml of each fraction in separate test tubes was diluted with 2.0ml of distilled water followed by addition of Fehling's solution (A + B) and warming. Appearance of brick red precipitate at the bottom of the test tube indicates presence of reducing sugar (Brain and Turner, 1976).

Test for saponins

0.5g of each of the extract was dispensed in a test tube each. 5.0ml of distilled water was added and shaken vigorously. A persistent froth that lasts for about 15 minutes would indicate the presence of saponins (Brain and Turner 1975).

Test for steroids

Two mls of the dry extracts were taken into separate test tubes. The residues were dissolved in acetic anhydride and chloroform was then added. This was followed by the addition of concentrated sulphuric acid by the side of the test tubes using a pipette. A brown ring at the interface of the two liquids and a violet colour in the supernatant layer denoted the presence of steroids (Ciulci 1994).

Test for tannins

Two mls of each of the extract was diluted with distilled water in separate test tube and 2 – 3 drops of 5% ferric chloride ($FeCl_3$) solution was added. A green – black or blue colouration would indicate tannin (Ciulci 1994).

Disc preparation

Improvised discs were punched from Whatman No. 1 filter paper, sterilized in bijou bottles by autoclaving at 121^0C for 15mins. Sensitivity disc were prepared by serial doubling dilution of the extract. In Dimethyl sulfoxide (DMSO) the paper disc were placed in the solution such that each disc took up 0.01m to make the disc potency of

Test isolates and inoculum Standardization

The test isolates were confirmed enterobacteriaceae isolates obtained from prevalence study at Lamco Diagnostic Laboratory in 2009. Few colonies of confirmed extended spectrum β-lactamase producers were dispensed in sterile normal saline to match the 0.5 McFarland standard for sensitivity tests as described by NCCLS (1999).

Bioassay

This was achieved by disc diffusion method (NCCLS, 1999). Standardized inocula of the confirmed ESBL producing isolates were swabbed onto the surface of prepared and solidified Mueller Hinton Agar in separate Petri-dishes. This was followed by placing the prepared discs of the extracts and standard antibiotic discs onto the surface of inoculated media at intervals. The plates were incubated at 37^oC for 24 hours before observation for and measurement of zones of inhibition formed.

Results and Discussion

Psidium guajava plant used in this research yielded extracts amounting to 5.45% and 12.0% when subjected to extraction using ethanol and methanol as solvents respectively with the extracts having gummy texture and brown appearance as shown in Table 1. The high yield of methanol extract may be due to the fact that the compounds present in the plant material are readily soluble in methanol than ethanol.

The results of phytochemical screening of the two extracts of revealed the presence of alkaloids, reducing sugars, saponins, steroids and tannins. These metabolites have been reported to possess antimicrobial activity (Cowan, 1999) particularly alkaloids and tannins are well documented for antimicrobial activity (Tschehe, 1971).

In general, the sensitivity test results showed that both *Psidium guajava* extracts were active against the test isolates when compared with the sensitivity of the isolates to standard ceftazidime disc with ethanol extract being active on both *K. pneumoniae* and *Proteus vulgaris* and methanol extract against *Proteus vulgaris* only at equal disc concentration of 30µg (Table 3). The antibacterial activity exhibited by both extracts may be associated with the presence of alkaloids and tannins in addition to flavonoids which was reported to be responsible for antimicrobial properties of some ethnomedicinal plants (Singh and Bhat, 2003).

Ethanol extract was more active than methanol extract with *E. coli* being insensitive to both extracts at 30 µg/disc concentration. The variation in the sensitivity of different isolates tested to both extracts may be as a result of the differences in the type of ESBLs harboured by these organisms since there were more than 200 different phenotypes identified worldwide (Jacoby and Muno-Price, 2005) and different ESBLs vary in their resistance to different antibiotic substances (Paterson and Bonomo, 2005).

Table 1: Physical properties of *P. guajava* extracts.

Physical parameters	Ethanol	Methanol
Weight extracted (g)	55	55
Weight of extract (g)	3.0	6.6
Percentage yield (%)	5.45	12.0
Colour	Brown	Brown
Texture	Gummy	Gummy

Table 2: Phytochemical properties of *P. guajava* extracts.

Extracts	Phytochemical Tests					
	Alkaloids	**Flavonoids**	**Reducing sugars**	**Saponins**	**Steroids**	**Tannins**
Ethanol	**+**	**-**	**+**	**+**	**+**	**+**
Methanol	**+**	**-**	**+**	**+**	**+**	**+**

Key: + - Present, **- -** Absent

Table 3: Sensitivity of ESBLs Producers to *Psidium guajava* extracts

Key: EE – Ethanol Extracts, **ME –** Petroleum Ether Fraction, **CAZ** – Ceftazidime disc

	EE					ME					CAZ
Isolates	**30**	**60**	**120**	**240**	**480**	**30**	**60**	**120**	**240**	**480**	**30**
	Inhibition Zones (*mm*)										
Escherichia coli	6	7	7	8	8	6	7	8	11	11	6
Klebsiella pnemoniae	7	8	8	8	8	6	7	8	8	8	6
Proteus vulgaris	8	8	8	9	9	8	8	8	9	10	6

Conclusion

From the results obtained in this work, it can be concluded that *Psidium guajava* has the potential for the production of drug for the treatment of urinary tract infections caused by antibiotic resistant pathogens.

Recommendations

In view of the results obtained in this work, it is recommended that researchers should;

(a) Isolate and identify the active compound(s) present in the ethanol extract and fractions.

(b) Determine the toxicity level of both crude extract and the active compound(s).

(c) Screen more plants with the view of finding alternative treatments to microbial infections.

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