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Single and Combined Effects of Antibacterial Activities of Fruit and Stem Bark Extracts of Tetrapleura Tetraptera on Streptococcus Mutans and Pseudomonas Aeruginosa

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Abstract: Single and combined effect of the antibacterial activities of fruit and stembark extracts of *Tetrapleura tetraptera* was carried out against *Streptococcus mutans* and *Pseudomonas aeruginosa*. Samples of *T.tetraptera* fruits were purchased in Wukari market, Taraba State. Stem bark samples were collected from Vandeikya, Benue State of Nigeria. Phytochemical analyses were carried out on both samples in the laboratory, Department of Biological Sciences, University of Agriculture, Makurdi. Phytochemical screening showed the presence of flavonoids, saponins, tannins, steroids, alkaloids, phlobatannins, anthraquinones, glycosides and reducing sugars in varying concentrations in fruit and stem bark samples. Antibacterial activity of extracts of fruit and stembark of *Tetrapleura tetraptera* singly and combined was studied against *Pseudomonas aeruginosa* and *Streptococcus mutans.* Results showed that extract of fruit of test plant gave an inhibition zone of 21.00mm against *Pseudomonas aeruginosa,* 08.33mm against *S. mutans* . Extract of stem bark gave 12.00mm against *S. mutans* and no antibacterial activity against *Pseudomonas aeruginosa.* Combined extracts of *Tetrapleura tetraptera* fruit and stembark showed antibacterial activity on *Pseudomonas aeruginosa* giving an inhibition zone of 16.67mm and *S. mutans* an inhibition zone of 10.33mm. There was significant difference ($p< 0.025$) between the antibacterial effects of extracts of fruit and stem bark of *Tetrapleura tetraptera* singly and combined as shown in this study. Minimum inhibitory concentration of test organisms subjected to single and combined extracts of fruit and stem bark was 100mg/ml except for *P.aeruginosa* which showed that concentrations (mg/ml) of stem bark extract used singly was not enough to inhibit its growth, except the combined extract which inhibited its growth at 100mg/ml.

Keywords: Pseudomonas aeruginosa, Streptococcus mutans Tetrapleura tetraptera, Phytochemicals flavonoids, saponins, tannins, steroids, alkaloids, phlobatannins, anthraquinones, glycosides, reducing sugar, antibacterial, ethanolic, combined, single

Introduction

The use of plants as medicines predates written human history. Many of the pharmaceuticals currently available to physicians have a long history of use as herbal remedies (Lichterman, 2004). The use of plants with medicinal values in treating human diseases is almost universal. Medicines from plant sources is more affordable than expensive modern drugs (Lai and Roy, 2004). Global estimates indicate that 80% of about 4 billion population cannot afford drugs from Western pharmaceutical industry and have to rely on medicinal plants which are mainly derived from plant materials (Joy *et al.*, 1998). In many of the developing countries, the use of plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world's population (Joy *et al.*, 1998).

In tropical and subtropical countries, microorganisms cause serious infections to both human and animal. Antibiotic resistance by this organisms has become a worldwide apprehension (Masud *et al.*,2014). Many researchers have reported that the effectiveness of natural plants against microorganisms cannot be over emphasized (Masud *et al.*,2014).

Against the above background, this study was undertaken to evaluate the susceptibility of

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Pseudomonas aeruginosa and *Streptococcus mutans* to extracts of fruit and stembark of *T.tetraptera* singly and combined for treatment of infections caused by these organisms.

MATERIALS AND METHODS

Collection of plant materials

Stem bark of the test plant *(Tetrapleura tetraptera)* for this study was collected in Vandeikya local government area of Benue State. Fruit samples were bought from railway market at Makurdi and from new market, Wukari local government area in Taraba state. The plant parts were package in sterile polythene bags and transported to the laboratory, Department of Biological Sciences, University of Agriculture, Makurdi for identification and analyses.

Preparation of plant materials: The collected plant parts were shade dried at 27 °C for a period of one week and crushed into small pieces using a clean mortar and pestle, crushing was done separately. They were later taken to the laboratory, Department Biological Sciences, University of Agriculture, Makurdi for extraction process.

Preparation of plant for extraction using ethanol as solvent: The extract of the plant materials were obtained using the cold maceration method described by Umeh *et al,* 2005. Fifty gram (50g) of powdered plant materials (fruit and stembark) was weighed into clean sterile bottles. Each weighed-out plant parts was extracted using 250ml ethanol separately in tightly covered bottles and left for 48hours at room temperature. The resultant suspensions were filtered into sterile beakers, and filtrates collected was re-filtered using Whatmans No. 1 filter paper into sterile sample bottles. They were labelled appropriately and stored in plastic bags at -20 °C for further analyses.

Phytochemical screening

Plant materials extracted using ethanol solution were subjected to phytochemical screening according to the method described by Odebiyi and Sofowora (1978); Okerulu and Ani (2001) to ascertain the presence or absence of some specific active metabolites such as tannins, saponins, flavonoids, reducing sugars, alkaloids, steroids, anthraquinones, glycosides and phlobatannins

Identification and confirmatory test on test organisms

Test organisms used for this study were *Streptococcus mutans* and *Pseudomonas aeruginosa* the stock isolates of these organisms were obtained from Tosema specialist diagnostic laboratory, Makurdi. A well isolated colony of the bacteria was picked using sterile inoculating wire-loop and transferred into nutrient agar and blood agar slant, and incubated at 37^{o}C for 24hours before susceptibility test. The agar slants were stored at 4^{o}C. Identification and confirmatory test were carried out on the organisms using appropriate biochemical tests like catalase, coagulase Pyrrolidonyl arylamidase (PYR) test, oxidase, bile solubility test, Optochin susceptibility test and Glucose fermenting test.

Determination of antibacterial activity

The disc diffusion method was used (Salie *et al*., 1996; Nostro *et al*., 2000). Stock solutions used contain 200mg/ml of each extract for both fruit and stem bark. Blood agar plates were inoculated with the organisms, within 15min of inoculation of the plates, the drug/extract-impregnated disc was placed on the agar surface, with at least 24mm (centre to centre) (Jorgensen and Turnidge, 2003). The disc was placed with a sterile forceps and then gently pressed down onto the agar surface to provide uniform contact. The plates were allowed to stand for few minutes to enable the extracts diffuse into the agar. Standard ofloxacin antibiotic discs (10microgram/disc) was used as control and was similarly applied on plates seeded with the organism. Sterile disc loaded with 0.1ml of sterile distilled water was used as negative control. Within 15minutes of applying the disc, the plates were inverted and incubated at 37^{o}C for 24hrs (Salie *et al*., 1996). All tests were performed in triplicate and the antibacterial activity was expressed as the mean diameter of inhibition zones (mm) produced by the plant extracts. The diameters of the zones of inhibition produced around the disc was measured with a transparent ruler to the nearest millimetre (Salie *et al*., 1996). The measurements taken were recorded. Extracts of the fruit, and stembark that inhibited bacterial growth were subjected to further (quantitative) tests to determine their minimum inhibitory concentrations (MICs).

Determination of minimum inhibitory concentration (MIC)

After phytochemical screening, the extract that showed potent antibacterial activity was further tested to determine the minimum inhibitory concentration (MIC) of the bacterial samples. The MICs of these extracts was determined by broth micro dilution method.

Dilution of extracts

The stock solution was serially diluted with the extraction solvent (ethanol), in sterile test tubes labelled and arranged from the highest to lowest concentration of extracts desired (400mg, 200mg,150mg, 100mg,50mg and 25mg). Using a sterile pipette (or 2ml needle and syringe), 1 ml of solvent was added to each of the 6 tubes, except the first and second tubes. 2ml of extract was added to the

first tube (400mg), 1ml of the extract (200mg/ml) was added to the second and third tubes, and the contents of the third tube agitated on a Vortex mixer. 1 ml of the solution in the third tube was transferred to the fourth tube, and the process continued through the next to the last tube from which 1 ml was removed and discarded. 0.25ml of extract was later added to the third tube to make the concentration 150mg. No extract was added to the 7th tube which served as a negative growth control, 10microgram of ofloxacin was used as positive control (that prevented bacterial growth). An equal volume of a fixed bacterial culture was added to the tubes and incubated at 37 °C for 24 hrs. After which tubes were examined for turbidity. The lowest concentration that shows no visible growth (turbidity) was noted and recorded as the MIC values (Salie *et al*., 1996).

Statistical analysis: Statistical package for the social sciences (SPSS) version 20 was used to analyse the data obtained

RESULTS AND DISCUSSION

TABLE 1: Phytochemical screening of *Tetrapleura tetraptera* stembark and fruit extracts

Phytochemicals	Stembark	Fruit
Saponins	++	+
Tannins	++	+
Reducing sugar	+	–
Phlobatannins	+	+
Anthraquinones	+	+
Steroids	+	+
Flavonoids	+	+
Glycosides	–	–
Alkaloids	+	+

Key: + = present, – = absent

TABLE 2: Antibacterial activity of single and combined fruit and stembark extract of *Tetrapleura tetraptera* on *P.aeruginosa and S.mutans*

Extracts	fruit extract		stembark extract		fruit &stembark extract combined	
	P.aeruginosa	S.*mutans*	*P.aeruginosa*	*S. mutans*	*P.aeruginosa*	*S. mutans*
Ethanol	21.00	08.33	0.00	12.00	16.67	10.33
D/water	0.00	0.00	0.00	0.00	0.00	0.00
Ofloxacin	31.00	11.67	26.00	23.00	12.00	0.00

T-cal =2.857, p<0.025, t- tab =2.776 (n-1=4)
Mean diameter of zone of inhibition are expressed in millimeter.
Key:D/water-distill water

TABLE 3: Minimum inhibitory concentration (MIC) of single and combined fruit and stembark extracts of *Tetrapleura tetraptera.*

Plant Extract	test organisms	concentration in mg/ml 400	200	150	100	50	25
Stem bark	*S. mutans*	–	–	–	*	+	+
Fruit	*S. mutans*	–	–	–	*	+	+
Stembark	*P. aeruginosa*	+	+	+	+	+	+
Fruit	*P. aeruginosa*	–	–	–	*	+	+
Combined	*S. mutans*	–	–	–	*	+	+
Combined	*P. aeruginosa*	–	–	–	*	+	+

Key: + = indicate growth, - = indicate no growth and * = indicate MIC

Discussion

Antibacterial activity of *Tetrapleura tetraptera* plant has been established in this study. *Uchechi and Chigozie (2010*) opined that *Tetrapleura tetraptera* plant has some antibacterial properties. Fruits of *Tetrapleura* tetraptera extracted with ethanol exhibited antibacterial activity against *P.aeruginosa* and *S. mutans* showing maximum zone of inhibition of 21.00mm against *Pseudomonas aeruginosa and 08.33 against S.mutans.* Susceptibility of both organisms to fruit extract could be that, concentrations of phytochemicals in fruit extract is in the required quantity to cause inhibition of the test organisms. Stembark extract showed no antibacterial activity against *P.aeruginosa*, but gave an inhibition zone of 12.00mm against *S. mutans*. The susceptibility of *S. mutans* to stembark extract could be as a result of varying concentrations of phytochemical components in the fruit and stembark of the plant. Combined extracts of fruit and stem bark in the same ratio also showed inhibition against *P.aeruginosa and S. mutans.* Highest zone of inhibition was observed against *Pseudomonas aeruginosa* (16.67mm). Least zones of inhibition was observed against *Streptococcus mutans* (10.33mm). Combined effect of fruit and stem bark extract was able to exert antibacterial activity against *P.aeruginosa* as opposed to stembark extract. This is because the bioactive substance that was lacking in the stembark to exert antibacterial activity against *P.aeruginosa* was found in the fruit after combining fruit and stembark extracts. Phytochemical analyses, revealed that saponins, tannins, steroids, phlobatannins, alkaloids, anthraquinones, and flavonoids were more in one extract than the other.These groups of compounds form the active principles that confer antibacterial activity on the plant. Minimum inhibitory concentration for test organisms subjected to single and combined extracts of fruit and stem bark was 100mg/ml except for *P.aeruginosa* which shows that concentrations (mg/ml) of stem bark extract used singly was not enough to inhibit its growth, except the combined extract which inhibited its growth at 100mg/ml. this could be attributed to the synergistic effect of the combined extract on *P.aeruginosa*.

Conclusion

It was therefore concluded based on the results of this study that extract of the test plant, *Tetrapleura tetraptera* possess antibacterial activity when they are used singly and combined. Extracts of Tetrapleura *tetraptera* stem bark could not exert antibacterial activity on *P.aeruginosa*, but combined extracts of fruit and stem bark was able to inhibit growth of *P.aeruginosa* as a result of the synergistic effect. Combined extracts of fruit and stem bark of the test plant should be used in treating infections caused by these organisms.

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