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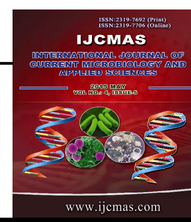

Original Research Article

Effect of Ethanol and Aqueous Solutions as Extraction Solvents on Phytochemical Screening and Antibacterial Activity of Fruit and Stem Bark Extracts of *Tetrapleura tetrapteraon Streptococcus salivarus* and *Streptococcus mutans*

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Keywords

Streptococcus mutans, Streptococcus salivarius, Tetrapleura tetraptera flavonoids, saponins, tannins, steroids, alkaloids, Phlobatannins, Anthraquinones, Glycosides reducing sugar

A B S T R A C T

Phytochemical screening and antibacterial activity of fruit and stem bark of *Tetrapleura tetraptera* using ethanol and aqueous solvents as means of extraction against *S. mutans* and *S. salivarius* was investigated. 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 of the test plants using ethanol and aqueous solutions. Antibacterial activity of ethanolic and aqueous extracts of *Tetrapleura tetraptera* was also studied against *Streptococcus mutans* and *Streptococcus salivarius.* Ethanol extracts showed strong antibacterial activity, aqueous extracts did not show any antibacterial activity on the test organisms, hence, no antibacterial activity with extracts using aqueous solution. Ethanol extract of fruit gave an inhibition zone of 08.33mm against *S. mutans* and 16.33mm against *S. salivarius*. Also ethanol extract of stem bark gave an inhibition zone of 12.00mm against *S. mutans* and no inhibition zone against *S. salivarius.* There was significant difference (p□ 0.025) between antibacterial effect of ethanolic and aqueous extracts of fruit and stem bark as shown in this study. Ethanolic extracts of fruit and stem bark of *Tetrapleura tetraptera* was more potent against the test organisms than the aqueous extracts.

Introduction

Plants have being the basis for medical treatments through much of human history (Nunn, 2002). Traditional medicine is valued in most part of the world and it is still widely practiced today (Hong, 2004). Medicines from plant source otherwise known as herbal or botanical medicine refers to the use of plant seeds, fruits, roots, leaves and stem bark for treatment of aliments (Robertson and Baek, 2009).

Tetrapleura tetraptera, belongs to the family Fabaceae, it is highly valued in Nigeria and

beyond for its medicinal properties (Essien *et al.,* 1994). The aqueous fruit extract has also been shown to possess hypoglycaemic properties (Ojewole and Adewunmi, 2004). Phytochemical screening revealed the presence of tannins, phenolic compounds, saponins, alkaloids, steroids and flavonoids which could be assumed to be responsible for its varied biological and pharmacological properties.

Streptococcus mutans is commonly found in the human oral cavity and a significant contributor to tooth decay (Ryan and Ray, 2004). *Streptococcus salivarius*is a prominent member of the oral microbiota (Ryan and Ray, 2004). Therefore subjecting these organisms to antibacterial activity of *Tetrapleura tetraptera* of aqueous and ethanolic extract of fruit and stem bark will be a head way for caring about the hygienic situation of the oral cavity.

Materials and Methods

Collection of plant materials

The stem bark of the 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 material

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, Biological Sciences Department, University of Agriculture, Makurdi for extraction process.

Preparation of plant for extraction using aqueous and ethanol as solvents

The extracts 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 aqueous and 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 Whatman 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

Plants material extracted using ethanol and aqueous solutions 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 *Streptococcus salivarius* 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°C for 24hours before susceptibility test. The agar slants were stored at 4°C.Identification and confirmatory test were carried out on the organisms using appropriate biochemical tests like catalase, coagulase Pyrrolidonylarylamidase (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 stembark. 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) were used as control and were 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 37ºC 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 were 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)

Extracts that showed potent antibacterial activity was further tested to determine the minimum inhibitory concentration (MIC) for 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 and aqueous), in sterile test tubes labelled and arranged from the highest to lowest concentration of extract 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

Phytochemical screening using ethanol on stem bark of *Tetrapleura tetraptera* showed appreciable amounts of phytochemicals than its aqueous counterpart. These result confirmed the evidence in previous studies that alcoholic solvents like ethanol and methanol are more suitable than other solvents such as water in extracting components of medicinal plants (Ahmad *et al.,* 1998; Cowan, 1999;Emad*et al.,* 2009).

The ethanolic extract of the fruit of *Tetrapleura tetraptera* exhibited activity against *S. mutans* and *S. salivarius* showing maximum zone of inhibition of 08.33 and 16.33 respectively. Ethanolic extract of stem bark of *Tetrapleura tetraptera* exhibited activity against *S. mutans*only showing maximum zone of inhibition of 12.00. There was no activity on *S. salivarius*.These also showed that the antibacterial activity and susceptibility test obtained in this study varied according to the extraction solvent and parts of the plant used. The variation may probably be due to the type of bioactive compounds present in the different extraction solvents as suggested by Abiodun *et al.* (2007). In the phytochemical analyses, saponins, tannins, steroids, phlobatannins, alkaloids, anthraquinones, and flavonoids were present in highest concentration in one extract than the other. These groups of compounds form the active principles that confer antibacterial activity on the plant.

Steroids and phlobatannins which were found to be present in all the extracts of the plant parts tested are steroidal compounds and as such they are of tremendous importance and interest in pharmaceutical research. Aqueous extracts of *T. tetraptera* did not show any antibacterial activity against the tested organisms. This is because water is not a good solvent for extraction. This contrast the work of Uchechi and Chigozie (2010), where aqueous extract of this same *Tetrapleura tetraptera* exhibited activity against some bacteria namely *Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*.

Tetrapleura tetraptera possess antibacterial activity as shown in this study. The ethanol extract of the fruit showed the highest inhibitory effect on the test organisms while aqueous extract did not inhibit any of the tested organisms. This is because ethanol extracted more phytochemicals that will inhibit growth of bacteria as opposed to aqueous solution.

Sensitivity pattern of test organisms to the fruit extracts (aqueous and ethanol) of *Tetrapleura tetraptera* was higher than the stem bark extract. Results showed that ethanol extract of fruit of *Tetrapleura tetraptera* produced clear zones of inhibition on the tested organisms (*Streptococcus mutans* and *Streptococcus salivarius).*The mean zones of inhibition were 8.33mm and 16.33mm respectively.

The aqueous extract of the plant produced no inhibitory effect on the tested organisms, hence no visible zone of inhibition. Sensitivity of test organisms to the stembark extracts of *Tetrapleura tetraptera* was not as high as fruit extract. It was clear from the data shown that there was no zones of inhibition on *Streptococcus salivarius* except against *Streptococcus mutans* and it gave a mean clear zone of inhibition of 12.00mm.

Table.1 Phytochemical screening of *Tetrapleura tetraptera* fruit extract using aqueous and ethanol as extraction solvents

Phytochemicals	extraction solvents	
	aqueous	ethanol
Saponins	+	+
Tannins	–	+
Reducing sugar	–	–
Phlobatannins	+	+
Anthraquinones	+	+
Steroids	+	+
Flavonoids	+	+
Glycosides	+	–
Alkaloids	+	+

Key: + = present, – = absent

Table.2 Phytochemical screening of *Tetrapleura tetraptera* stem bark extract using aqueous and ethanol as extraction solvents

Phytochemicals	extraction solvents	
	aqueous	ethanol
Saponins	+++	++
Tannins	++	+ +
Reducing sugar	+	+
Phlobatannins	+	+
Anthraquinones	+	+
Steroids	++	+
Flavonoids	–	+
Glycosides	–	–
Alkaloids	+	+

Key: + = low concentration, ++ = moderate concentration, +++ present in appreciable amount and – = absent

Table.3 Antibacterial activity of *Tetrapleura tetraptera* fruit extracted with aqueous and ethanol on *Streptococcus mutans and Streptococcus salivarius*

Extracts	*S.mutans*	*S.salivarius*
Aqueous	0.00	0.00
Ethanol	08.33	16.33
D/water	0.00	0.00
Ofloxacin	11.67	12.00

T-cal=4.255, p□ 0.025, (n-1 = 4) t-tab =2.776

Mean diameter of zone of inhibition are expressed in millimeter.

Key: D/water- distilled water.

Table.4 Antibacterial activity of *Tetrapleura tetraptera* stem bark extracted with aqueous and ethanol on *Streptococcus mutans and Streptococcus salivarius*

Extracts	*S. mutans*	*S.salivarius*
Aqueous	0.00	0.00
Ethanol	12.00	0.00
D/water	0.00	0.00
Ofloxacin	23.00	08.67

T-cal = 3.933, p □0.025 (t 4) t-tab =2.776

Key: D/water- distilled water. Mean diameter of zone of inhibition are expressed in millimeter.

Table.5 Minimum inhibitory concentration (MIC) of extracts of *Tetrapleura tetraptera* with ethanol solution

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

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

Table.6 Minimum inhibitory concentration (MIC) of extracts of *Tetrapleura tetraptera* with aqueous solution

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

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

The MIC of the ethanolic extracts of *Tetrapleura tetraptera* showed that at concentration of 100mg/ml, the stem bark extract of the plant inhibited the growth of *Streptococcus mutans* while at concentration of 400mg/ml, the fruit extract inhibited the growth of *Streptococcus mutans*. Also, the ethanolic fruit extract of *Tetrapleura tetraptera* inhibited the growth of *Streptococcus salivarius* at a very low concentration of 25mg/ml (indicating highest potency).

Based on the findings of this research study, ethanolic extract of the test plant, *Tetrapleura tetraptera* possess more antibacterial activity as opposed to aqueous extract. Ethanolic extract was found to be more potent than the aqueous extract against *S. mutans* and *S. salivarius*. Highest zone of inhibition of 16.33mm was observed against

S. salivarius with ethanolic fruit extract of *T. tetraptera*. There was no zone of inhibition with aqueous extracts of fruit and stem bark of the test plant. The findings of this study therefore demonstrates the effectiveness of alcoholic solvent like ethanol in extracting components of medicinal plants which can be used is the treatment of infections caused by these organisms.

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