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ANTIBACTERIAL ACTIVITY OF *TETRAPLEURA TETRAPTERA* TAUB.POD EXTRACTS

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ABSTRACT

Phytochemical composition and antibacterial activity of ethanolic and water extract of *Tetrapleura tetraptera* were studied. Four known human bacterial pathogens were used. They were *Escherichia coli ,Staphylococcus aureus, Salmonella typhi* and *Pseudomonas aeruginosa*.The test plant yielded 2.322% of extract with water and 3.180% of extract with ethanol. Also, phytochemical composition revealed the presence of tannin,0.36%:saponin,0.54%;flavonoid,0.84%;alkaloid,1.28%:phenol,0.42% and 9.86mg/kg of hydrocyanic acid (HCN).Both water and ethanol extracts showed strong antibacterial activity. The water extract gave inhibition zone of diameters 14.1mm,11.0mm,12.9mm and 20.4mm with *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi* respectively,while ethanol extract gave 20.1mm,26.4mm,24.8mm and 14.0mm of the same test organisms. It was recorded that ethanolic extracts were more potent against the test organisms than the water extract.The established antibacterial activities were attributed to the presence of phytochemical and the continuous use of the test plant was highly recommended.

KEYWORDS

Phytochemical composition, ethanol extract, water extract, test organisms, zones of inhibition

INTRODUCTION

It was discovered long ago that some plant materials exhibit antibacterial properties .The use of these plant materials and as preservative and as a means of preventing microorganism development in foods has become the subject of extensive studies (Gould,1996).Importantly ,the inhibitory effects of *Tetrapleura tetraptera* extracts against some human pathogens have been reported. Currently, there is a growing demand worldwide of consumers for minimizing chemical preservation that can be detrimental to human health consequently, spices, herbs and naturally occurring phenolics from various plants sources are being studied in detail in response to consumer requirements for fresher and more natural additive-free products (Nychas,1995)

Tetrapleura tetraptera is one of the medicinal plants in Nigeria. The documented biological or pharmacological activities are found to be molluscicidal, cardiovascular, neuromuscular, hypotensive, anti-conversant, anti

ulcerative,anti-inflammatory and anti-microbial. The pods notably have an appealing culinary use for mothers from the first day of delivery to post parturition and as a lactation aid (Enwere,1998).The antibacteial activity of this plant has been formulated into soap bases using palm kernel oil. At the same time, most of the folkloric chains agree in the traditional use of the fruit for management of convulsion,leprosy,inflammation and rheumatoid pains(Dalziel,1948).

Alcoholic and water extracts of *Tetrapleura tetraptera* inhibited the growth of *Staphylococcus aureus* (Salako,*et al*.,1990).The antibacterial activities of these plants has been exploited in the formulation of the dried powdered fruit of the plant. thus, dried powdered herbs have been formulated into soap bases using palm kernel oil, shea butter and mixture of bases. The formulated soaps were evaluated for organoleptic properties an and foaming ability. Soaps with mixture of these two bases were of better qualities than those with the individual base. Incorporation of powdered plant materials influenced both the foaming property and the hardness of the soaps. Except for the *Tetrapleura tetraptera* fruit powder which improved the foaming ability of these soaps all other herbs including *Acalypha wilkensia,Harugana madagascariensis* and *Ficus exasperate* depressed the foaming ability of the soaps.The extract from *Tetrapleura tetraptera* exhibited anti-convulsant activity, which could be linked to their ability to depress the central nervous system(Akah and Nwambie,1993). The ethanol extract and saponins from stem-bark of *Tetrapleura tetraptera* exerted an inhibitory effect on luteinizing hormone released by pituitary cells (El Izziet *et al.,*1990) suggesting its use as a contraceptive agent. The nutritive quality of the dry fruit of *Tetraptera tetraptera* used as spice was assessed. The fruit shell, fruit pulp and seed contained varying amount of nutrients such as proteins, lipids and minerals which were comparable and some were higher than popular spices such as red pepper, onion curry and ginger (Essien *et al.,*1994)

Previous researchers repeated the presence of glycosides and tannins in water and ethanolic extracts of *Tetrapleura tetraptera* and observed that such phytochemical metabolites were effective inhibitors of growth of bacterial.

Apart from food qualities, vegetables have been found t contain phytochemical with demonstrated medicinal usefulness (Ames,1983). Oguntana(1988) attributed the protection offered by some fruits and vegetables against diseases such as cancer and heart diseases to the presence of some phytochemical.

The molluscicidal activity of the extracts from the leaf, leaf-stalk, stem-bark and root-bark have been exploited for long, but studies on the antibacterial effects of the essential oil from its fruit are scarce. The purpose of this study was to examine the antibacterial effects of the essential oil of the fruit of *Tetrapleura tetraptera* extracted using different solvents to identify the chemical components of the extract and to determine at which concentration they were inhibitory to some human pathogens like *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi.* Also, to provide a guide or direction on the concentration of the fruit extracts to the populace who use them for the treatment of diseases and to prevent side effects associated with the diseases

MATERIALS AND METHODS

Collection and Identification

The test spice sample, *Tetrapleura tetraptera* was obtained from Umuahia Central Market and was identified by Mr. Garuba of the Department of Biological Sciences, Michael Okpara University of Agriculture, Umudike.

Sample Preparation

The test fruit of *Tetrapleura tetraptera* was first cut into small bits and dried in the oven at 65^oC for 24 hours before it was ground in a Laboratory attrition mill and sieved through a 1mm test sieve to obtain a ground (powdery)processed sample used in the work.

Collection of test Organisms

The test human bacteria pathogens *Staphylococcus aureus ,Escherichia coli ,Salmonella typhi and Pseudomonas aeruginosa* were sourced from Verterinary Diagnostic Laboratory of National Verterinary Research Institute ,South East Sub Station, Umudike Laboratory.The test organisms were further identified and the biochemical and morphological characteristics were confirmed by standard methods (Cheesebrough,2000)

Preparation of media

The media used for this work; Nutrient agar (NA), MacConkey agar and Salmonella-Shigella(SSA) were prepared according to manufacturer's instructions.

Sensitivity Test

Preparation of antibacterial sensitivity discs

This was done with the aid of an office perforator, circular discs were cut out of Whatman No.1 filter paper. The discs were boiled in distilled water for an hour to remove any preservative, which may contain antibacterial substances. The boiled discs were transferred to Aluminium foil and dried with oven at 100°C to remove the water. They were wrapped well in a foil and sterilized by autoclaving. The sterile discs were used for the tests.

Test on Bacterial Sensitivity Extract

The susceptibility of each test bacteria (pathogen) to the effect of the various extracts was determined through sensitivity test .The disc diffusion technique (Cheesbrough, 2000) was employed. A culture of each test bacteria was prepared, using the spread plate technique described by Pelczar and Chan (1977).A loopful of the inoculum was aseptically collected from a pure culture plate of each test bacteria placed on the surface of sterile agar plate. A flamed glass rod was used to spread the inoculum evenly over the surface of the plate .Then very carefully, the extract embedded paper discs were picked with sterile forceps and placed at some distance from one another. The plates were allowed to stand for a few minutes to enable the extract diffuse into the medium. The plates were incubated at 37°C in an incubator for 24hours.They were observed for growth and the presence of inhibition zones as a mark of sensitivity to the test extract. Extent of sensitivity was determined by the diameter of the inhibition zone as measured with a transparent ¼ meter rule.

Determination of Extract Yield

The amount of extract obtained from each of the test solvents(water and ethanol) was determined gravimetrically. The total weight of the extract was obtained by evaporating the extract to dryness in a weighed evaporation dish. subsequently, the dish and its content were reweighed. By difference, the weight of extract was calculated using the formula below:

$$\text{Extract yield in mg/g} = \frac{w2-w1}{\text{Wt. of sample}} \times \frac{1000}{1}$$

w1 = weight of empty evaporation dish

w2 = weight of dish + extract

The weight of extract (w2-w1) was multiplied by 1000 to convert it from grams to milligrams.

Reconstitution of Extract

To facilitate the process of conducting the extract into the sterile diffusion discs and to be able to estimate an approximate concentration of the extract used per disc, the dry extract was reconstituted in minimum volume of sterile distilled water.0.5g of the extract was mixed with 0.5ml of sterile distilled water and with a glass rod; it was reconstituted to a uniform viscous mixture. The sterile discs were counted into the mixture and with the aid of sterile forceps the discs were mixed well with the reconstituted extract until the disc absorbed all the extracts .More sterile discs were added until the extract was used up. The approximate concentration of the extract on each paper discs was calculated using the formula below:

$$\frac{CE}{N} = Cp\ D$$

Where

CE = concentration of reconstituted extract

N = Number of discs used
Cp D = Concentration per disc

Determination of Minimum Inhibition Concentration of the Extract

The minimum inhibition concentration (MIC) of the different extracts was determined as the least concentration that inhibited each test organism. Different concentrations of the extract was prepared by reconstituting different weights of the dry extract (0.1 ,0.2, 0.3, 0.4 and 0.5g) in 0.5ml sterile distilled water. Discs were incubated with the resulting extract and used for the sensitivity test. After incubation, the plates were examined. The least concentration to cause inhibition was recorded as the Minimum Inhibition Concentration (MIC)

PHYTOCHEMICAL SCREENING

Qualitative Analysis

The processed sample was subjected to screening to establish the presence or absence of some specific active principles.

Test for Tannins

The ferric chloride test described by Harborn (1973) was used. An aqueous extract of the sample was obtained by shaking 10g of the powdered sample in 100ml of distilled water for 30minutes.After filtration, the extract was used for the test as described below. 2mls of the aqueous extract mixed with equal volume of distilled water in a test tube and drops of diluted ferric chloride solution. The presence of dark precipitate gave an indication of the presence of tannin in the extract.

Test for saponins

The combined froth and emulsion test was used to test for the presence of saponin in the sample.2mls of the aqueous extract was mixed with 5mls of distilled water in a test tube. The mixture was shaken well and observed. The formation of a stable froth (foam) gave a positive result. However, this was confirmed by the addition of few drops of olive oil and shaken again. The formation of an emulsion confirmed the presence of saponin in the test sample.

Test for Flavonoid

The alkaline acid test (Harborn, 1973)was used. A drop pf bench ammonia solution was added to 3mls of the aqueous extract of the sample in a test tube. The formation of yellow coloration which clears on the addition of concentrated acid solution was taken as a positive result for the test.

Test for Cyanogenic glycoside

An alkaline picrate colorimetric method was used. One gram of the processed sample was dispersed in 150mls of distilled water in a conical flask. An alkaline picrate paper was suspended over the mixture and held in place by the rubber bung (stopper).Care was taken to avoid the paper touching the surface of the mixture. The arrangement was allowed to stand for 18hours (over-night) at room temperature. The picrate paper was examined for colour change from yellow to orange as a positive test for the presence of cyanogenic glycoside(HCN).

Test for Alkaloid

The Mayors and Haglens test (Harborn,1973) was used to test for alkaloid in the sample.2mls of an ethanolic extract was mixed with equal volume of Mayors and Haglens reagents in separate test tubes. The formulation orange-brown precipitate indicated the presence of alkaloid.

Test for Steroids/Triterpens

The Dragendert's test was used. A portion of the ethanolic extract was mixed with acetyl anhydride in a test tube. The presence of a brown colouration at the interface between the two layers indicated a positive result for steroid.

Quantitative Analysis

The processed sample was analyzed to determine the quantity of the various phytochemical present in the test sample

Determination of Flavonoid

The gravimetric method following ethyl lactate precipitation was used (Harborn,1973).A measured weight of the sample 5.0g was mixed with 100mls of 2mls of hydrogen chloride solution and boiled for 30 minutes. It was then allowed to cool and then filtered through Whatman No.42 filter paper. The filterate was treated with dropwise addition of ethyl lactate until full precipitation was obtained. The filterate was recovered using a weighed filter paper and dried in the oven at 100°C for 30minutes.It was cooled in a desiccator and reweighed by difference; the weight of flavonoid was obtained and expressed as a percentage of the sample weight analyzed.the formula below was used to calculate the flavonoid content:

$$\% \text{ Flavonoid} = \frac{100\ (w2-w1)}{\text{Wt. of sample}}$$

W1 = wt. of empty filter paper
W2 = wt. of paper + Flavonoid precipitate

Determination of Tannins

Tannin content of the test sample was determined by the Folin-Dainas spectrophotometric method. A measured weight of the processed sample (5g) was mixed with 50mls of distilled water and allowed to stand for 30 minutes at room temperature. It was filtered and the filterate (extract) was used for the analysis.

5mls of the extract and 5mls o f standard tannic acid solution were put in separate 50mls volume flask. One ml of Folin-Dainas reagent was added to each followed by 2.5mls of saturated Na_2CO_3 solution. The mixture was diluted to 50mls with distilled water and the absorbance of the dark color, which developed, was measured in a spectrophotometer after incubation at room temperature for 90 minutes. Absorbance was read at a wavelength of 760nm with a reagent blank at zero. The formula below was used to calculate the tannin content.

$$\% \text{ Tannin} = \frac{100}{W} \times \frac{AU}{AS} \times \frac{VF}{VA} \times D$$

W = Weight of sample analyzed
AU = Absorbance of standard tannin solution
AS = Concentration (mg/ml) of standard tannin solution
VF = Total volume of filterate (extract0
VA = Volume of extract analyzed
D = Dilution factor where necessary

Determination of Phenol

Phenol content of the sample was determined using Folin-Ciocaltean spectrophotometer method.0.2g of the sample was extracted with 10mls of pure methanol. One milliliter of the filtrate (extract) was mixed with equal volume of Folin-Ciocaltean reagent in a test tube. Also,1ml of standard phenol solution was treated the same way. One milliliter of saturated sodium bicarbonate solution was added to each tube and their respective content was calculated using the formula below:

$$\% \text{ Phenol} = \frac{100}{W} \times \frac{AU}{AS} \times \frac{C}{1000} \times \frac{VF}{VA} \times D$$

Determination of hydrocyanic Acid

This was determined using the alkaline picrate colorimetric method .One gram of the sample was soaked in 150ml of the water and a strip of alkaline picrate paper was hung over it with the aid of a rubber bung used as a stopper for the flask. Similarly, the same arrangement was made using 1ml of standard cyanide solution in place of the sample. In each case, the flask with their respective picrate papers, were incubated for 18hours (overnight) at room temperature.

The next day, the picrate papers were eluted in 60mls of distilled water and their negative absorbance were measured at 540nm in a spectrophotometer with the reagent blank at zero. The hydrocyanide content was calculated as shown below:

$$\text{HCN mg/kg} = \frac{100}{W} \times \frac{AU}{AS} \times C \times D$$

Where
W = weight of the sample
AU =absorbance of sample
AS = absorbance of standard cyanide solution
C = Concentration (mg/ml) of standard cyanide solution
D = Dilution factor where necessary

RESULTS AND DISCUSSION

The result of extract yield and qualitative phytochemical test showed saponin, tannin, flavonoid and cyanogenic glycosides were found present in the aqueous extract while alkaloids, steroids, tannin, flavonoid and saponin were found present in the ethanolic extract. (Table 1).These groups of plant chemicals and others form the active principles that confer antibacterial activity on the extract from Table 1, it was observed that the ethanolic extract was 3.18% as against 2.32% of the aqueous extract.In the quantitative phytochemical composition of the test fruit *(Tetrapleura tetraptera*), tannin, flavonoid and saponin content were found to be 0.36, 0.82 and 0.54% respectively (Table 2).These phytochemicals were present in the aqueous extract Cyanogenic glycosides are plants substances which hydrolyze to form hydrogenic acid (HCN)(Urich,1970).The test plant contains the acid (HCN) at 9.8mg/kg.Alkaloid content (1.28%) was found to be fairly higher (Table 2) .Harbone(1973) reported that alkaloids are often toxic and many has dramatic physiological activities hence their wide use in medicine. Again, the test fruit contained 0.42% of phenols (Table 2). Phenols exist in plants as phenolic acid, and glycosidy (Harborn, 1973). As acids they possess antibacterial potency. Levels of antibacterial activity of the aqueous and ethanolic extracts of *Tetrapleura tetraptera* on the four test pathogenic bacteria *(Escherichia coli, Staphyloccoccus aureus, Pseudomonas aeruginosa and Salmonella typhi*) were carried out. Both water and ethanolic extracts were found to possess antibacterial activity and affected the test organism to varying degees. The water extract inhibited the Gram negative pathogens; *Escherichia coli, Salmonella typhi and Pseudomonas aeruginosa* to diameter of 14.0mm,20.0mmand 12.9mm respectively (Table 3).The ethanolic extract showed more antibacterial activity than the water extract on *Escherichia coli*, ethanolic extract inhibited the growth to a diameter of 20.0mm as against 14.1mm diameter with the water extract. Similarly, for *Staphylococcus aureus* and *Pseudomonas aeruginosa* the inhibition zones were 26.4mm and 24.8mm respectively as against 11.0mm and 12.9mm obtained with water extract. However, water extract showed greater potency against *Salmonella typhi* than the ethanolic extract. The water extract inhibition zone for *Salmonella typhi* was 20.0mm against 14.0mm obtained with ethanolic extract (Table 3).From the above, it was observed that both the water and ethanol extracts acted more against the Gram negative enteric pathogen (*Escherichia coli* and *Salmonella typhi*) than the Gram positive *Staphylococcus aureus.*

Table 4 shows the minimum inhibition concentration of extracts of the test plant, *Tetrapleura tetraptera*. Extract was effective against *Staphylococcus aureus* at a minimum concentration of 100mg while *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* were inhibited at the minimum concentrations of 180mg, 100mg and 200mg respectively.

Table 1
Extract Yield and Qualitative Phytochemical Screening of Tetrapleura tetraptera Fruit

Sample	Saponin	Tannin	Alkaloid Steroid	Triterpens	Cyanogenic glycoside	Flavonoid	%Yield of extract
Water	+ve	+ve	+ve	-ve	+ve	+ve	2.32
Ethanol	+ve	+ve	+ve	+ve	-ve	+ve	3.18

Key : +ve = present -ve = absent

Table 2
Quantitative Phytochemical composition of Tetrapleura tetraptera fruit

Tannin%	Alkaloid %	Saponin %	Flavonoid %	HCNmg/kg (Hydrocyanic acid)	Phenol%
0.36	1.28	0.54	0.82	9.86	0.42

Table 3
Antibacterial Properties of Ethanol and Water extract of Tetrapleura tetraptera

	Escherichia coli	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Salmonella typhi*
Water	14.0	11.0	12.9	20.0
Ethanol	20.0	26.4	24.8	14.0

Note: The diameters of inhibition zones are in millimeter and include the diameters of the paper disc

Table 4:
Minimum inhibition concentration of aqueous and ethanolic extracts of Tetrapleura tetraptera

Conc. of the extract	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomomas aeruginosa*	*Salmoinella typhi*
0	Nil	Nil	Nil	Nil
50	Nil	Nil	Nil	Nil
100	10.80	Nil	9.50	Nil
150	14.60	11.30	12.00	Nil
200	18.20	18.00	17.90	9.50
250	21.40	22.10	20.80	11.70
300	21.40	24.10	24.80	14.00
MIC	100mg	180mg	100mg	200mg

CONCLUSION

Based on the findings of this research, water and ethanolic extract of the test plant (*Tetrapleura tetraptera*) possess antibacterial activity. Also ethanolic extract was found to be more potent than the water extract against three of the four human pathogens tested. Only Salmonella typhi was found to be more susceptible to water extract than ethanolic extract. It was also observed that the test yield more active principles with ethanol than water. Since traditionally, *Tetrapleura tetraptera* is used as spices in foods, it may confer some protection against these human bacteria pathogens. It is therefore recommended that the use of *Tetrapleura tetraptera* in food preparation should not be discouraged. There is also need for further studies on the plant extract and its activity against other known human pathogens. Indepth biochemical studies of the implicit may be necessary to implicate the actual phytochemicals responsible for such antibacterial activity. This may increase the utilization of the plant especially in the pharmaceutical aspect

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