



Research Article

Phytochemistry and Antimicrobial Properties of 2:1 and 1:2 Ethanol-methanol Extracts of *Tetrapleura tetraptera*

Samuel Adeniyi Adeleye^{*}, OI Oguoma, Wesley Braide
and Egbadon Emmanuel Osogbase

Department of Microbiology, Federal University of Technology Owerri, Nigeria

Abstract

Tetrapleura tetraptera (Aidan)-an African medicinal plant was analysed for its phytochemicals and its antimicrobial activity using 1:2 and 2:1 ethanol-methanol as solvents for extraction. Amongst the phytochemicals present in the plant, oxalates, cyanogenic glycosides, tanins and phytic acids were quantified. The solvents produced different degrees of extraction of these phytochemicals as well as their antimicrobial activity. The 2:1 methanol: ethanol solvent extracted 1.425%, 0.70 mg/100g, 0.315 mg/100g, 10.805 mg/100g and 0.50% while the 2:1 ethanol: methanol solvent extracted 5.43%, 0.705 mg/100g, 0.52 mg/100g, 7.365 mg/100g and 0.635% of oxalates, cyanogenic glycosides, tanins and phytic acids respectively. Considering the antimicrobial activity of the 1:2 ethanol: methanol extracts. There was a corresponding decrease in susceptibility with decrease in the concentration of the extract indicating that the concentration of the plant extract has an effect on the isolates. The results also revealed that the isolates were all sensitive to high concentrations of 250 mg/ml and 125 mg/ml of the plant extract but demonstrated varied response to the 62.5 mg/ml and 31.25 mg/ml of the plant extract. On the other hand, the 2:1 ethanol: methanol extract was also characterized by a low activity at a low concentration of 31.25 mg/ml and 62.5 mg/ml. The Ethanol: methanol 2:1 extract of *T. tetraptera* is a better extraction method than the 1:2 ethanol: methanol extract as demonstrated by the phytochemistry and antimicrobial activity of the plant.

Keywords: phytochemicals; extract; Aidan; susceptibility; antimicrobials

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***Correspondence to:** Samuel Adeniyi Adeleye, Department of Microbiology, Federal University of Technology Owerri, Nigeria

Email: adeleyesamuella@gmail.com, +2347031297068

Introduction

The need for new drugs is on the increase due to the drastic increase in the antibiotic resistance (Raghunath, 2008). This resistance is caused by various activities ranging from the activities of health care professionals or practitioners to the behaviour of patients such as indiscriminate use of antibiotics, wrong disposal of antibiotic waste, excessive and under dosage (Okeke *et al.*, 1999; Mourad *et al.*, 1993). Plants have demonstrated their capacity to treat a wide range of infections and diseases and have been quite promising recently. (Ahmad *et al.*, 1998; Sooad *et al.*, 2013; Silva and Fernandes, 2010; Mahuya *et al.*, 2014). The capacity of herbal plants to demonstrate medicinal values has been attributed to some phytochemicals present in them (David, 2001; Mamta *et al.*, 2013; Okorondu *et al.*, 2015). One importance of medicinal plants is that they also have additional nutritional benefits they confer to their patients. This nutrients include macro and micro-nutrients, amino acids, carbohydrates, oils, etc. (Ari *et al.*, 2012; Richard and Ayomadewa, 2014; Verla *et al.*, 2014).

Tetrapleura tetraptera also called trivially as Aidan tree in Nigeria is a deciduous tree that belongs to the family fabaceae. This plant does not only have medicinal properties as reported but also is commonly used as a spice for some traditional foods (Uyoh *et al.*, 2013; Woode *et al.*, 2008; Sunday *et al.*, 2010). However, one important aspect of medicinal plant usage is the ability to extract the medically important phytochemicals from the plants.

Ethanol and methanol are common choices of solvents in phytochemistry due to their high polarity. Ethanol has been reported to penetrate the cellular membrane to extract the intracellular ingredients from the plant material (Wang, 2010). Since nearly all of the identified phytochemical components of plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction (Cowan, 1999).

This research reports on the medicinal values of this African spice, *Tetrapleura tetraptera*, against selected medically important human gastrointestinal tract pathogens.

Material and Methods

Preparation of Inoculums

The young actively growing cells were generated by growing cells in nutrient broth for 24 hours at 37⁰C. The cell suspensions were diluted with nutrient broth to provide initial cell counts of about 3×10^8 CFU/ml, while an aliquot of 1ml was used for antimicrobial test.

Kirby-Bauer Disc Diffusion Technique

This was done in line with the protocol described by Cheesbrough (2006) and Mohamed *et al.* (2010). *In vitro* antimicrobial activity was screened by using Mueller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petri dishes. The plates were allowed to solidify for 5 min and 0.1 % inoculum suspension was swabbed uniformly and allowed to dry for 5 min. Sterile disc impregnated with different concentrations of the plant extracts was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes. The plates were kept for incubation at 35-37⁰C for 16–18 hours. Negative control was prepared using respective solvent. Gentamycin (10 µg/disc) and ciprofloxacin (10 µg/disc) was used as positive control. At the end of incubation, zones formed around the disc were measured with transparent ruler in millimeter.

Quantitative Analysis of Phytochemicals

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

Determination of Tannins

Tannin content of the test sample was determined by the Folin-Dainas spectrophotometric method. A measured weight of the processed sample (5 g) was mixed with 50mls of distilled water and allowed to stand for 30 minutes at room temperature. It was filtered and the filtrate (extract) was used for the analysis. Five milliliters (5 ml) of the extract and 5 ml of standard tannic acid solution were put in separate 50 ml volume flask. One ml of Folin-Dainas reagent was added to each followed by 2.5 ml of saturated Na₂CO₃ solution. The mixture was diluted to 50 ml with distilled water and the absorbance of the dark colour, 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.

$$\%Tannin = \frac{100}{W} \times \frac{A_U}{A_S} \times \frac{V_F}{V_A} \times D$$

W = Weight of sample analyzed

A_U = Absorbance of standard tannin solution

A_S = Concentration (mg/ml) of standard tannin solution

V_F = Total volume of filtrate (extract)

V_A = Volume of extract analyzed

D = Dilution factor where necessary

Determination of total alkaloids

Five grams (5 g) of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hrs. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Hangerman *et al.*, 2000).

Determination of hydrocyanic Acid

This was determined using the alkaline picrate colorimetric method. One gram of the sample was soaked in 150 ml 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 (Hangerman *et al.*, 2000).

The next day, the picrate papers were eluted in 60 ml 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:

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

Where

W = weight of the sample.

A_U = absorbance of sample.

A_S = absorbance of standard cyanide solution.

C = Concentration (mg/ml) of standard cyanide solution.

D = Dilution factor where necessary.

Determination of Oxalates

The oxalate was determined by the method of Munro and Basir (1969). The oxalate was extracted with dilute Hydrochloric acid (HCl) at 50°C and treated with ammonium hydroxide and glacial acetic acid. Further treatment with CaCl₂ solution, precipitated calcium oxalate, which was solubilized with hot H₂SO₄ and titrated against KMnO₄ as equivalent to 2.2 mg of oxalates.

Test for phytic acids

The litmus test was used to detect the presence of phytic acids. This test was confirmed when the extract turns blue litmus paper red. The phytic acids were quantified titrimetrically (Harborn, 1973).

Results

The phytochemical analysis of the plant extract reveals that the plant material contains oxalates, cyanogenic glycosides, tannins and phytic acids (Figure 1). These components were extracted in different degrees using the two different extraction methods adopted. The 2:1 methanol: ethanol extract contains 1.425%, 0.70 mg/100g, 0.315 mg/100g and 10.805 mg/100g and 0.50% respectively of oxalates, cyanogenic glycosides, tannins, phytic acids and alkaloids. while the 2:1 ethanol : methanol extract contains 5.43%, 0.705 mg/100g, 0.52 mg/100g, 7.365 mg/100g and 0.635% of oxalates, cyanogenic glycosides, tannins, phytic acids and alkaloids respectively (Figure 1)

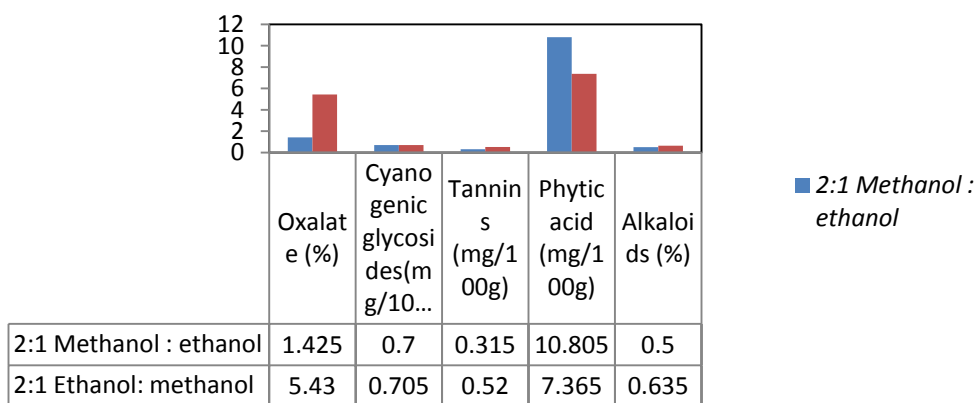


Figure 1 Quantitative phytochemical screening of the plant extracts

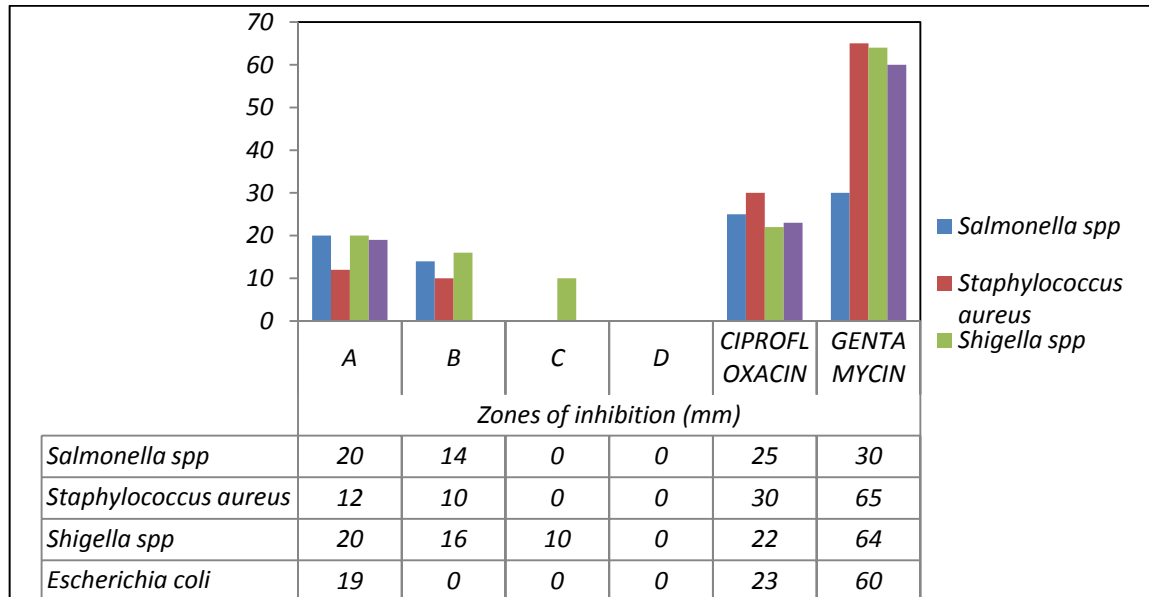


Figure 2 Antimicrobial activity of the ethanol: methanol 1:2 extract of *Tetrapleura tetraptera*.
A, 250 mg/ml; B, 125 mg/ml; C, 62.5 mg/ml and D, 31.25 mg/ml

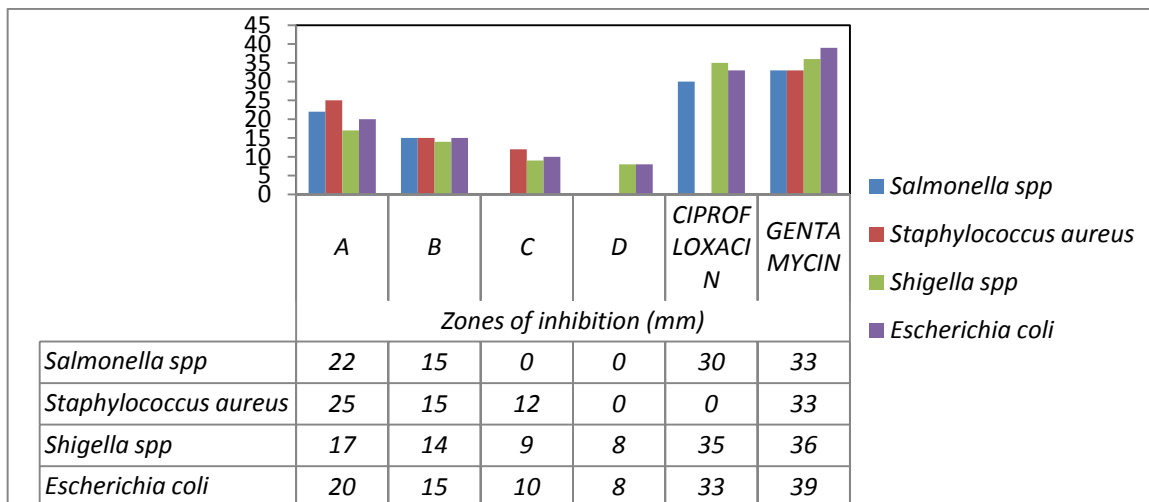


Figure 3 antimicrobial activity of ethanol: methanol 2:1 extract of *Tetrapleura tetraptera*.
A, 250 mg/ml; B, 125 mg/ml; C, 62.5 mg/ml and D, 31.25 mg/ml

Figure 2 shows the antimicrobial activity of the ethanol: methanol 1:2 on the test isolates. There was a corresponding decrease in the antimicrobial activity with decrease in the concentration of the extract indicating that the concentration of the plant extract has an effect on the isolates. The results revealed that the isolates were all sensitive to high concentrations of 250 mg/ml and 125mg/ml of the plant extract but demonstrated varied response to the 62.5 mg/ml and 31.25mg/ml of the plant extract. *Salmonella spp* was

resistant to treatment of the plant extract, *Escherichia coli* was more susceptible and was inhibited by a concentration of 31.25 mg/ml. This extraction method demonstrates a good activity.

The Figure 3 shows the antimicrobial activity of the ethanol: methanol 1:2 extract on the test isolates. The 2:1 ethanol: methanol extract was also characterized by a low activity at a low concentration of 31.25 mg/ml and 62.5 mg/ml. *Escherichia coli* being more resistant to the extract having an activity only at 250 mg/ml was susceptible and was inhibited by a concentration of 31.25 mg/ml of 1:2 ethanol: methanol extract. *Shigella* spp is more susceptible and was inhibited at a concentration of 62.5 mg/ml compared to the 1:2 ethanol: methanol extract that produced an activity at 31.25 mg/ml.

Discussion and Conclusions

The phytochemical analysis of the plant extract reveals that the plant material contains oxalates, cyanogenic glycosides, tannins and phytic acids in varied quantities. These active components were extracted with ethanol and methanol. The 2:1 methanol: ethanol extract contains 1.425%, 0.70 mg/100g, 0.315 mg/100g, 10.805 mg/100g and 0.50% respectively while the 2:1 ethanol: methanol extract contains 5.43%, 0.705 mg/100g, 0.52 mg/100g, 7.365 mg/100g and 0.635% respectively. Considering this results, it could be observed from the histogram that the 2:1 ethanol: methanol extract was more efficient than the 2:1 methanol: ethanol extract in the extraction of the components except phytic acids. Therefore, the process of extraction and the solvent used varies depending on the target phytochemical (Prashant *et al.*, 2011, Okorondu *et al.*, 2015). The presence of phytochemicals suggests that the extract and/or its metabolites may possess antibacterial potential against several pathogens. This is because the different phytochemical compounds have been linked with various bioactivities (Evans *et al.*, 1989; Periyasamy *et al.*, 2010; Jalander and Gachande, 2014; Swathi *et al.*, 2014).

Olusimbo *et al.* (2011) reported that aqueous extracts of *Tetrapleura tetraptera* contains flavonoids, glycosides, terpenoids and saponins but no alkaloids and tannins. Therefore organic solvents such as ethanol and methanol could boost the hidden potentials of alkaloids and tannins present in the plant material with vast antimicrobial activities.

The glycosides detected are non-toxic but can get hydrolyzed to release phenolics which are toxic to microbial pathogens (Aboaba and Efuwape, 2001). Several phenolic compounds like tannins present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as pectolytic macerating enzymes used by plant pathogens (Abd El Rahman *et al.*, 2003, Osbourn, 1996).

Plant based antimicrobials have enormous therapeutic potentials as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu *et al.*, 1999).

A large number of constituent plant components have been reported to have antimicrobial activity. Well known examples include phenols, unsaturated lactones, saponins, cyanogenic glycosides and glucosinolates (Adejumobi *et al.*, 2008). The influence of solvent for extraction on the inhibitory capacity of the extract on the test organism has been reported by Al-Bayati and Sulaiman (2008).

The susceptibility testing of the 1:2 ethanol: methanol extracts shows a corresponding decrease in susceptibility with decrease in the concentration of the extract, indicating that the concentration of the extract have an effect on the isolates. Figure 1 reveals that the isolates were all sensitive to high concentrations of 250mg/ml and 125mg/ml of the plant extract but demonstrated varied response to the 62.5mg/ml and 31.25mg/ml of the plant extract. *Salmonella* spp demonstrated resistant to treatment of the

plant extract whereas *Escherichia coli* was more susceptible and was inhibited by a concentration of 31.25mg/ml.

The 2:1 ethanol: methanol (see figure 2) extract was also characterized by a low activity at a low concentration of 31.25 mg/ml and 62.5 mg/ml. *Escherichia coli* being more resistant to the extract having an activity only at 250 mg/ml was susceptible and was inhibited by a concentration of 31.25 mg/ml of 1:2 ethanol: methanol extract. More so, *Shigella* spp is more susceptible and was inhibited at a concentration of 62.5 mg/ml compared to the 1:2 ethanol: methanol extract that produced an activity at 31.25 mg/ml. The reason for this variation could be attributed to the varied extraction capability of the extracting solvents. The antimicrobial properties of substances are desirable tools in the control of infections and in food spoilage (Aboaba, *et al.*, 2005). The active components of these extracts usually interfere with the growth and metabolism of microorganisms in a negative manner and are quantified by determining the minimum inhibitory concentration and the minimum bactericidal activity. These values are used as guide for the treatment of most infections (Aboaba, *et al.*, 2005).

Recommendation

- Ethanol:methanol 2:1 extract of *T. tetraptera* is a more better extraction method than the 1:2 Ethanol:methanol extract as demonstrated by the phytochemistry and antimicrobial activity of the plant.
- *T. tetraptera* is a good source of remedy for GIT pathogens used in this research but depending on the target organism by diagnosis, an extraction method can be recommended.
- More research is needed to assay for the individual activity of the quantified phytochemicals in the plant material.

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