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Chemical Constituents of *Tetra Pleura Tetraptera* (Fabaceae) and their Antimicrobial Activities.

Udobre[1], A.S. Etim[1], E.I. Udoh[2] A.E. and Johnson[1] E.C.

[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, University of Uyo, Nigeria.

[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Nigeria.

ABSTRACT

Tetrapleura tetraptera (Fabaceae) is a medicinal plant used traditionally in Nigeria to treat skin infection. It is also used in cooking soup and pepper soup for mothers of newly born babies to prevent post-partum contraction. This study dealt with the identification of five bioactive chemical constituents from the n-butanol Extract of the fruit of the plant. The extract contained some secondary metabolites and also demonstrated antimicrobial and antifungal activities. The column, Thin Layer Chromatography and the Gas Chromatography in tandem with Mass Spectrometer (GC-MS) analysis of these substances afforded five compounds namely: Hexadecanoic acid-ethyl ester, Ethyl Oleate, Hexadecanoic acid butyl ester, Octadecanoic acid butyl ester and Docosanoic acid. Antimicrobial and antifungal activities of ethyl oleate as well as the antioxidant and antimicrobial activities of Hexadecanoic acid Butyl ester had been reported in previous studies.
Key Words: Chemical constituents, *Tetrapleura tetraptera*, Antimicrobial activity

INTRODUCTION

Medicinal plants are most important source of life saving drugs for the majority of World's population Tripathi and Tripathi. *Tetrapleura tetraptera* is a deciduous plant widely distributed in the Middle East and some West African countries such as Ghana and Nigeria. In Nigeria *T. tetraptera* is known traditionally as Aridan in the South West, Oshosho in the South East and Uyayak in the South-South.

The back of the tree is fairly smooth. The Leaves are sessile, the flowers are pinkish-cream in colour. The fruits are prominent and their seeds are dispersed by small animals. The fruit pulp is rich in sugars and maybe used in flavouring food. Fruits and flowers are used as perfumes and in pomades prepared from palm oil (Udorioh and Etokudoh). The Leaf is said to be moluscidal (Adewunmmi 1991). Extracted constituents showed anti-ulcer effect (Noamesi *et al* 1992). The aqueous extract of this plant exhibits anticonvulsant activities
(Aderibigbe 2010).

Isolated and characterized scopoletin in the previous study from the fruit possessed hypotensive effects in anaesthetized rats. Fruits are used to prepare soups for mothers from the first day of delivery to prevent post-partum contraction in eastern part of Nigeria (Nwanu and Akali 1986). *T. tetraptera* is an excellent source of anti-oxidants (Nkwenye and Okorie 2010).

METHODS

Collection of Plant Materials

The fruits of *T. tetraptera* (Fabaceae) were bought from a local market at Obot Akara Local Government Area of Akwa Ibom State. The fruit was authenticated by Dr. Mrs. Margaret Bassey, a taxonomist in the Department of Botany, University of Uyo, Akwa Ibom State, Nigeria.

treatment of sample and storage

The fresh fruits of *T. tetraptera* were thoroughly washed with clean water and chopped into small particles. They were dried for seven days to reduce moisture content and to ease pounding. The dried samples were pulverized using mortar and pestle into coarsely powdered form. These was again dried for another two days to reduce moisture content and hence prevent enzymatic action. They were then preserved in an airtight polythene bag until required.

Extraction

600g of pulverized fruits of *T. tetraptera* was cold-macerated with n-butanol (750ml) for 72 hours at room temperature 25^{o}C with agitation three times daily to enhance extraction process. The extract was filtered out using filter paper (Whatman no. 1) into a beaker and then evaporated to dryness in water bathe at 40^{o}C

***Corresponding author: E-mail:** aniefiokudobre@uniuyo.edu.ng **Phone:** +2348027672367

Chromatographic Techniques

An open glass (gravity) column was used for this analysis. 50g of silica gel of 60-120 mesh particle size was constituted into slurry with 150ml of dichloromethane and stirred for uniformity. The slurry was poured into a glass column of 3cm wide and 53cm long with persistent tapping. A mixture of the extract and 10g silica gel 60-120 mesh was made by crushing the mixture in a mortar until a fine powder was obtained before pouring it into the column. The compounds in the column were eluted with the following solvent system (dichloromethane, acetone, n-butanol) and 46 eluates were collected into labeled sterile bottles. The eluates were spotted on a pre-coated aluminum foil thin layer chromatographic (TLC) plate and observed under ultra-violet (UV) light at a wave length of 254nm. The isolated compounds were identified using the modern GC-MS QP-2010.

Sources of Micro-Organisms

The standard strains of the microbes used for the antimicrobial test were obtained from the pharmaceutical microbiology laboratory, faculty of Pharmacy University of Uyo.

Culturing Media

The media used in culturing the microbes were Nutrient agar (NA). Nutrient broth (NB), Sabroaud dextrose agar (SDA) and Sabroaud dextrose broth (SDB). The media were prepared according to the manufacturer's instructions.

Standardization of Microorganisms

The test organisms were standardized and pure isolates obtained. The organisms were cultivated overnight in a nutrient broth and sabroaud dextrose broth respectively. The organisms were sustained on a slant at 4 degrees before use. Exactly 0.2ml of the overnight culture of each organism were dispensed into 2ml sterile nutrient broth (bacteria) and sabroaud dextrose broth (fungi) and incubated for 3-5 hours to standardize the culture to 10cfu/ml. a loopful of the standard culture was used for the antimicrobial assay.

Plate Preparation, Inoculation and Incubation

The plates (petri-dishes), pipettes, test tubes, pasteur pipettes, cork borer and other apparatus to be used were sterilized and allowed to cool before use. 300mg of n-butanol extract of *Tetrapleura tetraptera* was weighed into 10ml of tween 80 to produce a stock concentration of 30mg/ml. Tween 80 was used because the extract was insoluble in water. Two-fold serial dilution was carried out from the stock concentration to give further concentrations of 15mg/ml, 7.5mg/ml and 3.75mg/ml respectively. 25ml of nutrient agar and sabroaud dextrose agar each in different plates representing a particular organism was seeded with 0.2ml of the test organisms respectively by gently swirling for proper mixing of the agar and the organisms. The mixtures in the plates were allowed to solidify in an aseptic environment. The plates were divided into quadrants and labeled. A sterile cork borer with the diameter size of 4mm was used to make five holes at equidistant in solidified plates. The antimicrobial activities of the extract and standard drugs (chloramphenicol 250mg and Nystatin 500,000IU) were tested using agar diffusion technique by pour plate method as described above (Alves et al 2013) .1mg/ml of chloramphenicol (antibiotic) and 50,000IU/ml of nystatin (antifungal) were used as standards. Each standard drug (0.2ml) was introduced at the central hole of each plate representing a particular organism, while 0.2ml of different concentrations of the extract were carefully poured into the holes corresponding with the labeled concentrations using a sterile Pasteur pipette. The prepared plates with the extracts and standard drugs were allowed to remain at room temperature for an hour to enhance the proper diffusion of agents into the medium. The bacterial cultures were incubated for 24hours at 37^0C while the fungal cultures were incubated at 25^0C from 24 hours before examination for zones of inhibition. Readings were taken by measuring the zones of inhibition of the organism with metre rule.

RESULTS

Table 1: Phytochemical Screening of n-Butanol Extract of *Tetrapleura Tetraptera*

S/N	CONSTITUENTS	N-BUTANOL
1	Alkaloid	+ +
2	Steroidal glycoside	+ + +
3	Deoxy sugar	+
4	Terpenes	+ +
5	Carbohydrate	+
6	Tannins	+ +
7	Balsams	+ +
8	Protein	+
9	Coumarin	+ + +
10	Polyphenols	+ +
11	Steroidal ring	+ +
12	Resins	+ + +
13	Saponins	-

KEY: +++ = present in abundance, ++ = moderately present, + = present - = absent

Antimicrobial Test Results

Table 2: Zones of Inhibition(mm) of n-butanol extract *of tetrapleura tetraptera* and standard drugs excluding the diameter (4mm) of the borer.

Organisms	**n-butanol extract (3.75mg/ml)**	**Chloramphenicol (1.0mg/ml)**	**Nystatin (50,000IU/ml)**
Escherichia coli (NCTC 10418)	8	25	-
Staphylococcus aureus (NCTC 6571	10	23	-
Bacillus subtilis (NCTC 8853)	5	26	-
Pseudomonas aeruginosa (ACTC 27853)	10	30	-
Salmonella typhi (NCTC 8571)	5	30	-
Candida albicans (Clinical isolate)	4	23	25

Data from GC-MS Spectra Showing Fragmentation Pattern of Isolated Compounds

Isolate 1

Hexadecanoic acid ethyl ester: Base peak is 88 at 100%, Molecular ion (M^+) is 284 and Formula is $C_{18}H_{36}O_2$. M/Z, R.I.(%) and Interpretation is as follows: 43, 35%, M-241 ($C_{16}H_{33}0$); 74, 25%, M-227 ($C_{16}H_{19}0$); 88, 100%, M-196 ($C_{14}H_{12}0$); 89, 20%, M-195 ($C_{13}H_{23}0$); 101, 55%, M-183 ($C_{12}H_{23}0$); 115, 5%, M-169 ($C_{11}H_{21}0$); 143, 5%, M-143 ($C_9H_{19}0$); 157, 10%, M-127 ($C_8H_{15}0$); 284, 5%, M^+

Isolate 2

Ethyl oleate : Base peak is 55 at 100% , Molecular ion (M^+) is 310 and Formula is $C_{20}H_{38}0_2$ .M/Z, R.I.(%) and Interpretation is as follows: 69, 70%, M-241 ($C_{16}H_{33}0$); 83, 55%, M-227 ($C_{16}H_{19}0$); 101, 45%, M-209 ($C_{14}H_{25}0$); 111, 25%, M-199 ($C_{13}H_{27}0$); 222, 15%, M-88 ($C_5H_{12}0$); 264, 30%, M-46 (C_2H_60); 310, 6%, M^+

Isolate 3

Hexadecanoic acid butyl ester: Base peak is 56 at 100% , Molecular ion (M^+) is 212 and Formula is $C_{20}H_{40}O_2$. M/Z, R.I. (%) and Interpretation is as follows:73, 25%, M-239 ($C_{16}H_{31}0$); 116, 10%, M-196 ($C_{14}H_{12}0$); 129, 15%, M-183 ($C_{12}H_{23}0$); 239, 20%, M-73 (C_4H_90); 257, 25%, M-55 (C_3H_30); 312, 7%, M^+

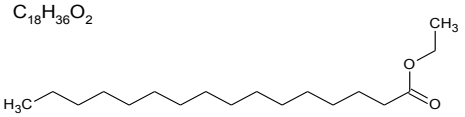
Isolate 4

Butyl Octadecanoate: Base peak is 67 at 100%, Molecular ion (M^+) is 336 and Formula is $C_{22}H_{44}0_2$. M/Z, R.I.(%) and Interpretation is as follows: 95, 70%, M-241 ($C_{16}H_{33}0$); 109, 35%, M-227 ($C_{16}H_{19}0$); 123, 25%, M-213 ($C_{14}H_{29}0$); 135, 20%, M-201 ($C_{14}H_{17}0$); 135, 20%, M-201 ($C_{14}H_{17}0$); 150, 15%, M-186 ($C_{12}H_{28}0$); 263, 25%, M-73 (C_4H_90); 279, 5%, M-57 (C_4H_9); 336, 8%, M^+

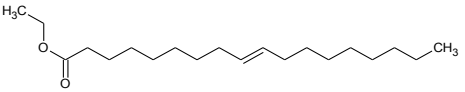
Isolate 5

Docosanoic acid: Base peak is 56 at 100%, Molecular ion (M^+) is 340 and Formula is $C_{22}H_{44}O_2$. M/Z, R.I. (%) and Interpretation is as follows: 129, 20%, M-211 ($C_{14}H_{27}0$); 185, 10%, M-155 ($C_{10}H_{19}0$); 267, 20%, M-73 (C_4H_90); 285, 40%, M-55 (C_3H_30); 340, 4%, M^+

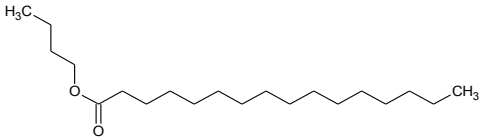
Hexadecanoic acid ethyl ester
(Ethyl hexadecanoate)
$C_{18}H_{36}O_2$



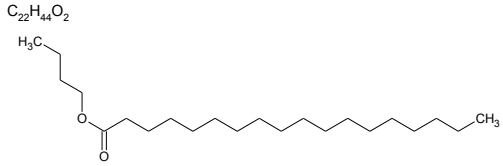
Ethyl Oleate
$C_{20}H_{38}O_2$



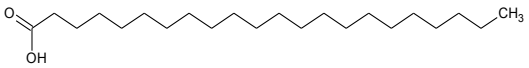
Hexadecanoic acid butyl ester
(Butyl hexadecanoate)
$C_{20}H_{40}O_2$



Butyl octadecanoate
$C_{22}H_{44}O_2$



Decosanoic acid
$C_{22}H_{44}O_2$



DISCUSSION

The phytochemical screening n-butanol extract of *Tetrapleura tetraptera* fruit showed the presence of alkaloids, cardiac glycosides, terpenes, carbohydrates, polyphenols and tannins. Tannins are astringent that either bind and precipitate or shrink protein. They have been known to constrict blood vessels and provide protective coverings to wounds. Tannins have antidiarrhoea and anti-HIV activities (Edeoga *et al* 2001). Terpenes comprise essential oil and fragrance which have been employed in perfume industry and aromatherapy. The presence of coumarin is believed to be responsible for the aroma the fruit imparts on food (Okwu 2006).

Flavonoids are one of the groups of phenolics with antimicrobial activity (Abdou *et al* 2010)**.** The chromatographic separation of the n-butanol extract yielded 46 eluates. (K_1-K_{46}). The eluates were monitored using thin layer chromatography (Dichloromethane – Ethyl acetate, 6:4). The R_f values of isolates 1, 2, 3, 4, and 5 were 0.64, 0.56, 0.68, 0.72 and 0.59 respectively. These eluates yielded yellow amorphous substances. The GC-MS analysis of these substances afforded the following compounds: Hexadecanoic acid-ethyl ester, ethyl oleate, Hexadecanoic acid, -butyl ester, Octadecanoic acid, butyl ester and Docosanoic acid. These compounds are believed to be responsible for the antimicrobial activity of the extract.

The antimicrobial assay showed that *Escherichia Coli*, *staphylococcus areas*, *Bacillus subtilis, pseudomonas aeruginosa, salmonella typhi* and *candida albicans* were all susceptible to the n-butanol extract of *Tetrapleura telraptera*.. Fatty acid esters accumulate in the lipid layer of the cell membrane and mitochondria (Solorzanosantos and Miranda-Novales 2012). They carry out their antimicrobial activity by altering the integrity of the cell structure and make them permeable to antibiotics (Prakash et al 2011).

Conclusion

Findings from this study show that:The n-butanol extract of *Tetrapleura tetraptera* contained important bioactive compounds such as alkaloids, polyphenols, terpenes, tannins, carbohydrates, cardiac glycosides, proteins and coumarin. The n-butanol extract of the plant had antibacterial activity against *staphylococcus* areas, *Escherichia coli, Bacillus subtilis, salmonella typhi, pseudomonas aeruginosa* and antifungal activity against *candida albicans*. The n-butanol extract of the plant also yielded five bioactive compounds which are believed to be responsible for the antimicrobial and antifungal activities of the plant.

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