INVESTIGATION OF ANTI-INFLAMMATORY ACTIVITY OF FRACTIONS FROM THE METHANOL EXTRACTS OF THE LEAF OF *TETRAPLEURA TETRAPTERA* (SCHUMACH & THONN) TAUB

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ABSTRACT

Background: *Tetrapleura tetraptera* (Schumach & Thonn) Taub has many folklore uses mainly in the management of convulsion, leprosy, inflammation and rheumatic pains, schistosomiasis, asthma and hypertension. This study was focused at investigating the anti-inflammatory activitys of various fractions from the methanol extract of *Tetrapleura tetraptera.*

Methods: The methanol extract was obtained by cold maceration, and the fractions were carried out by liquid-liquid extraction procedure. Anti-inflammatory activities were evaluated using two acute anti-inflammatory models: Inhibition of albumin denaturation and Membrane stabilization test.

Results: The results indicate that the different solvent fractions (n-hexane, ethylacetate, chloroform, butanol and aqueous) of*Tetrapleura tetraptera*leaf possess varying anti-inflammatory activity; at stabilizing the Red Blood Cells membrane at concentration of 200 and 1000 µg/ml respectively. The aqueous extract exhibited maximum inhibition (77.84%) at the concentration of 1000 µg/ml followed by (75.33%) at 200µg/ml then n-hexane fraction (70.58%) at 1000µg/ml while the ethyl acetate fraction was the least active at both concentrations. Only the aqueous fraction was active atinhibiting the heat induced albumin denaturation with a maximum inhibition of 63.91% at 200 µg/ml.

Conclusion: These findings offer pharmacological support to the suggested folkloric uses of *Tetrapleura tetraptera*leaf in the management of inflammatory conditions, in south-western communities in Nigeria

Keywords: Anti-inflammatory, albumin denaturation, fractions, membrane stabilization, *Tetrapleura tetraptera*leaf

INTRODUCTION

Inflammation is an acute reaction by living tissue to any kind of lesion. There can be four primary index of inflammation: pain, redness, heat or warmness and swelling. When there is injury to any part of the human body, the arterioles in the surrounding tissue dilate. This gives a raised blood circulation towards the area (redness).[1] Steroids such as betamethasone and the non-steroidal anti-inflammatory drugs (NSAIDs) such as acetylsalicylic acid are the foundation in the treatment/management of inflammation and inflammatory disease conditions. However, these drugs have severe adverse effects such as adrenal suppression for steroids and gastric ulceration and perforation for NSAIDs. Most NSAIDs are known to exercise potentially adverse effects on the gastrointestinal tract,[2,3] these have seriously limited the utilization of these agents in inflammation and inflammatory diseases therapy.

Medicinal plants and their extracts have long been used as therapeutic agents for inflammatory medications by traditional medicine practitioners in most parts of the globe.[4,5] Most of the commonly used herbal solutions by traditional medical practitioners have not been scientifically make valid, therefore this calls for conscious and joint efforts to collect, document and scientifically confirm medicinal plants use in our communities.[6]

Tetrapleura tetraptera(Schumach & Thonn) Taub (Fabaceae) locally known in Yoruba tribe, as Aridan, has a wide natural spread over a large part of tropical Africa, especially in the rain forest belt of West, Central and East Africa. The fruit consists of a fleshly pulp with small, brownish – black seeds.[7] The plant has many traditional uses mainly in the management of convulsion, leprosy, inflammation and rheumatic pains, schistosomiasis, asthma and hypertension.[8] The aqueous extract of *T. tetraptera* fruit exhibited anti-inflammatory and hypoglycaemic effects in rats.[14]The fruit extract has been reported to possess analgesic and anticonvulsant properties in mice.[15,16]The root extract has been established to be useful for the treatment of gastrointestinal related clinical problem.[9] Toxicological reports have shown that *T. tetraptera* has no cytotoxic and genotoxic effects in Chinese hamster ovary cells.[10]GC-MS analysis of essential oil from the leaves of *Tetrapleura tetraptera* confirmed the presence of forty-one compounds representing 89.5% of the essential oil. . The essential oil was dominated by 1,8-cineole (19.4%), 6,10,14-trimethyl-2-pentadecanone (13.6%), phytol (9.1%), alpha-pinene (8.1%) and geranylacetone (6.7%).[11]Two new oleanane type saponins,Tetrapterosides A and B, has been isolated from *Tetrapleura tetraptera* stem bark.[12] The phytochemical screening confirmed the presence of tannins, alkaloids, phenolic compounds, saponins, steroids and flavonoids which could be presumed to be responsible for its varied biological and pharmacological properties. [14] From the documentation on this plant, several works had been done on the fruit, root and stem back,however to the best of our knowledge there is dearth of informationon the anti-inflammatory activity of the leaf of this plant. This present study investigates the anti-inflammatory activities of fractions from the leaf of *Tetrapleura tetraptera*.

METHODS AND MATERIALS

Collection and identification of plant material

The leaves of *Tetrapleura tetraptera* were obtained and identified by an agronomistat National Horticulture Research Institute Idi-Ishin, Jericho Reservation area, Ibadan Oyo State, Nigeriawith voucher number NH 07. The leaves were collected in fresh condition, washed with water to remove all contaminants and debris, dried under shade for seven days.Then ground into powder using a laboratory roller miller (Christy 8000 RPM; Serial NUMBER50158). It was stored at room temperature prior to experiments.

Extraction procedure

The powdered *Tetrapleura tetraptera*leaves (2200 g) was then subjected to cold maceration in 7.5 L of methanol (Analar grade) for 72 h. The crude extract was filtered first through cotton wool, then through Whatman's filter paper of pore size, 125 mm. The filtrate was then concentrated using rotary evaporator at 35°C and further dried in the oven at 35°C. The dry extracts were weighed, stored in a sample bottle and preserved in the freezer before fractionation.

Fractionation of the methanol extract of *Tetrapleura tetraptera*

Of the methanol extract, 50g was dissolved in 750 ml of distilled water and placed in a separating funnel to be fractionated with 750 ml of each solvent three times in order of increasing polarity, starting with the least polar, n-Hexane, followed by ethyl acetate,chloroform, butanol and distilled water successively. The fractions were dried in the oven at 35°C. The dried fractions were weighed and stored in a glass sample bottle for further experiment.

Phytochemical Screening

Phytochemical screenings were carried out on the methanol extract using standard procedures to identify the following secondary metabolites alkaloids, cardiac glycosides, reducing sugars, terpenoids, saponins, tanins, flavonoids, anthraquinones and steroids as described by Tease and Evans. [17]

In vitro Anti-inflammatory activity

Inhibition of Albumin Denaturation:

Methods of Mizushima and Kobayashi, and Sakat*et al*[18,19] were followed with minor modifications. The test solutions consist of test fractions (200 and 1000 µg/ml) and 1% aqueous solution of bovine albumin fraction. The pH of the reaction mixture was adjusted using a small amount of HCl at 37°C. The test solutions were incubated at 37°C for 20 min and then heated to 51°C for 20 min. After cooling the test solutions the turbidity was measured with UV-Visible spectrometer at 660nm. The experiment was done in triplicate. Aspirin was used as a standard drug. Percentage inhibition of protein denaturation was calculated using the equation 1:

Membrane Stabilization Test:

$$\text{inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100\% \quad \text{----------Equation 1}$$

Where $\text{Abs}_{\text{control}}$ is the absorbance without sample, $\text{Abs}_{\text{sample}}$ is the absorbance of sample extract/ standard.

Preparation of red blood cells (RBCs) suspension

A volunteer participant was briefed on the study goals, risks, inclusion and exclusion criteria and volunteer was asked to sign a written, informed consent form before participation. The participant gave informed consent and completed a comprehensive questionnaire and ethical approval was appropriately obtained from the College of medicine, University of Lagos Health Research ethics committee with approval details CMUL/HREC/08/17/232. Fresh whole human blood (10 ml) was collected from a volunteer and transferred to the centrifuged at 3000 rpm for 10 min and washed three times with equal volume of normal saline and reconstituted to10% v/v suspension with normal saline. [19,20]

Heat Induced Haemolytic: The test solutions (2 ml each) consist of 1ml of test fractions (200 and 1000 µg/ml) and 1ml of 10% RBCs suspension. Substitute to the test fractions, saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing the test solutions were incubated in water bath at 56°C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500rpm for 5 min and the absorbance of the supernatants was taken at 560nm. The experiment was conducted in triplicates for all the test samples. Percentage membrane stabilization activity was calculated by the formula mentioned in equation 1 above. [18,21]

Statistical analysis

The data were expressed as mean ± standard error of mean (SEM).

RESULTS

The weight of the methanol extract of *T. tetraptera* leaf was 149.49 g which translate to 6.79 % yield, while the weight of each fraction was n-hexane (11.65 g), ethyl acetate (2.37 g), chloroform (4.26 g), butanol (12.43 g) and water (8.24 g).The phytochemical screening revealed the presence of alkaloids,reducing sugars, cardiac glycoside,Terpenoids, tannins, flavonoids and saponins while steroidsand anthraquinones were absent (Table 1).

Table 1: Qualitative phytochemical constituents of methanol extract of *T. tetraptera*leaf

Phytochemicals	Extract
Alkaloids Reducing sugars	+ ve
Cardiac glycoside	+ ve
Terpenoids Tannins	+ ve
Flavonoids	+ ve
Saponins	+ ve
Anthaquinones	+ ve
steroids	+ve -ve -ve

+ve = present -ve = absent

Inhibition of albumin denaturation

Aspirin, a standard anti-inflammation drug showed the maximum inhibition of 61.2 % at the concentration of 1000 µg/ml, while only the aqueous fraction was active with a maximum inhibition of 63.91 % at 200 µg/ml(Table 2)

Membrane stabilization test

The fractions inhibited the heat induced haemolysis of RBCs to varying degree (Table 3). The maximum inhibition was recorded from aqueous fraction (77.84%) at the concentration of 1000 µg/mlas well as 75.33 % at 200µg/ml and n-hexane fraction gave maximum inhibition of 70.58%, 70.45% at 200 µg/ml and 1000µg/ml respectively, while the ethyl acetate fraction was the least active (Table 3). However, the aspirin had

Table 2: Effect of fractions fromthe *T. tetraptera* leaf on albumin denaturation inhibitory activity

Test fractons	% Inhibition at 200 µg/ml	% Inhibition at 1000 µg/ml
n–Hexane	na	Na
Ethyl acetate	na	Na
Chloroform	na	Na
Butanol	na	Na
Water	63.91 ± 0.0014	63.15 ± 0.0021
Aspirin	60.7 ± 0.0005	61.2 ± 0.0005

Note: na = not active

the highest inhibition of 85.96 %, 86.92 % at 200 and 1000 µg/ml respectively.

Table 3: Effect of fractions from the *T. tetraptera* leaf on membrane stabilization inhibitory activity

Test fractons	% Inhibition at 200 µg/ml	% Inhibition at 1000 µg/ml
n–Hexane	70.58 ± 0.0014	70.45 ± 0.0007
Ethyl acetate	22.30 ± 0.0098	27.30 ± 0.0035
Chloroform	67.68 ± 0.0007	54.35 ± 0.0007
Butanol	44.33 ± 0.0028	68.21 ± 0.0021
Water	75.33 ± 0.0014	77.84 ± 0.0063
Aspirin	85.96 ± 0.002	86.92 ± 0.002

DISCUSSION

Inflammatory diseases are common in the aging society of developed and developing countries; yet, most of the drugs in clinical use for treatment of of inflammatory diseases often have serious side-effects. Hence, in recent years, researchers are focusing more on investigation of on anti-inflammatory agents from plants. In the current study, anti-inflammatory activity of fractions from *T. tetraptera* leaf was investigated using two different experimental methods. Denaturation of proteins method has been a well-documented cause of inflammation. As part of the examination on the mechanism of the anti-inflammatory activity, the protein denaturation capacity of different fractions was studied. The inflammatory drug (Aspirin) has shown dosage dependent ability to thermally induce protein denaturation. [18] Similar results were observed from this study inthe aqueous fraction of the plant with inhibition of 63.91 % and 63.15 % at concentrations of 200 and 1000 µg/ml respectively (table 2). The fractionmay possibly inhibit the release of lysosomal content of neutrophils (which includes bactericidal enzymes and proteinases), at the site of inflammation .[22]

Stabilization of RBC membrane was studied to further establish the mechanism of anti-inflammatory action of the different fractions of *T. tetraptera* leaf. The maximum inhibition was also recorded from the aqueous fraction (77.84%) at the concentration of 1000 µg/ml followed by (75.33 %) at 200µg/ml and n-hexane fraction (70.58%, 70.45%) at 200 µg/ml and 1000µg/ml respectively(Table 3). These results provide confirmatin for membrane stabilization as an additional mechanism of their anti-inflammatory effect. However, the precise mechanism of this membrane stabilization is yet to be explained; it is possible that the active fractions of the plant leaf produced this effect surface area/volume ratio of the cells, which could be brought about by an enlargement of membrane or the shrinkage of cells and an interaction with membrane proteins. [20]

Furthermore, in the study of Ojewole and Adewunmi, [14]on the anti-inflammatory effects of *Tetrapleuratetraptera* fruit in rats, were fresh egg albumin-induced pedal oedema was used as experimental test model.. The aqueous extract of *T. tetraptera* (50-800 mg/kg p.o.) produced dose-related, significant reductions of the fresh egg albumin-induced acute inflammation of the rat hind paw oedema. . The phytochemical result indicates that the leaf contain an appreciable amount of secondary metabolites which includes alkaloids, reducing sugars, cardiac glycoside, terpenoids, tannins, flavonoids and saponins. These were also confirmed to be present in the fruit part in the study of Ojewole and Adewunmi[14].These secondary metabolites from the leaf of *Tetrapleura tetraptera* may be directly responsible for the anti-inflammatory activity.

CONCLUSION

In this study, results indicate that the aqueous fraction of *Tetrapleura tetraptera* leaf possesses anti-inflammatory properties based on the Inhibition of albumin denaturation and membrane stabilization experimental model. Other fractions (n-hexane, ethylacetate, chloroform and butanol) were only active in the membrane stabilization model, and these activities may be due to the presence of phytochemicals.

ACKNOWLEDGEMENTS

We thank the laboratory Staff of the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos for their technical support.

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