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Evaluation Of Anti-Inflammatory Effect Of Methanol, Ethylacetate, And N-Hexane Fraction Extract Of Combretum Obanense Stem Bark Plant Using Experimentally Induced Inflammatory Models In Rats.

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Abstract
Evaluation of the anti-inflammatory effect of methanol, ethylacetate and N – hexane fraction extract of combretum Obanense stem bark plant using experimentally induced inflammatory models in rats. Inflammation (oedema) of the hind paw was induced by injecting 0.1 mL of fresh egg-albumin into the subplantar surface of the left hind paw.After 30 minutes, animals in groups 1, 2 and 3 received equal doses of 200 mg kg^{-1},orally of methanolic fraction, ethyl acetate fraction and N- hexane fraction of extracts respectively, while group 4 received acetyl salicylic acid (100 mg kg^{-1} per Os) andqroup 5 received only distilled water.After 5hours of oral administration of the extracts and the control, it is observed that N – Hexane, methanol fraction and ethyl acetate fraction extract of C. Obanense was able to reduce the acute inflammation ($p<0.05$)significantly, just like the positive control drug acetyl salicylic acid (100 mg kg^{-1} per Os) whilethe negative control distilled water could not reduce the acute inflammation. This could be attributed to the presence of flavonoids, tannins and alkaloids in the phytochemical constituent of the plant extract. The screening of the phytochemical constituents of the plant shows the presence of Tannins, saponin, flavonoids, steroids, alkaloids, carbohydrate, cardiac glycosides, reducing sugar, resin and proteins. However, the ability of N – Hexane and methanol fraction of the plant extracts to reduce the inflammation significantly indicates that they have anti – inflammatory properties in their profile.

Keywords*: combretum Obanense, Anti – inflammation, phlogistic agent, phytochemical screening, Acetyl salicylic acid.*

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I. INTRODUCTION

INFLAMMATION

Over the years medicinal plants have been known as important sources of therapeutically active compounds (Adebayo, et al., 2015). To a greater extent plants have played a prominent role in the well-being of human health and improving the quality of life for many years and serve as a valuable component of medicines, seasonings, beverages, cosmetics, and dyes (Santhosh Kumar1, et al., 2014). Medicinal plants are considered a rich resource of ingredients that can be used in drug development and synthesis (Yudharaj, et al., 2016). The medicinal value of plants lies in some chemical substances or groups of compounds, known as Secondary Metabolite which produces a definite physiological action in the human body that can promote good health and alleviate illnesses. The main value of these bioactive groups of plants are alkaloids, terpenoids, steroids, flavonoids, tannins, and phenolic compounds.These plants have boasted confidence and give serious hope for a better template in the discovery of new drug development (Oguntoye, et al., 2019). Although plants are unique

in their activities, it has also been found that a particular plant may be used by different tribes or countries for different ailments. This shows that plants possess a wide range of healing powers that are attributed to their chemical composition.Humans dependon the healing powerof plant materials to meet their medical needs to maintain health andcure diseases (Jamshidi-Kia, et al., 2017).

Despite the wealth of human experience and folklore concerning the medicinal uses of plants, proper scientific investigation has only been applied to a small fraction of the world's plants. *Combretum obanense* is a rainforest shrub, with flower spikes up to 5 cm long. It is a forest liana or scandent shrub. The leaves are glabrous and glandular beneath with pits in the axils of the main nerves, lamina elliptic, coriaceous, and about 4 cm broad. Main lateral nerves are prominently looped. (Eloff, et al., 2008) The plant is found in rainforests of South Eastern part of Nigeria and is easily distinguished from other indigenous Combretumspecies by the coriaceous leaves with looped lateral veins. (Hutching, et al., (1996) With increasing chemical and pharmacological investigations, it has been reported that crude extract of Combretum species has shown various sources of secondary metabolites, such as alkaloids, steroids, terpenoids, flavonoids, tannins, phenolic compounds, saponins, Glycosides, oil and Reducing Sugar. Also, it was found to be an effective anti-inflammatory therapy at the crude extract level. However, to the best of my literature survey search, it has not been reported at the fractionation level. The present work is poised to evaluate the anti-inflammatory effect of methanol, ethyl acetate, and n-hexane fraction extract of *combretum obanense* stem bark plant using experimentally induced inflammatory models in rats.

II. Materials and Methods

Plant material

The stemof *Combretum obanense* was collected at Okutu in Nsukka Local Government Area of Enugu State, Nigeria in June 2014. It was identified and authenticated by a taxonomist, Mr. Alfred Ozioko together with Mr.Felix Dibia of the International Centre for Ethnomedicine and Drug Development located at Nsukka, Enugu State of Nigeria. The voucher specimen is INTERCEDD 022013. It was deposited at the same center. The plant material was air-dried and powdered by a machine. It was weighed to be 5 Kg and was macerated using MeOH 45 L analytical grade. It was allowed to stay for 24 hours. The solvent was removed in a rotary evaporator and freeze-dried which provide an organic extract of 200 g. Adopted from (Emmanuel, et. al., 2022).

Animal

Male and female Wistar albino rats weighing between 200 – 250g were used. They have sheltered in clean polypropylene cages under standard conditions of humidity (50 ± 5 %), temperature (25±2°C), and light (12 h light/12 h dark cycle) and fed with a standard diet (Amrut laboratory animal feed) approved by Committee for Control and Supervision on Experiments on Animals (CPCSEA). All animals were handled with humane care.

Phytochemical screening

The phytochemical screening of the extracts was carried out using standard phytochemical procedures and tests (Harborne, 1973; Trease and Evans, 1989)

Table 1: *Phytochemical screening* of C. Obanense stem bark

S/NO	PHYTO-CONSTITUENTS	PRESENCE
1	**Reducing Sugar**	**++**
2	**Resins**	**++**
3	**Cardiac Glycosides**	**++**
4	**Saponins**	**++**
5	**Steroids**	**+++**
6	**Terpenoids**	**+++**
7	**Proteins**	**+++**
8	**Alkaloids**	**+++**
9	**Carbohydrate**	**+++**
10	**Flavonoids**	**+++**
11	**Tannins**	**+++**

Note: ++ = moderate in abundant; +++ = high in abundant.

Adopted from Emmanuel. et. al., (2022).

Methodology.

Fresh egg albumin-induced inflammation in rats:

Rat hind paw oedema induced by sub-plantar injection of a phlogistic agent was used as the measure of acute inflammation (Akachukwu, *et al*., 2018). The phlogistic agent employed in this study was fresh egg albumin (Akah and Nwanbie, 1994). Adult albino rats of either sex were used for this study. They were fasted for 24 hours before use and were only deprived of water during the experiment. Inflammation (oedema) of the hind paw was induced by injecting 0.1 mL of fresh egg albumin into the subplantar surface of the left hind paw. After 30 minutes, animal groups 1, 2, and 3 received equal doses of 200mg kg^{-1}, orally of methanolic fraction, ethyl acetate fraction, and N- hexane fraction of extracts respectively, while group 4 received acetylsalicylic acid (100 mg kg^{-1} per Os). Group 5 received only distilled water. The anti-inflammatory effect of the administered extracts was assessed as the difference in the size of inflammation in the paw between the control and the treatment group at 1, 2, 3, 4, and 5 hours after administration of the phlogistic agent (Jude and Paul, 2010). The volume of fluid displaced by the inflamed paw was measured after every hour for 5 hours.

Table 2: Effect of methanolic fraction, ethyl acetate fraction, and N-hexane fraction extract on fresh egg albumin-induced hind paw oedema in rats

Fractions	**Time**			
	2nd hr	**3rd hr**	**4th hr**	**5th hr**
Methanol fraction	1.00 ±0.00^{a}Ccm	5.49 ±2.27 ^{a}Ccm	20.63 ±6.24^{b}Ccm	23.41 ±7.66^{b}Ccm
Ethylacetate fraction	6.34 ±3.11^{ab}Ccm	12.53 ±0.45^{ab}Ccm	18.94 ±4.14^{ab}Ccm	21.16 ±6.26^{b}Ccm
N-hexane fraction	13.49±5.77^{b}Ccm	17.96 ±6.87 ^{b}Ccm	22.42 ±8.44^{b}Ccm	34.82 ±5.43^{b}Ccm
acetyl salicylic acid	2.75 ±1.75^{a}Ccm	6.58 ±3.32^{ab}Ccm	12.98 ±3.20^{ab}Ccm	21.79 ±3.21^{b}Ccm
Distilled water	1.00 ±0.00^{a}Ccm	1.00 ±0.00^{a}Ccm	2.52 ±1.52 ^{a}Ccm	3.94 ±1.47^{a}Ccm

Note: a or b = there is a significant difference across the group while ab = No significant difference across the group.

Fresh egg albumin-induced inflammation in rats: The *C. Obanense* extract showed significant anti-inflammatory activity against acute inflammation ($p<0.05$).

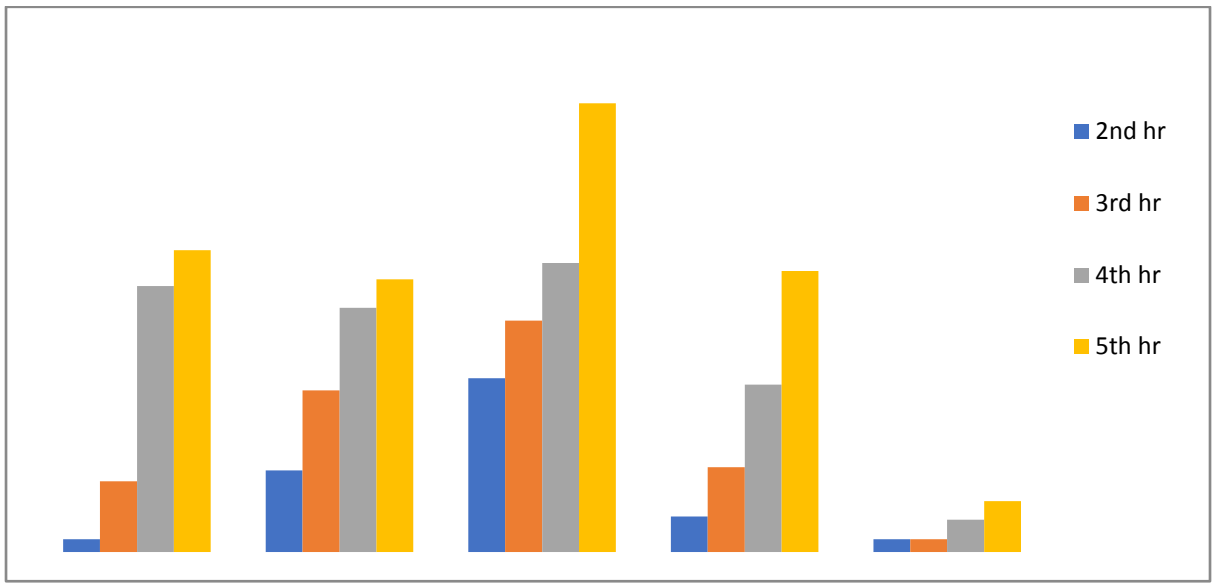

Figure1: showing the effect of methanol fraction, ethylacetate fraction, N – Hexane fraction extract, acetyl salicylic acid and distilled water on fresh egg albumin-induced hind paw oedema in rats

$$\text{Percentage reduction of oedema} = \frac{\text{Initial size} - \text{Final size}}{\text{Initial size}} \times \frac{100}{1}$$

III. DISCUSSION

It is observed as shown in Table 2 above that N – Hexane, methanol fraction, and ethyl acetate fraction extract of *C. Obanense* was able to reduce the acute inflammationsignificantly ($p<0.05$) just like the positive control drug acetylsalicylic acid (100 mg kg^{-1} per Os) whilethe negative control distilled water were unable to reduce the acute inflammation. It was noted that N – Hexane and methanol fraction extract of *C. Obanense* seems to reduce the inflammation more than the positive control drug acetylsalicylic as shown in table 1 above having 34.82 Ccm, 23.41Ccm, and 21.79 Ccm while ethyl acetate fraction and distilled water have 21.16Ccmand 3.94Ccm. The anti-inflammatory effect of the administered extracts was assessed as the difference in the size of inflammation in the paw between the control and the treatment group at 1, 2, 3, 4, and 5 hours after administration of the phlogistic agent (Jude and Paul, 2010). The volume of fluid displaced by the inflamed paw was measured after every hour for 5 hours. However, the *C. Obanense* fraction extract showed significant anti-inflammatory activity against acute inflammation ($p<0.05$). Similarly, the anti-inflammatory activity of the *Commelinaascendens* by (Akachukwu, et al., 2018) shows that the extract significantly ($P <0.05$) reduced the granuloma tissue formed in the treated groups as compared to the control. The presence of flavonoids and tannins in the phytochemical constituents of the plant seems to suggest a possible reason for the significant reduction in acute inflammation. The phytochemical screening of the plant shows the presence of the following: Tannins, saponins, flavonoids, steroids, alkaloids, carbohydrates, cardiac glycosides, Reducing sugar, resin, terpenoid, and proteins.

IV. CONCLUSION

From the result above, the ability of N – Hexane and Methanol fraction extract of *C. Obanense* to reduce the acute inflammation of the rats more than the positive control.This depicts that they have anti-inflammatory properties that are of economic Important. However,the presence of flavonoids and tannins in the phytochemical constituents of the plant may be responsible for the significant reduction in acute inflammation.

V. RECOMMENDATION

Massive plantation of *Combretum Obanense* plants should be encouraged across Nigeria because of its huge therapeutic importance to mankind. More research works need to be done on the isolation of N – hexane and methanol fraction extract of this plant because there are many more health benefits of this plant that are yet to be discovered for therapeutical purposes. Tetfund, PTDF, or other scholarship sponsorship should fund most of these research works because they are capital intensive.

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