

## The effect of Methanol, Ethylacetate and N-hexane fractions of Combretum Obanense stem bark on rats in ethanol induced gastric ulcer and the phytochemistry constituent of the plant.

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### Abstract

The research work was conducted to assess the effect of methanol, ethylacetate and n-hexane fraction extracts of Combretum obanense on ethanol-induced gastric ulcer in rats and also to assess the phytochemical constituent of the plant for identification of biochemical components present in the plant. The rats which was fasted for 24 hours was orally administered with distilled water and Omeprazole (20 mg/kg). 100 mg/kg each of Methanol, Ethylacetate and N-hexane fractions were administered respectively. After an hour, 1 mL/kg of 96 % ethanol was administered orally to all the rats. Omeprazole (20 mg/kg) was used as a positive control. The antiulcer activity was assessed by determining and comparing the ulcer index in the test group with that of the standard drug treated group. From the result, the effects of Combretum Obanense were more pronounced after administration of methanol fraction followed by ethyl acetate and N-hexane fraction. They were all statistically significant at  $p < 0.05$ . This shows that the effect seems more effective in the plant extract fractions than in the commercial standard drug in the market. However, the result of the phytochemistry of the plant shows that methanol fraction contain thirty compounds, ethyl acetate has thirty three compounds while n-hexane contain sixteen compounds. Therefore it contains seventy nine (79) compounds from the three different fractions and they all have so many interesting applications in our health. Moreso, the gastro-protective activity of C. obanense may be due to their ability to synthesize the aromatic substances such as flavonoids and tannins which serve as a defense mechanism against different forces and also reduces the reduction in the damage of the mucosa induced by free radicals that causes cells and DNA damage.

**Keywords:** Combretum obanense, Phytochemistry, Omeprazole, Antiulcer.

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### I. Introduction

Over the years there has been a long search for cure of ulcer and the search continue to be effort in futility by researchers and medical practitioners. However, sorting relief seemed to be on the way. Ulcers is a tough illness as it occur when there are disruption or imbalance between the protective factors (mucus, bicarbonate, nitric oxide and normal blood flow, etc) and aggressive factors (acid, pepsin, leukotrienes, endothelins, bile) in the stomach. Majority of ulcers are caused by bacterial infection of the stomach called Helicobacter pylori (It is a spiral shaped, gram negative bacteria).[1] This infection in most cases is subclinical and it may be acquired in childhood. Other causes may be over use or excessive use of aspirin and anti-inflammatory medicines accounting for approximately 25% of gastric ulcer cases. Some of the symptoms of ulcers include upper abdominal pain, nausea and vomiting, and black, tarry stools (a sign of a bleeding ulcer). Current treatment of ulcers in developing countries has been largely suppression of pain, with little or no strategy aimed at a cure. [2-3] Responding to the cry by WHO to develop drugs from plant origin, which will be inexpensive, accessible particularly to the rural people in the developing countries, and show less or no side effects.[4] Thus, herbal medicine came becoming alternative treatment to available synthetic drugs for treatment of ulcer possibly due to lower costs, availability, fewer adverse effects and perceived effectiveness. Many

tropical herbs have been scientifically reported to possess potent antiulcer activity [5,6,7,8] therefore, this study is meant to assess the effect of methanol, ethylacetate and n-hexane fraction extract of combretum obanense stem bark of gastric ulcer and to study the phytochemistry of the fractions extract of the plant.

## II. Materials And Methods

### Plant material

The stem of *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 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 h. The solvent was removal in a rotary evaporator and freeze dried which provide an organic extract of 200 g.

### Phytochemical screening

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

### Acute Toxicity Test (LD50)

This study was done using Lork's procedure (1983) of LD<sub>50</sub> determination. In this study, 13 albino mice were used throughout. The following assumption were made during the assay, Substances more toxic than 1 mg/kg are so highly toxic that it is not important to calculate the LD<sub>50</sub> exactly. LD<sub>50</sub> values greater than 5000 mg/kg are of no practical interest. An approximate figure for the LD<sub>50</sub> is usually adequate to estimate the risk of acute intoxication. The experiment involved first, a preliminary trial using three different doses of the plant extract. The mice were divided in to three groups of three animals per group and marked at the head, tail and trunk in the first group. In the second group it was marked at Right hind, left hind and at both hind while the third groups were marked at right side, left side and both side. The first group (a) received 10 mg/kg of the extract, Second group (b) received 100 mg/kg of the extract, while the third group (c) received 1000 mg/kg of the extract as well. Under close observation, the mice were noticed to be calm during 1.50 minus after administration. It was also calm and depressed within the first 2h after administration. The observation was intermittent done for the next 4 h and overnight. From the result obtained from first phase. The second phase of the study test were administered using doses of 1600 mg/kg which was marked at Right Fore, 2900 mg/kg marked at Left Fore and 5000 mg/kg at Both Fore given to each mice. The last mice were unmarked and were only given distilled water and served as the positive control. There was no dead animal recorded within 24h of the experiment. This shows that the extract is safe and has a wide range of effective dose.

**Table 1:** phytochemical analysis of *Combretum Obanense*

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	Tanins	+++

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

### Effect of methanol, ethylacetate and n-hexane extracts of combretum obanense on ethanol-induced gastric ulcer in rats.

Ethanol-induced ulcer in rats was evaluated as described by Ode and Asuzu (2011). For each extract, adult wistar albino rats of either sex were used. They were carefully marked and weighed to be between 180-200 g. They were randomly assorted into five different groups with each group containing five rats. The rats were fasted for 24h. One group was orally administered with distilled water and another with Omeprazole (20 mg/kg) and serve as the positive control. For the remaining three groups, 100 mg/kg each of Methanol, Ethylacetate and N-hexane fractions were administered respectively. After an hour, 1 mL /kg of 96 % ethanol was administered orally to all the rats. Two hours after drenching the rats with ethanol, they were sacrificed humanely using

chloroform and their stomach were carefully removed and rinsed with distilled water. Each was cut open through the greater curvature with a scalpel blade and again rinsed with distilled water. Each stomach was pinned to a white background on a wooden board for examinations and assessment of ulcer. The stomachs were examined for ulcer with the aid of a magnifying lens (x10). The ulcer index was assessed as follows:

**Less than (<) 1.0 mm = 1,**

**Between 1 and 2mm = 2,**

**Greater than or equal to (≥) 3mm = 3.**

The sum of the scores were divided by 10 (magnification of the lens) to obtain the ulcer index in rat (Main and White, 1975). The mean ulcer index for each group was subjected to Anova and Duncan's multiple range test and the effectiveness of each extract and drug was calculated using the equation below.

$$\text{Preventive index} = \frac{U_1 - U_2}{U_1} \times 100$$

where,  $U_1$  = Ulcer index of control

$U_2$  = Ulcer index of treated

### III. Results

Effect of Methanol, Ethylacetate and N-hexane fractions of *combretum obanense* on rats in ethanol induced gastric ulcer.

The effect of Methanol, Ethylacetate and N-hexane fractions of *combretum obanense* on the ulcer index and percentage inhibition against ulcer in experimental animals are shown in table 2.

**Table 2: Effect of Methanol, Ethylacetate and N-hexane fractions of *combretum obanense* on Ethanol Induced Gastric Ulcer in Rats.**

Treatment	No. of animals	Mean ulcer index ± SE	Preventive index %
Methanol fraction (100 mg/kg)	5	3.00 ± 0.00 <sup>a</sup>	82.35
Ethylacetate fraction	5	3.33 ± 1.33 <sup>a</sup>	80.41
N-hexane fraction	5	11.00 ± 7.02 <sup>ab</sup>	35.29
Omeprazole (20 mg/kg)	5	3.20 ± 0.0 <sup>a</sup>	81.18
Distilled water as control	5	17.00 ± 1.53 <sup>b</sup>	0

Superscript indicates significant difference at  $P < 0.05$  when compared with the control.

**Table 3: PI = Preventive index mean ulcer index**

S/NO	Treatment	No. of animals	Mean ulcer index ± SE	Preventive index %
A	Methanol fraction (100 mg/kg)	5	3.00 ± 0.00 <sup>a</sup>	82.35
B	Ethylacetate fraction	5	3.33 ± 1.33 <sup>a</sup>	80.41
C	N-hexane fraction	5	11.00 ± 7.02 <sup>ab</sup>	35.29
D	Omeprazole (20 mg/kg)	5	3.20 ± 0.0 <sup>a</sup>	81.18
E	Distilled water as control	5	17.00 ± 1.53 <sup>b</sup>	0

Superscript indicates significant difference at  $P < 0.05$  when compared with the control.

NOTE: A, B, C are the treated groups treated with *combretum obanense*.

D serve as the positive control treated with omeprazole

E is the negative control administered with normal saline

### IV. Discussion

It is known that the effect of ethanol on administration to animal causes severe ulcerogenic effect, as ethanol is noted to increase gastric acid secretion which is involved in the formation of ethanol induced mucosal lesions, suppression of prostaglandin synthesis which results in increased susceptibility to mucosal injury and gastro duodenal ulceration.

From the result in the table, It was observed that when ethanol was administered on the animal of methanol sample the effect of ethanol on the animal was not much (82.35 %) suggesting that the effect was not significant. The effect of ethanol on the animal of ethyl acetate sample was (80.41 %) suggesting that the impart/effect of the ethanol was not much on the animal also. However, the N-hexane sample was (35.29 %) suggesting that the effect of ethanol was much (destructive) on the animal. This indicate that there is a serious ulcerogenic effect in the intestine of the animal as a result of ethanol induced mucosal lesions, suppression of prostaglandin synthesis which results in increased susceptibility to mucosal injury and gastro duodenal ulceration. While the effect on omeprazole that serve as the positive control was 81.18 %. From the look of the result generally, it seemed that methanol fraction of the plant extract was as effective as the omeprazole (positive control) or more than the control. Moreso, the gastro-protection was observed on the methanol fraction, ethyl acetate fraction of *Combretum Obanense* on the animals as it protected the rat gastric mucosa

against hemorrhagic lesions produced by absolute ethanol. This were statistically significant at  $p < 0.05$  as shown in Table. This gastro-protective activities of *C. obanense* may be due to the presence of flavonoids and tannins that serve as defense mechanism against different forces and it also reduce the reduction in the damage of the mucosa induced by free radicals.

#### **GC-MS Machine For Analysis Of The Fraction Extract Of Combretum Obanense**

The Atomx, an Agilent 7890A GC and a 5975C inert XL MSD were used for this study while employing the following conditions: J&W Scientific DB-VR of the column (30 m × 0.25 mm × 1.4 μm ), the carrier gas used was helium gas of 1.02mL/min of constant column flow rate. The oven temperature was programmed as 35°C for 4 min, 16°C/min to 85 °C for 0min, 30 °C/min to 210°C for 3 min. the total run time is 14.29 min. the inert temperature is 220°C. The solvent delayed for 0.5 min only and scanned at 5.19 scans/sec with scan range of m/z 35-300 at threshold of 400 and pressure of 20.14 psi. The chromatogram obtained from gas chromatography was then analyzed in mass spectrometry to get the mass of all fractions. The identification of phytochemical components was achieved through retention time and mass spectrometry by comparing the mass spectra of unknown peaks with those stored in Wiley 9 GC-MS library.

#### **V. Discussion**

The phytochemical analysis of the fraction of *C-Obanense* stem bark indicate that it contains the following; reducing sugar, resins, cardiac glycosides, saponin, steroids, carbohydrate, proteins, flavonoids, terpenoids, alkaloids, and tannins. The effect of methanol, ethylacetate and n-hexane fractions of combretum obanense stem bark on rats in ethanol induced gastric ulcer could be attributed to the present of favonoids and tannins in the sample extract.

#### **VI. Conclusion**

The present study shows that they contain numerous phyto constituents and that the stem bark of *Combretum obanense* possess significant anti-ulcer activity in animal models that seems to be safe for consumption. It shows that the effect seems more effective in the plant extract fractions than in the commercial standard drug. The result of the phytochemistry of the plant indicate that it contain seventy nine (79) compounds from the three different fractions and they all have so many interesting applications in our health. However, the gastro-protective activity of *C. obanense* may be due to their ability to synthesize the aromatic substances such as flavonoids and tannins which serve as a defense mechanism against different forces and also reduces the reduction in the damage of the mucosa induced by free radicals that causes cells and DNA damage.

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