

Original Research Article

Evaluation of Diuretic, Anti-Inflammatory and Antioxidant Potential of Ethanol Extract of *Combretum Platypterum* (Welw) Leaves

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Abstract: Folk medicines enjoy a respectable position today, especially in the developing countries. Medicinal plants have been source of wide variety of biologically active compounds used extensively as crude material or as pure compounds for treating various disease conditions. This present study was designed to evaluate the diuretic, antioxidant and anti-inflammatory potential of ethanol extract of *Combretum platypterum*. Twenty eight animals were used in the diuretic and anti-inflammatory study, and they were randomly divided into VII groups of four animals per group. Inflammation was induced using egg albumin together with a normal saline, and aspirin administered to each experimental animals paw before measurements were taken every 30 min interval. Group I (negative control) received standard laboratory feed and portable water *ad libitum*. Group II (positive control) received feed, water and fruseamide. Group III to VII received the extract ranging from 500mg/ml to 100mg/ml. The antioxidants activity was also evaluated using DPPH, Antilipid Peroxidation, and Nitric Oxide method. The results of the antioxidant activity of *C. platypterum* extract showed significant ($p < 0.05$) antioxidant potential. In the diuretic study, the concentration at 500mg/kg and 400mg/kg of the ethanol extract had the highest significant dose dependent increase in urinary excretion and urinary sodium loss but no effect on urinary potassium loss. The anti-inflammatory results showed that the extract significantly ($p < 0.05$) reduced inflammation. These findings suggest that the *Combretum platypterum* is highly potent diuretic, antioxidant and anti inflammatory agents.

Keywords: diuretic, antioxidant, anti inflammatory, *Combretum platypterum*, paw, fruseamide.

INTRODUCTION

Diuretics are the drugs that increase the rate of urine flow; clinically useful diuretics also increase the rate of excretion of sodium (Na) (natriuresis) and an accompanying anion, usually chorine (Cl) [1]. Most clinical applications of diuretics aim to reduce extracellular fluid volume by decreasing total body NaCl content. Although continued administration of diuretic causes a sustained net deficit in total Na⁺, the time course of natriuresis is finite because renal compensatory mechanisms brings Na excretion in line with the Na⁺ intake, a phenomenon known as diuretic braking [1]. Diuretics alter the excretion of other cations (e.g. K⁺, H⁺⁺), anions (e.g. Cl⁻, HCO₃⁻ and H₂PO₄) and uric acid. In addition diuretics may alter renal hemodynamic indirectly mediated by local prostaglandins synthesis. The study of plant species with diuretic effects is still a fruitful research for new diuretics with minimal or no side effects.

Inflammatory process is known to play a major role in most chronic disorders including neurodegenerative, cardiovascular, pulmonary,

metabolic, autoimmune etc. Various current treatments including steroids and Non-Steroidal Anti-inflammatory Drugs (NSAIDs) are clinically important therapeutic agents being used for inflammatory disease treatments. Prolonged use of such drugs causes undesirable and severe side effects. *Combretum Platypterum* leaves are used in Ogun State, Nigeria for the treatment of inflammatory diseases like rheumatoid-arthritis [3].

Medicinal plants are important sources of chemical substances with potential therapeutic effects. Since ages, there is exclusive use of plant drugs in traditional medicines as they represent a large source of natural medicines [2]. The World Health Organization has estimated that over 75% of the world's population still relies on plant-derived medicines, usually obtained from traditional healers, for basic health-care needs.

Also, the use of natural remedies has a long traditional history with minimum or no side effects, therefore making naturally originated agents with medicinal potential enviable to surrogate the use of chemical therapeutics.

Combretum Platypterum (Welw) belongs to Combretaceae family found in various regions of Africa. Its leaf used in night blindness and in treatment of ulcer. Flower used as antiseptic, antioxidant, emollient, astringent, and in relieving pain in folkloric medicinal use. Flower also used in obesity, thirst, headache, ozoena, dim vision, indigestion, anemia gout, bronchitis, nyctalopia, quarantine fever also stimulate milk secretion, and libido. A large number of compounds with great structural diversity have shown dependable promise in several animal bioassay systems [4]. Some of the compounds, found in some of those plants, which include flavonoids, alkaloids and vitamins have been identified in ethanol leaf extract of *Combretum Platypterum* [5].

Despite its wide traditional use in ethnobotany, there are a very few reports in literature on the diuretic and anti-inflammatory activity of these plants.

Therefore, the present study was designed to evaluate the diuretic, antioxidant and anti-inflammatory potential of ethanol extract of *Combretum Platypterum* (Welw).

MATERIALS AND METHOD

Sample Collection and Authentication

The leaves of *C. Platypterum* (Welw) were collected in April, 2016, from National Root, Crop and Research Institute, Umudike, Umuahia, Abia State, Nigeria. The leaf was identified by Dr. M.A. Jimoh of the department of plant science and biotechnology, Michael Okpara University of Agriculture, Umudike. The fresh leaves were plucked out from the plant stalk, rinsed with clean water and air-dried for 14 days. The dried leaves were pulverized with a mechanical grinder, packaged in air-tight glass jar and stored at room temperature until analysis was carried-out.

PREPARATION AND CONCENTRATION OF EXTRACT

500g of the pulverized sample was weighed, and soaked with 2L of absolute ethanol for 72hrs at room temperature. The mixture was then filtered into a beaker using Whatman filter paper No. 1 (125mm) and then allowed to stand in a water bath at 40°C for concentration of the crude extract.

Animals

Twenty eight wistar albino rats weighing between 80-130g were obtained from the department of zoology, university of Nigeria, Nsukka and kept in the animal house of the biochemistry department of Michael Okpara University of Agriculture, Umudike. The animals were allowed access to feed and water *ad libitum* for two weeks of acclimatization before the commencement of the experiment. The animals were

kept in a well-ventilated aluminum cages at room temperature and under natural light/darkness cycles. They were maintained in accordance with the recommendation of the Guild for the care and use of laboratory animals [6].

Experimental design

- i)** Group I (Negative Control): The animals were provided with standard laboratory feed and portable water *ad libitum*.
- ii)** Group II: furosemide group (Positive Control): Apart from feed and water, they were administered with fruseamide.
- iii)** Group III: The animals were administered 500mg/kg of *C. platypterum ethanol* extract.
- iv)** Group IV: The animals were administered 400mg/kg of *C. platypterum ethanol* extract.
- v)** Group V: The animals were administered 300mg/kg of the plant extract.
- vi)** Group VI: The animals were administered 200mg/kg of *C. platypterum ethanol* extract.
- vii)** Group VII: The animals were administered 100mg/kg of *C. platypterum ethanol* extract.

ACUTE TOXICITY TEST

The acute toxicity of the extract was done using white mice of both sexes. The animals were of average weight of 33g. Five groups of four animals each were used. The plant extract was administered to the animals intra-peritoneally at the doses of 0.2mg/kg, 125mg/kg, 250mg/kg, 500mg/kg and 1000mg/kg body weight respectively. The animals were observed for a maximum of 72 hours separately.

Evaluation of diuretic activity

The method of Lipschitz *et al.*, [8] was employed for the assessment of diuretic activity. The urine was collected using a syringe. The volume of the urine collected was measured at the end of 24hrs. During this period, no food and water was made available to the animals. The parameters taken were total urine volume, concentration of sodium ion, potassium ion, chloride and bicarbonate in the urine.

Sodium and potassium ion concentration were determined by flame photometer, chloride concentration and bicarbonate was estimated by titration with silver nitrate solution using three drops of 5% potassium chloride solution as indicator.

ANTI-INFLAMMATORY STUDY USING EGG ALBUMIN

Egg albumin induced paw edema was employed in the study. Before induction of

inflammation, they were given the ethanol leaf extract. Acute inflammation was produced by injecting the fresh egg albumin (0.1ml) into the plantar surface of all the rats right hind paw according to a modified method [7]. The size of the right hind paw of the rats was measured using vainer caliper, and measurements of the diameters of the paw was taken in a 30 min interval for 3 hours.

EVALUATION OF ANTIOXIDANT ACTIVITIES

DPPH radical scavenging assay

2,2-diphenyl-1-1-picrylhydrazine (DPPH) scavenging activity was quantified in the presence of stable DPPH radical on the basis of 2,2-diphenyl-picrylhydrazine (DPPH) assay system [9].

➤ Preparation of DPPH solution

- 1mmol/L of DPPH = 0.394g OF DPPH
- 0.5mMol = 0.197g(197mg)
- 1000ml = 197mg
- 150ml = ×

$$\times = \frac{150 \times 197}{1000} = 29.55\text{mg}$$

2ml of *Combretum playpterum* extract dissolved in ethanol was mixed at different concentrations (12.5 – 200µg/ml) with 1ml of DPPH solutions in test tube and incubated for 30minutes in the dark at room temperature. 1ml of ethanol + 2ml of test extract were used as negative control. The degree of discoloration indicates the scavenging efficacy of the extracts and absorbance was measured at 517nm. The experiment was performed in triplicate and percentage of scavenging activity was calculated using the following equation:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

NITRIC OXIDE INHIBITION ACTIVITY:

The nitric oxide scavenging activity was conducted based on the Greiss assay method [19] which involves generating nitric oxide from sodium nitroprusside by the Greiss reaction.

2.0ml of 10mM sodium nitroprusside and 5.0ml of phosphate buffer were mixed with 0.5ml of different concentrations (12.5-200µg/ml) of plant extract and incubated at 25°C for 150 minutes. The samples were run as above but the blank was replaced with the same amount of water. After the incubation period, 2ml of the incubated sample was added to 2ml of Greiss reagent (1% sulphanilamide, 0.1% α-naphthyl-

ethyldiaminedihydrochloride and 3% phosphoric acid) and then incubated for a period of 30 minutes. The absorbance of the pink chromophore formed by the diazotization of nitrite with α-naphthyl-ethyldiaminedihydrochloride was measured at 540nm. Ascorbic acid was used as positive control. The experiment was performed in triplicate and the capacity to scavenge the nitric oxide was calculated using the following calculation:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

ANTI-LIPID PEROXIDATION ACTIVITY

The determination of anti-lipid peroxidation activity was according to the method of Dinakaran *et al.*, [10]. The ethanol extract of *Combretum playpterum* were used at different concentrations (200, 100, 50, 25 and 12.5µg/ml) individually. 3ml of liver homogenate was added to 100µl of 15mM ferric chloride and was shaken for 30 minutes. From collected mixture, 100µl was added with 1ml of different concentrations of plant extract individually in different test tubes. Ascorbic acid was used as the standard (100µg/ml). All the test tubes were incubated for four (4) hours at 37°C.

After incubation, 1.1ml of 30% trichloroacetic acid (TCA) and 1.1ml of 0.65% thiobarbituric acid (TBA) were added to all tubes containing the mixture. After 30minutes of incubation in a shaking water bath and subsequent cooling in ice-cold water for 10 minutes, the tubes were centrifuged at 800g for 15 minutes. The absorbance was measured at 530nm. The percentage inhibition of lipid peroxidation was calculated by using the equation below:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical Analysis

All statistical analysis was performed using SPSS (version 22.0). Data was analyzed using one way analysis of variance (ANOVA). The mean values of each of the parameters measured in all the groups were compared with those of Group I (Control) for any significant difference using Duncan Multiple Range Test (DMRT). P values of 0.05 were taken as statistically significant.

RESULT

Table-1:Antioxidant activity of *Combretumplatypterum*

Sample	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	Vitamin C
DPPH	85.27±4.06 ^b	51.80±2.86 ^c	34.53±3.11 ^d	20.77±5.00 ^e	0.00±0.00 ^f	97.70±0.49 ^a
Antilipid.Peroxidation	81.17±3.13 ^b	43.23±53.24 ^c	28.01±2.63 ^d	18.70±2.60 ^e	8.87±4.43 ^f	99.37±0.29 ^a
Nitric.Oxide	63.17±1.94 ^b	50.03±1.18 ^c	37.70±0.26 ^d	21.50±1.29 ^e	19.90±4.12 ^f	98.13±0.15 ^a

Note: Values having the same superscripts are not significantly different from one another, while values having different superscripts are significantly difference by Duncan Multiple Range Test (DMRT), significant at (P ≤ 0.05)

Table 2 above shows the results of the antioxidant activity of *C. platypterum*, tablets containing 200, 100, 50, 25, and 12.5 mg/ml. All three

formulations were evaluated for antioxidants activity by using DPPH, Antilipid.Peroxidation, and Nitric.Oxide models.

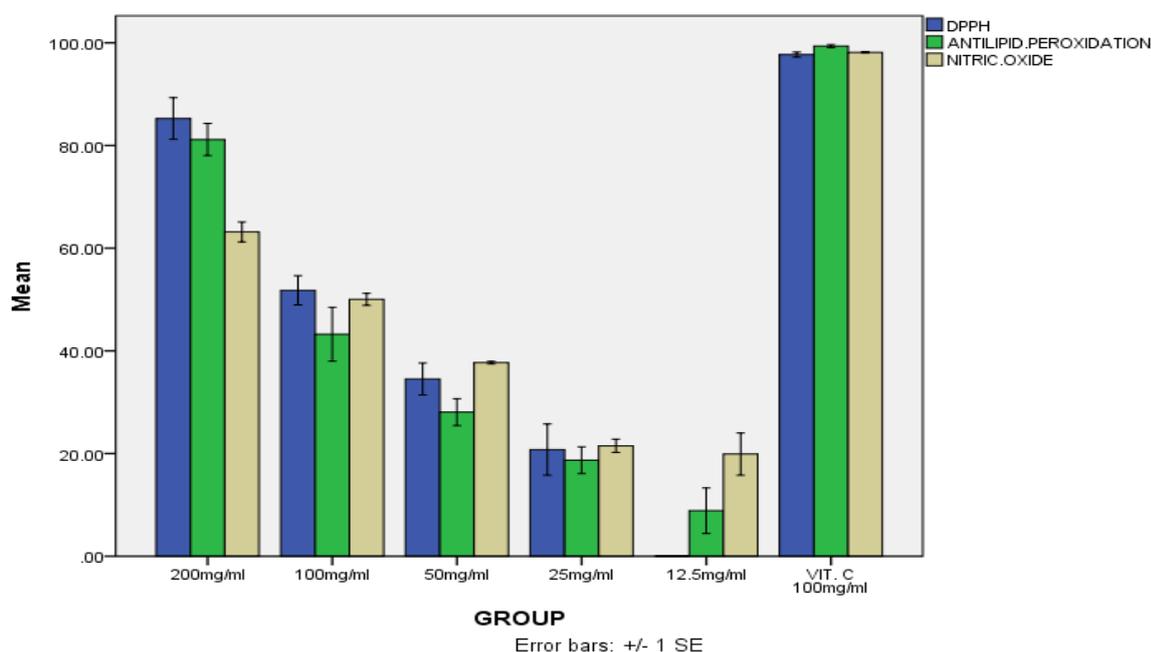


Fig. 1: Graph showing Antioxidant analysis

The first bar shows the DPPH (Blue), the second bar shows Antilipid.Peroxidation (Green) and the third bar shows Nitric.oxide (Brown).

Table-2: Diuretic activity of *Combretumplatypterum*

	Group 1 (Negative Control)	Group 2 Furosemide (Positive Control)	Group 3 (500mg/kg)	Group 4 (400mg/kg)	Group 5 (300mg/kg)	Group 6 (200mg/kg)	Group 7 (100mg/kg)
Urine Volume	3.25±0.07 ^a	0.15±0.49 ^e	2.50±1.23 ^b	2.00±0.63 ^c	1.05±0.41 ^d	1.00±1.07 ^e	0.10±0.10 ^e
Sodium	87.25±70.10 ^a	40.60±22.81 ^f	79.15±67.08 ^b	68.65±50.23 ^c	53.37±37.80 ^d	48.00±35.29 ^e	27.95±15.88 ^g
Chloride	84.75±66.33 ^a	38.40±18.07 ^e	77.15±53.64 ^b	66.90±42.17 ^c	50.90±26.76 ^d	46.85±32.24 ^d	25.55±19.83 ^f
Potassium	33.70±24.81 ^a	13.35±7.63 ^e	31.75±22.22 ^a	27.00±14.29 ^b	19.45±12.46 ^c	16.15±12.24 ^d	11.45±4.46 ^e

Note: Values having the same superscripts are not significantly different from one another, while values having different superscripts are significantly difference by Duncan Multiple Range Test (DMRT), significant at (P ≤ 0.05)

Table 2 show the urine volume collected in 24 hours for all the groups. It is evident that test extract treated groups excreted more urine than the control groups. The extract at 500 mg/kg exhibited comparable

effect with reference drug furosemide 20mg/kg and the results was statistically significant. Table-2 shows the sodium and potassium content of the urine for all groups. The amount of Sodium excreted was increased

for Furosemide treated group; statistically significant rise in Na⁺ excretion was also noticed for ethanol extract treated groups. The potassium content excreted in the urine was statistically insignificant for all the groups.

The Natriuretic effect was calculated by employing the formula Na⁺ / K⁺. It was found that the extract treated groups possess favourable Natriuretic effect. The results showed that the ethanol extract of *Combretum platypterum* significantly increases the urine output and excretion of urinary sodium and had no effect on the urinary potassium excretion.

Table 3: Results of Anti-inflammatory Analysis

Groups	Initial Size	Mean Change in Paw (mm)					
		30 mins	60 mins	90 mins	120 mins	150 mins	180 mins
Group 1	2.90±0.10 ^b	2.85±0.10 ^b	2.75±0.10 ^c	2.20±0.00 ^b	2.45±0.10 ^b	2.20±0.00 ^b	2.05±0.10 ^c
Group 2	3.20±0.20 ^a	3.10±0.10 ^b	2.95±0.10 ^c	2.75±0.10 ^a	2.95±0.10 ^a	2.60±0.10 ^a	2.40±0.00 ^a
Group 3	3.45±0.10 ^a	2.15±0.10 ^b	2.95±0.10 ^c	2.70±0.00 ^a	2.80±0.10 ^a	2.60±0.10 ^a	2.35±0.10 ^b
Group 4	3.10±0.10 ^a	3.10±0.00 ^b	2.75±0.10 ^c	2.55±0.10 ^c	2.80±0.10 ^a	2.65±0.15 ^a	2.25±0.15 ^c
Group 5	2.95±0.10 ^b	2.85±0.10 ^b	2.75±0.10 ^c	2.75±0.10 ^a	2.65±0.10 ^a	2.50±0.00 ^b	2.80±0.20 ^a
Group 6	3.10±0.10 ^b	2.85±0.10 ^b	2.85±0.10 ^c	2.90±0.10 ^a	2.70±0.00 ^a	2.65±0.10 ^a	2.35±0.10 ^b
Group 7	2.90±0.10 ^b	3.00±0.30 ^b	2.95±0.15 ^c	2.85±0.15 ^a	2.80±0.20 ^a	2.65±0.10 ^a	2.65±0.15 ^a

Note: Values having the same superscripts are not significantly different from one another, while values having different superscripts are significantly difference by Duncan Multiple Range Test (DMRT), significant at (P ≤ 0.05)

Table 3 above showed the anti-inflammatory activity test, *C. platypterum* ethanol extract (100-500mg/kg), normal saline as control (0.1ml/kg) 1 hour before the induction of inflammation. Acute inflammation was produced by the sub-planter administration of 0.1ml fresh egg albumin into the right hind paw of each rat 3hour after administration of respective extracts. The paw volume was measured at 0min and 180mins, taking the readings at 30mins intervals, after the egg- albumin administration by displacement technique using digital Phlethysmometer [11].

DISCUSSION

Screening of traditionally used plants and discovery of their active components with combined antioxidant properties would be beneficial in the treatment of various disorders. Studies have shown that increased production of reactive oxygen species (ROS) may be one of the underlying causes of most degenerative diseases [12,13] including cancer.

Combretum platypterum is an important plant which is used traditionally as a medicine. Various fractions of *C. platypterum* were prepared and tested for their antioxidant, anti-lipid.peroxidation and Nitric.Oxide effects.

In the DPPH radical scavenging assay the different concentrations of the active samples were calculated in upper and lower bound, the values were found to be significant as 97.70±0.49^a for Vitamin. C and 0.00±0.00^f at 12.5mg/ml respectively.

Anti-lipid peroxidation activity of *Combretum platypterum* revealed great efficacy as compared to standard inhibitor egg albumin. Among these samples Vitamin. C (100mg/ml) and *C. platypterum* ethanol extract (200mg/ml) showed relatively good activity of inhibition at a concentration 0.1ml.

Nitric oxide radical scavenging assay; Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions, which can be measured at 546 nm spectrophotometrically in the presence of Griess reagent. The oxidant potency of the extract was showed to be dose dependent as shown in table 1 above.

Diuretics have two separate connotations; increase urinary phrase and net loss of solute (i.e. electrolyte) and water (i.e. saluretic). These two processes are involved in the suppression of renal tubular reabsorption of electrolytes, water and low molecular weight organic compounds into the blood stream and a consequence; promote the formation of urine. An attempt to extrapolate the diuretic action of plant extract from rats to man using the activity of furosemide in an organism as a guideline has been reported [14-16].

The 500 and 400mg/kg was found to be the most potent in increasing the urinary output: the effect was comparable to that of the standard drug (furosemide). The results clearly shows that the ethanol extracts at doses of 500 and 400 mg/kg produced significant (p<0.05) dose dependent increase in urinary excretion and urinary sodium loss but no effect on

urinary potassium loss with respect to control and standard drug treated groups. The data demonstrates that the extract has diuretic effect and natriuretic effect but no potassium sparing effect and is as potent as furosemide.

The most widely used primary test to screen anti-inflammatory agent measures the ability of a compound to reduce local oedema induced in the rat paw by injection of an irritant agent [17]. Egg albumin induced oedema has been commonly used as an experimental animal model for inflammation. Table 3 shows the effect of the various treatment groups with regards to the mean paw circumference. There is significance difference ($P < 0.05$) between the mean paw circumferences of group treated with 500mg/kg concentration of the ethanol extract and the negative control. The development of oedema in the right paw of the rat after injection of egg albumin is due to release of pro-inflammatory mediators like histamine and prostaglandin [18]. The highest percentage inhibition activity was found in the 500mg/kg concentration of the ethanol extract. Egg albumin-induced inflammation model is a significant predictive test for anti-inflammatory activity [11]. These results are an indication that *Combretum platypterum* can be effective in the amelioration of acute inflammatory disorders.

CONCLUSION

Combretum platypterum which is traditionally used by tribes showed significant diuretic and anti-inflammatory activity and it was non toxic at the concentration administered on acute toxicity evaluation. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as diuretic and anti-inflammatory agent.

With the emerging need for herbal therapies in our society to combat life threatening ailments, it is important to conduct further studies to understand the potential of these plants as sources of compounds responsible for their actions.

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