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Proceeding Paper

Modulatory Action of Phenolic-Enriched *Combretum paniculatum* Vent Ethanolic Extract on Oxidoinflammatory Anomalies in Experimental Animals †

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Abstract: Medicinal plants with favorable therapeutic effects have gained interest over conventional drugs in treating oxidative stress and inflammatory-mediated diseases. The antioxidant and anti-inflammatory activities of *Combretum paniculatum* ethanolic extract (CPEE) were investigated in this study using in vitro and in vivo analysis. The results of phytochemical screening recorded in mg/100 g revealed that CPEE is phenolic rich and also contains high abundance of alkaloids, reducing sugars and flavonoids. Terpenoids, and tannins were recorded in moderate quantity/Our in vitro analysis revealed that CPEE inhibited nitric oxide, phospholipase A2, and thiobarbituric acid reactive substance activities with half-maximal inhibitory concentration (IC_{50}) values of 6.55, 361.1 and 2.28 µg/mL, respectively. Furthermore, the in vivo study showed that implantation of cotton pellets elicited an increase in granuloma tissue formation, levels of malondialdehyde (MDA), and C-reactive protein (CRP) while decreasing the activities of superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) in the untreated groups compared to normal rats. Interestingly, the groups treated with 100 and 200 mg/kg of CPEE had decreased granuloma tissue, MDA, and CRP with an increase in the activities of SOD, CAT, and GSH. These findings suggest that CPEE ameliorated chronic inflammatory-induced oxidative stress in the experimental animals. Thus, it could be applied as an effective remedy for developing antioxidant and anti-inflammatory drugs.

Keywords: antioxidant; inflammation; medicinal plants; oxidative stress; phenols and phytochemicals

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1. Introduction

Inflammation is a physiological process saddled with the role of destroying injurious agents and harmful stimuli [1]. Based on the duration and mediators mobilized, inflammation could be acute or chronic. However, chronic inflammation leads to an unregulated release of activated mediators, cells, and oxidant species implicated in the pathogenesis of several diseases [2]. Consequently, several orthodox anti-inflammatory agents are prescribed in clinical settings to modulate inflammation [1]. Regrettably, most of these drugs relieve symptoms transiently and present severe side effects on the liver, gastrointestinal tract, and kidney [3].

The recent quest for the use of medicinal plants with favorable therapeutic effects in the treatment of oxidative stress and inflammatory-mediated diseases is due to their safety profile, availability, biocompatibility, and multiple targeted approaches. *Combretum paniculatum* is a flowering plant in the *Combretaceae* family used as a treatment option

for pain, dysentery, and enlarged liver, as well as an anti-cancer, antimicrobial, and anti-diarrhea agent [4]. This study investigated the extract's efficacy in modulating oxidative stress and inflammatory anomalies using in vitro and in vivo approaches.

2. Materials and Methods

2.1. Plant Collection, Preparation and Extraction

Fresh leaves of *C. paniculatum* were shade dried, pulverized, and extracted with 2 L of ethanol (70%) for 72 h. The macerate was filtered with Whatman filter paper and concentrated with a rotary evaporator to get the *C. paniculatum* ethanol extract (CPEE) used for this research.

2.2. Chemicals and Reagents

This study procured analytical grade chemicals from these companies: Sigma-Aldrich Inc., UK, Teco USA, British Drug Houses, (BDH) England, and Evans Pharmaceutical, England.

2.3. Phytochemical Screening, In Vitro Antioxidant and Anti-Inflammatory Activities

The amount of phytochemicals in the CPEE was estimated with the Harbone [5] method. The inhibitory effects of CPEE on nitric oxide, scavenging activity, lipid peroxidation, and phospholipase A2 activity were investigated with the methods of Sreejayan and Rao [6] Banerjee et al. [7] and Vane [8], respectively.

2.4. Induction of Inflammation

This was done using twenty five Wistar rats randomized into five groups (n = 5) according to the protocol described by Mosquera et al. [9]. The rats in group 1 served as baseline (no induction and treatment), group 2 rats were implanted, and administered distilled water, the standard drug diclofenac sodium (100 mg/kg b.w.) was given to group 3, while groups 4–5 were treated with 100, and 200 mg/kg b.w. of CPEE, respectively. The treatment period lasted for seven days, and on the eighth day, the pellets were removed, and blood samples for measurement of biochemical parameters were collected. Ethical approval of the study with approval number UNN/FBS/EC/1082 was obtained from the Faculty of Biological Sciences, University of Nigeria, Nigeria Ethics and Biosafety Committee.

2.5. Biochemical Parameters

The following biochemical parameters were measured from the serum: MDA level, activities of SOD and CAT, and levels of GSH, vitamin E, and C using standard methods as reported by Chukwuma et al. [10].

2.6. Statistical Analysis

Data were analyzed using GraphPad Prism version 6.5 (GraphPad Software, Inc., San Diego, CA, USA), and the results were presented as mean ± S.D. Data were considered statistically significant when * $p < 0.05$, ** $p < 0.001$, and *** $p < 0.001$.

3. Results and Discussion

3.1. Quantitative Phytochemical Screening of the CPEE

The use of plants as therapeutic agents started from human evolution due to the presence of phytochemicals that have significant biological and pharmaceutical actions [11–13]. This study recorded a high abundance of phenols, alkaloids, flavonoids, and reducing sugar with moderate amounts of tannins and terpenoids in CPEE. (Table 1). These phytochemicals identified in the extract have proven to be effective as antioxidants, anti-inflammatory, immunomodulatory, neuroprotective, and anti-diabetic agents [13]. Hence, their

presence in the extract suggests that it could have a plethora of activities against the aforementioned diseases.

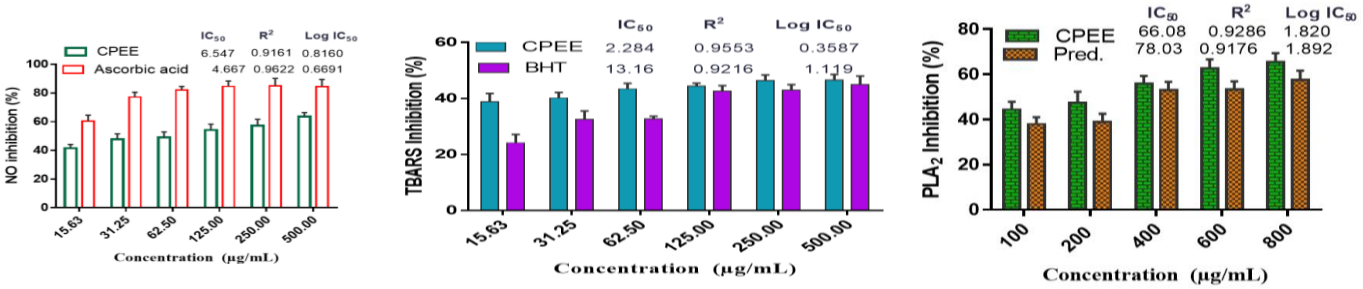
Table 1. Quantitative phytochemical screening of the CPEE.

S/N	Phytochemicals	Amount (mg/100 g)
1	Phenols	2711.02 ± 60.66
2	Tannins	21.12 ± 0.41
3	Flavonoids	49.00 ± 6.74
4	Alkaloids	605.83 ± 10.10
5	Steroids	0.64 ± 0.06
6	Terpenoids	12.17 ± 0.55
7	Reducing sugar	57.03 ± 0.12
8	Glycosides	2.59 ± 0.82

Values are recorded as mean ± SD of triplicate at 95% confidence intercal.

3.2. Antioxidant and Anti-Inflammatory Activities of CPEE

The results of the in vitro studies revealed that the extract demonstrated potent antioxidant and anti-inflammatory potentials by inhibiting NO, and TBARS, PLA_2, as shown in Figure 1. Natural products with antioxidant activity can mop up the excess oxidant species and regulate inflammatory response [2]. The high antioxidant and anti-inflammatory activities of CPEE is a pointer that it has a multi-therapeutic approach needed to avert cellular damages under oxidative stress and abrogated inflammatory response.



(**A**) (**B**) (**C**)

Figure 1. Antioxidant and anti-inflammatory activities of CPEE. The values are reported as mean ± S.D. (**A**) PLA_2 inhibition, (**B**) TBARS inhibition, and (**C**) NO scavenging activities

3.3. Effect of CPEE on Wet and Dry Granuloma Tissue Weight

Cotton pellet implantation elicits the proliferation of neutrophils, fibroblasts, and macrophages, leading to granuloma tissue formation to ward off the external agent, cotton pellet [1]. Herein, treatment with varied doses of CPEE (100 and 200 mg/kg b.w.) inhibited granuloma tissue formation significantly ($p < 0.05$) compared with the untreated group 2 (Figure 2). Inhibition of granuloma formations suggests that the extract might have reduced angiogenesis, collagen synthesis, and excessive exudation of inflammatory cytokine, which limited the amount of granuloma formed [1].

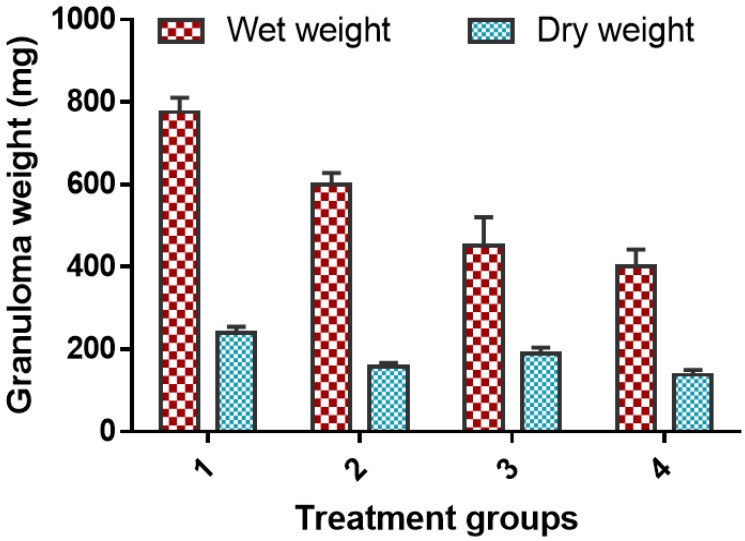


Figure 2. Effect of CPEE on wet and dry granuloma tissue weight. The values are presented as the mean ± S.D. (n = 5). Group 1 was implanted and given distilled water, and groups 2–4 were administered diclofenac sodium (100 mg/kg b.w.), 100 and 200 mg /kg b.w. of CPEE, respectively.

3.4. Effect of CPEE on Lipid Peroxidation and Antioxidant Markers

Oxidative stress and inflammation are closely related pathophysiological processes implicated in the etiology and pathogenesis of several diseases. In this study, CPEE demonstrated in vivo antioxidant effect by inhibiting inflammatory mediated peroxidation of biomembrane as evidenced in the significant ($p < 0.06$) decrease in MDA level in the treated groups relative to group 2, administered distilled water after cotton pellet implantation. More so, it restored the activities of SOD and CAT as well as levels of GSH, vitamin E, and C (Table 2). This pharmaceutical activity validates the antioxidant potency of the extract. Inhibitions of MDA and restoration of antioxidant markers are immensely beneficial in preserving tissue and cellular integrity [10]. This action could result from the high amounts of phenols and other antioxidant phytochemicals in the extract. Phenols attenuate oxidative damage by scavenging and chelating radical species, activating the expression of endogenous antioxidants, and terminating the peroxidation reaction [12].

Table 2. Effect of CPEE on lipid peroxidation and antioxidant markers.

Group	MDA (mg/dL)	SOD (iU/L)	CAT (iU/L)	GSH (mg/dL)
1	1.11 ± 0.45	11.22 ± 0.21	1.48 ± 0.24	0.60 ± 0.07
2	4.81 ± 0.01	9.30 ± 0.12	0.19 ± 0.03	0.18 ± 0.01
3	0.90 ± 0.07	11.36 ± 0.04	0.10 ±0.10	0.47 ± 0.09
4	2.12 ± 0.15	11.16 ± 0.19	1.19 ± 0.28	0.34 ± 0.02
5	0.96 ± 0.84	11.23 ± 0.02	1.24 ± 0.35	0.45 ± 0.01

The values are presented as the mean ± S.D. (n = 5). Group 1 was implanted and given distilled water, and groups 2–4 were administered diclofenac sodium (100 mg/kg b.w.), 100 and 200 mg /kg b.w. of CPEE, respectively.

4. Conclusions

These findings from this study show that CPEE ameliorated chronic inflammatory-induced oxidative stress in the experimental animals. This pharmaceutical action could be attributed to the rich phytoconstituents identified in the extract. Thus, the leaves of *C. paniculatum* could be employed as an effective remedy for developing antioxidant and anti-inflammatory drugs.

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