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# In vivo Antioxidant Evaluation of *Combretum platypterum* (Welw.) Hutch. and *Combretum racemosum* P. Beauv. (Combretaceae) in Rodents

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## ABSTRACT

**Aim:** The aim of this study was to evaluate the *in vivo* antioxidant effect of the aqueous leaf extract of both plants. **Methods:** Seven groups of 4 animals each were used. Groups 1 – 3 were administered *C. platypterum* extract at various doses. Groups 4 – 6 were administered *C. racemosum* extract while group 7 received distilled water. The animals were sacrificed after 21 days using chloroform as anesthesia. Vital organs; hearts, livers and kidneys were isolated and homogenized for antioxidant assays. **Results:** *C. platypterum* significantly ($P<0.05$) increased the level of SOD and peroxidase in the heart at (200 and 400) mg/kg. SOD level was also increased in the liver at 400 mg/kg and peroxidase in the kidney at all doses improved. *C. racemosum* significantly ($P<0.05$) increased the level of peroxidase in the kidney at doses (100, 200 and 400) mg/kg. MDA and catalase level were unaffected by aqueous leaf extracts of both plants. *In vivo* antioxidant activity of C. *platypterum* was more in *C. racemosum*. However, there was a marked boost of peroxidase in the kidney of animals treated with *C. racemosum*. **Conclusion:** *C. platypterum* demonstrates superior antioxidant activity in the heart and liver compared to *C. racemosum.*

KEY WORDS: *Combretum platypterum, Combretum racemosum, in vivo* antioxidant.

## INTRODUCTION

Medicinal plants have been used to treat various illnesses including diabetes, hypercholesterolemia, hypertension, arthritis, tumors, pain, inflammation, colds, coughs, many but a few to mention. Plants that have medicinal properties are said to be rich in phytochemical compounds such as saponin, alkaloid, tannins, phenolic compounds and antioxidants [1].

*Combretum platypterum* (Welw) Hutch & Dalziel belongs to the family of Combretaceae. It is widely distributed from Guinea to DRC, Southern Sudan and Angola [2]. C. *platypterum* is abundant in rain forests, secondary forests and Savanna regions, occasionally in swampy localities, from sea-level up to 450 m above sea level. In Nigeria, it is commonly called "mmanyanza" or "achichanza" by the Igbos while the Yorubas refer to it as "Ogan Ogandudu" or "Ogan ibule" [3]. C. *platypterum* is ethno-therapeutically used to treat lower back-ache, fever, eye problems, malaria, swellings, lumps, conjunctivitis, coughs, sexually transmitted diseases, helminthiasis and diarrhea. It is also used as tonic, febrifuge and to stop post-partum bleeding [4-6]. Despite its traditional uses, pharmacological studies have not been carried out on this species.

*Combretum racemosum* P. Beauv belongs to family Combretaceae. It is common called Christmas rose. In Nigeria, it is called "okoso" by the Edos, "Alagame" in Igbo (Umuahia) and "ogan pupa" in Yoruba [7]. Traditionally the young leaves are used as anti-helmint [2]. It is also used to treat roundworm in children [8]. In Congo the plant is used to treat genito-urinary and gastro-intestinal infections accompanied by bleeding. The macerated root or decoction, is used to treat dysenteries. Leaf-sap is used for haemorrhoids, and bark-pulp is used to control bleeding during pregnancy. Powered leaves or roots are used for haematuria, convulsion, cough and tuberculosis. Powdered bark or leaves are used to treat circumcision wounds [9]. Okwuasa *et al.* [10] have reported the anti-ulcer and antimicrobial effects of C. *racemosum* leaf extract. C. *racemosum* also possesses protective effect on the liver and bone marrow [11]. Phytochemicals present are alkaloids saponins, flavonoids, terpenoids, glycosides, resins, carbohydrate and steroid [11]. Schepetkin *et al.* [12] reported the immunomodulatory and hemaglutinating activities of aqueous extract of C. *racemosum*. The methanol extract possess antioxidant activity [13]. Okwuasa *et al.* [11] reported that the $LD_{50}$ of C. *racemosum* is greater than 5500 mg/kg. This study was aimed at evaluating the *in vivo* antioxidant activities of the aqueous leaf extracts of *Combretum platypterum* and *Combretum racemosum*.

## MATERIALS AND METHODS

### Collection and Preparation of Plant Material

Fresh leaves of *Combretum platypterum* and *Combretum racemosum* were collected from Sakponba Forest, Edo State, Nigeria. The plants were identified and authenticated by Dr. H. Akinnibosun of the Department of Plant and

Biotechnology, Faculty of life Sciences, University of Benin City, Edo State, Nigeria. The leaves were copiously washed in clean water and air-dried for one week in the Department of Pharmacognosy, University of Benin, Benin City. Leaves were further subjected to another 24 h of drying in an electric oven maintained at 40 $^{O}$C. Leaves were powdered using an automated mill. The pulverized material (200 g) was mixed with distilled water (5 L) and left for 72 h though stirred at 6 h intervals. At the end of the 72 h the mixture were filtered using a clean muslin cloth. Filtrate was concentrated over a water bath. Both concentrates yielded 40% w/w each. The concentrated extracts were stored in air-bottles; labeled and refrigerated at 4 $^{O}$C prior to use (Experiments commenced 24hrs after extracts were refrigerated). 10 g of plant extract was weighed and dissolved in 100 ml of distilled water to give a concentration of 100 mg/ml. The final volume that was administered from the prepared stock was calculated based on individual body weight of the animal.

### Experimental Animals

Sixteen weeks old Albino rats of both sexes weighing (100-250) g were purchased from a commercial farm in Benin City and housed in the animal facility of the Department of Biochemistry, University of Benin, Benin City. The animals were allowed to acclimatize for 2 weeks with 12-h light/dark cycle at room temperature. They were fed with standard rodent pellets and water *ad libitum*. The animals were handled according to standard protocols for the use of laboratory animals (National Institute of Health USA: Public Health Service policy on humane care and use of Laboratory Animals 2002).

### Experimental Protocol

Seven groups of 4 rats each weighing between 150 to 250g were used in this study. Group 1 received distilled water (2 ml/kg p.o.). Groups 2 to 4 received aqueous extract of *C. platypterum* and groups 5 to7 received *C. racemosum* (100, 200 and 400 mg/kg p.o.) for 21 days using oral-gastric tube (C.U. FNC-16-3). On the 21$^{st}$ day of administration, the animals were sacrificed after being anesthetized with chloroform. The hearts, livers and kidneys were excised, weighed and normalized for antioxidant activity.

(**N.B**: 21 days administration was preferred as authors aimed at evaluating the sub-acute *in vivo* antioxidant activities of the plant extracts).

### Antioxidant Enzymes Assay

**Superoxide dismutase (SOD)**: This assay was done following the method described by Misra and Fridovich [14]. Carbonate buffer (2.5 ml) was measured into test tubes. 0.2 ml of tissues homogenate was added to appropriately labeled test tubes containing the buffer. While 0.2 ml of distilled water was added to test tube labeled reference. 0.3 ml of 0.3 mM of adrenaline solution was then added to the reference and each of the solution. Absorbance was read at 420nm with UV-Visible Spectrophotometer (Model: T80+UV/Vis Spectrometer, PG Instruments Ltd), distilled water was used to zero the machine.

**Catalase:** Catalase acts to prevent the accumulation of noxious $H_2O_2$ by converting it to oxygen and water [15]. Distilled water (0.5 ml) was measured into blank test tubes while 0.5 ml of sample was measured into labeled test-tubes. Hydrogen peroxide (2.5 ml of 30 M) was added into the labeled sample test tubes and blank tube. After 3 min, 1 ml of 6 M $H_2SO_4$ and 3.5 ml of 0.01 M potassium permanganate were added to the test and the blank tubes. Absorbance was read within 30-60 s. Spectrophotometric standard was prepared by adding 3.4 ml of 0.01 M potassium permanganate to a mixture of 5.5 ml of 0.05 M phosphate buffer pH 7.0 and 1.0 ml of sulphuric acid solution. The spectrophotometer was zeroed with distilled water.

Moles of $H_2O$ consumed/min (units/mg of tissue) =

$$\frac{2.3}{\Delta t} \times \text{In} \left(\frac{E\ Initial}{E\ Final}\right) \times 1.63 \times 10^{-3}$$

Where E= optical density at 240 nm,

$\Delta t$ = time required for a decrease in the absorbance [16].

**Malondialdehyde:** Malondialdehyde (MDA) formed from the breakdown of poly unsaturated fatty acids, (PUFA) serves as a convenient index for determining the extent of peroxidation reaction. When heated with 2-thiobabituric (TBA) under acid condition, malondialdehyde forms a pink coloured product which absorbs at 535 nm. Malondialdehyde was estimated by the method of Buege and Aust [17]. A volume (1 ml) of the tissue homogenate (0.5 ml) was added to 1:1(v/v) TCA-TBA-HCI reagent (thiobarbituric acid 0.375 % w/v, 15% TCA w/v and 0.25 HCl) and mixed. The solution was heated for 15 min in a boiling water bath. After cooling the precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the clear supernatant was measured against a reference blank at 535 nm. The malondialdehyde concentration of the sample was calculated using extinction co-efficient of 1.56 x $10^5$ $M^{-1}$ $cm^{-1}$

**Peroxidase:** Peroxidase converts $H_2O_2$ to $H_2O$ and $O_2$ in the presence of the hydrogen donor pyrogallol [18]. 3.0 ml of pyrogallol solution, 0.1 ml of tissue homogenate 0.5 ml of $H_2O_2$ was added and mixed. Change in absorbance was read at 430 nm at 30 second and 3 minutes.

### Statistical Analysis Data

Data are expressed as Mean ± Standard Error of Mean (SEM) and "n" represents the number of rats or mice per experimental group. One-way analysis of Variance (ANOVA) was performed with Newman Keuls' post hoc test. All data were analyzed using Graph Pad Prism (UK) software version 6.0. Significant differences were determined at $P<0.05$. $P<0.05$ indicates significant difference between compared data.

## RESULT

The aqueous leaf extract of *C. platypterum* significantly ($P<0.05$) increased the level of peroxidase and superoxide dismutase (SOD) at 200 and 400 mg/kg on the heart when compared to control. However, the extract showed no significance on Malondialdedyde (MDA) and catalase activities compared to control as shown on Table 1.

Table 2 shows that the aqueous leaf extract of *C. platypterum* significantly ($P< 0.05$) increased the activity of SOD at 400 mg/kg on the liver. In contrast to the control group, catalase, MDA and peroxidase were not significantly different from control.

In the kidney, aqueous extract of *C. platypterum* at 100, 200 and 400 mg/kg significantly ($P<0.05$) increased peroxidase level compared to control. Catalase MDA and SOD was unaffected compared to control ($P > 0.05$) as reported in Table 3.

Aqueous extract of *C. racemosum* showed no activities on antioxidant enzyme in the heart at all dose levels as shown on Table 4.

Table 5 shows that the aqueous extract of *Combretum racemosum* on the liver showed no significant effect in the levels of SOD, catalase, MDA and peroxidase at all dose level compared to control.

Aqueous extract of *C. racemosum* on the kidney shows significant ($P <0.001$) increase in the level of peroxidase at all dose levels compared to control. There were no significant effect in the level of SOD, MDA and catalase compared to control (Table 6).

**Table 1.** The effect of 21 days daily oral administration of aqueous leaf extract of *C. platypterum* on the heart.

| Dose (mg/kg) | Catalase ( $\times10^{-2}$) | Peroxidase($\times10^{-3}$) | SOD ( $\times10^{-2}$) | MDA( $\times10^{-6}$) |
|---|---|---|---|---|
| Control | 3.42 ± 0.72 | 0.18 ±0.09 | 25.27±13.46 | 3.45±1.38 |
| 100 | 1.42 ± 0.49 | 1.85±0.62 | 46.32±10.86 | 8.31 ± 2.08 |
| 200 | 2.82± 0.68 | 4.22±1.59* | 64.13±2.01* | 2.72 ± 0.80 |
| 400 | 2.51± 1.51 | 4.27± 0.55* | 73.81±8.96* | 5.57±2.19 |

Key: Values are presented as Mean ± SEM, n = 4.* significant difference at $P < 0.05$ compared to control; SOD - Superoxide dismutase; MDA - Malondialdedyde;

**Table 2.** The effect of 21 days daily oral administration of aqueous leaf extract of *C. platypterum* on the Liver.

| Dose (mg/kg) | Catalase ($\times10^{-2}$) | Peroxidase($\times10^{-3}$) | SOD ( $\times10^{-2}$) | MDA($\times10^{-6}$) |
|---|---|---|---|---|
| Control | 6.82±3.62 | 1.05±0.23 | 36.95±6.15 | 3.16±1.54 |
| 100 | 1.72±1.05 | 1.76±0.66 | 25.36±4.72 | 2.72±0.58 |
| 200 | 3.44±0.65 | 1.20± 0.27 | 42.14±6.51 | 2.57±0.57 |
| 400 | 1.35±0.35 | 2.77±0.40 | 61.84±6.59* | 1.27± 0.28 |

* $P < 0.05$ Compared to control SOD: Superoxide dismutase MDA: Malondialdehyde. Value are presented as mean ± SEM, n = 4.

**Table 3.** The effect of 21 days daily oral administration of aqueous Leaf extracts *C. platypterum* on the Kidney.

| Dose (mg/kg) | Catalase ( $\times10^{-2}$) | Peroxidase( $\times10^{-3}$) | SOD ( $\times10^{-2}$) | MDA( $\times10^{-6}$) |
|---|---|---|---|---|
| Control | 30.76± 14.44 | 5.62± 4.23 | 54.67± 17.87 | 3.85 ± 0.92 |
| 100 | 07.43± 3.46 | 20.61± 4.41* | 65.79± 6.10 | 4.32± 0.56 |
| 200 | 20.28 ± 2.19 | 27.95± 6.54* | 14.09± 1.75 | 6.14± 0.50 |
| 400 | 20.39± 4.76 | 20.37± 3.73* | 13.95± 3.16 | 3.30± 0.27 |

* $P < 0.05$ Compared to control; SOD: Superoxide dismutase; MDA: Malondialdehyde; Values are presented as Mean ± S.E.M, n = 4.

**Table 4.** The effect of 21 days daily Oral administration of aqueous leaf extract of *C. racemosum* on the heart.

| Dose (mg/kg) | Catalase ( $\times10^{-2}$) | Peroxidase ($\times10^{-3}$) | SOD ( $\times10^{-2}$) | MDA ($\times10^{-6}$) |
|---|---|---|---|---|
| Control | 3.26±0.72 | 1.82±0.86 | 25.27±13.46 | 1.51 ±0.38 |
| 100 | 1.48±0.35 | 5.65±3.59 | 47.03±7.61 | 3.47±1.92 |
| 200 | 4.34±0.45 | 5.65±0.62 | 34.13±0.15 | 1.27±0.14 |
| 400 | 2.11±0.44 | 3.50±0.95 | 57.47±4.12 | 1.71±0.17 |

$P > 0.05$ Compared to control; SOD: Superoxide dismutase; MDA: Malondialdehyde; Values are presented as Mean ± S.E.M, n = 4.

**Table 5.** 21 days daily oral administration of aqueous leaf extract of *C. racemosum* on the Liver.

| Dose (mg/kg) | Catalase ($\times10^{-2}$) | Peroxidase ($\times10^{-3}$) | SOD ( $\times10^{-2}$) | MDA($\times10^{-6}$) |
|---|---|---|---|---|
| Control | 6.82± 3.62 | 10.50±1.37 | 36.95±6.15 | 3.16±1.78 |
| 100 | 3.04±0.76 | 3.97±0.34 | 54.05±5.84 | 1.66±0.25 |
| 200 | 6.32±2.56 | 5.78±2.38 | 41.44±3.37 | 2.18± 0.76 |
| 400 | 11.16±5.00 | 14.99±5.48 | 47.76±4.16 | 3.09±0.84 |

P > 0.05 Compared to control; SOD: Superoxide dismutase; MDA: Malondialdehyde; Values are presented as Mean ± S.E.M, n = 4.

**Table 6.** The effect of 21 days daily administration *Combretum racemosum* on the Kidney.

| Dose (mg/kg) | Catalase ($\times10^{-2}$) | Peroxidase ($\times10^{-3}$) | SOD ( $\times10^{-2}$) | MDA($\times10^{-6}$) |
|---|---|---|---|---|
| Control | 31.94±14.95 | 5.62±4.27 | 54.67±20.63 | 3.85±0.9 |
| 100 | 11.71±4.65 | 37.52±7.85*** | 25.83±4.37 | 5.82±1.05 |
| 200 | 0.83±0.11 | 41.88±1.89*** | 29.52±1.23 | 4.05±1.03 |
| 400 | 2.35±0.79 | 29.09±0.50** | 23.11±3.40 | 5.09±0.84 |

*** P <0.001, ** P <0.01 Compared to control; SOD: Superoxide dismutase, MDA: Malondialdehyde; Values are presented as Mean ±S.E.M, n = 4.

Figure 1 to 4 shows the comparison of both plants on heart, liver, and kidney. In figures 1and 2, *C. platypterum* show a better antioxidant result by elevating the level of SOD and peroxidase in the heart and the level of SOD in the liver than *C. racemosum* which showed no activity on the heart and liver. Both plants elevate the level of peroxidase in kidney. However, *C. racemosum* demonstrated a higher antioxidant activity compared to control (figure 4).

## DISCUSSION

Antioxidants help to track the activity of oxidative stress. Oxidative stress is known to be involved in many disease conditions such as: neurodegenerative diseases (Alzheimer's disease, Parkinson's disease), atherosclerosis, rheumatoid arthritis, Crohn's disease, certain cancer and contribute to aging process [19]. This study showed that the aqueous leaf extract *C. platypterum* enhances the activity of Superoxide dismutase (SOD) and Peroxidase in the heart (Table 1). *C. platypterum* promotes the activities of Superoxide dismutase (SOD) at higher doses in the liver; the activities of peroxidase were also enhanced in the kidney (Tables 2 and 3). Superoxide dismutase (SOD) is a vital antioxidant which helps to breakdown superoxide anions into oxygen and hydrogen peroxide which is further converted to oxygen and water by antioxidant catalase and peroxidase [19, 20]. Table 3 shows that SOD level is significantly more at 100 mg/kg and less at 400 mg/kg. This may perhaps be that the *C. platypterum* elicits a dose-dependent anti-oxidant/ anti-inflammatory activity in the kidney. Seguí *et al* (2004) suggested that treatment with SOD decreases reactive oxygen species generation and oxidative stress and, thus, inhibits endothelial activation

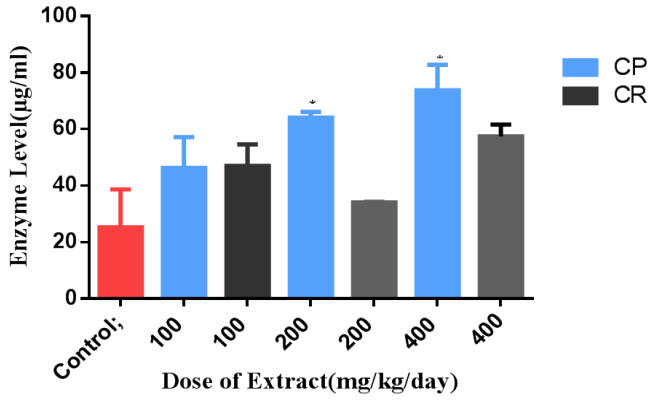


**Figure 1.** Superoxide dismutase levels in heart homogenates of rats following 21 days administration of *C. platypterum* (CP) and *C. racemosum* (CR). The level of Superoxide dismutase of *C. platypterum* extract was significantly (P<0.05) increased at 200 mg/kg and 400 mg/kg compared to control. However, *C. racemosum* showed no activity. Value are presented as mean ± SEM; n = 4.

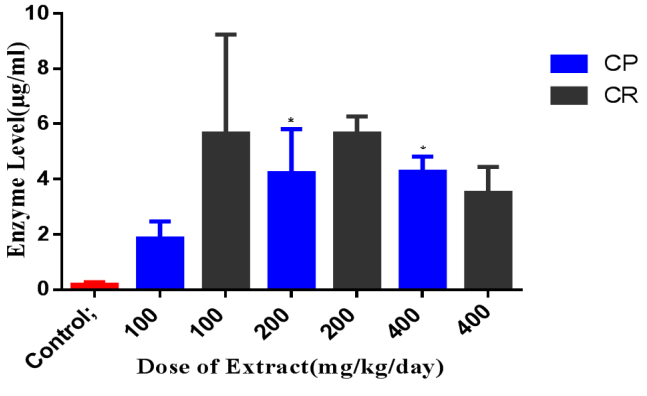


**Figure 2.** Peroxidase levels in heart homogenates of rats following 21 days administration of *C. platypterum* (CP) and *C. racemosum* (CR). The level of peroxidase was (P<0.05) increased at 200 mg/kg and 400 mg/kg of *C. platypterum* extract compared to control. However, *C. racemosum* show no effect. Value are presented as mean ± SEM, n = 4.

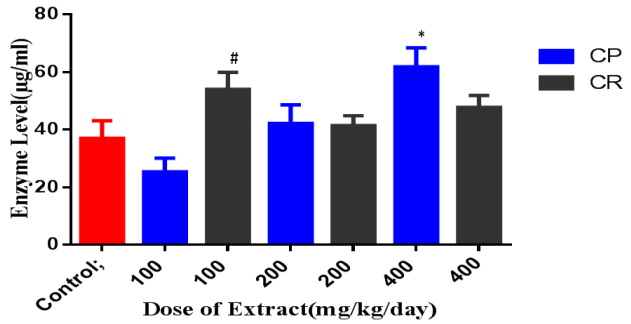


**Figure 3.** Superoxide dismutase levels in liver homogenates of rats following 21 days administration of *C. platypterum* (CP) and *C. racemosum* (CR). The level of Superoxide dismutase was increased at 400 mg/kg of *C. platypterum* extract compared to control. However, at 100 mg /kg, *C. racemosum* increased the level of superoxide dismutase compared to 100 mg/kg of C. platypterum. Value are presented as Mean ± SEM, n = 4.

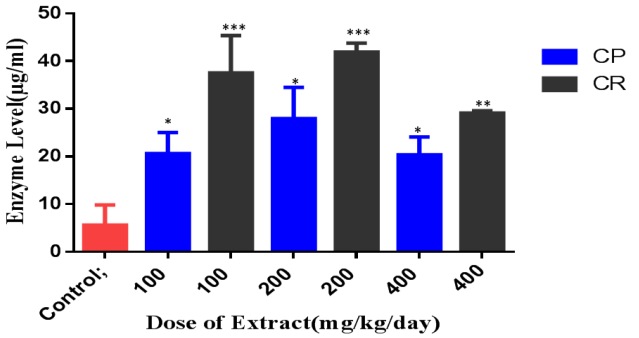


**Figure 4.** Peroxidase levels in kidney homogenates of rats following 21 days of administration of *C. platypterum* (CP) and *C. racemosum* (CR). The level of peroxidase was significantly (P<0.05) increased at 100 mg/kg, 200 mg/kg and 400 mg/kg of *C. platypterum* extract compared to control. *C. racemosum* significantly (P<0.001) increased the peroxidase level at 100 and 200 mg/kg and 400 mg/kg compared to control. Value are presented as mean ± SEM, n = 4.

and indicate that modulation of factors that govern adhesion molecule expression and leukocyte-endothelial interactions. Therefore, such antioxidants may be important new therapies for the treatment of inflammatory bowel disease [21]. When Superoxide dismutase (SOD) molecules from plants are absorbed into the blood, it boosts detoxification of harmful substances and reduces oxidative stress that contributes to aging and maladies such as heart failure, stroke, atherosclerosis and arthritis [22-24]. The increase of superoxide dismutase and peroxidase activities by the extract of C. *platypterum* in the heart may promote the body defense system against the risk of heart failure, stroke, atherosclerosis and aging. The antioxidant potential of C. *platypterum* supports its traditional use as tonic and lower backache treatment [6]. An increase in superoxide dismutase and peroxidase level in the liver and kidney reduces oxidative stress on liver and kidney, thereby enhancing functions and prolonging the life span of the organs. C. *platypterum* showed no activity on MDA and catalase in the heart, liver and kidney.

In this study, C. *racemosum* enhanced the activities of peroxidase on the kidney. But, had no effect on the heart and liver. Superoxide dismutase, MDA and catalase were not affected by the aqueous leaf extract of C. *racemosum*. Francine *et al*. [13] reported *in vitro* antioxidant activities of C. *racemosum*. *In vivo* antioxidant study (Tables 4, 5 and 6) shows an increase in the Superoxide dismutase, and catalase, peroxidase but the increase were not significant, this may be due to metabolic processes or fragility of its molecular structure as suggested by Joanny [19]. However, peroxidase activities in the Kidney indicate the presence of antioxidant in aqueous leaf extract C. *racemosum*.

In conclusion, although C. *platypterum* and C. *racemosum* belong to the same genus, C. *platypterum* demonstrates a better antioxidant activity in the heart and liver compared to C. *racemosum* (Figures 1, 2, 3 and 4). C. *platypterum* increases the Superoxide dismutase on the heart and the liver. It also increases the level of peroxidase on heart and kidney. Further study in the light of antioxidant activities of both plants is recommended.

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## REFERENCES

1. Fawzi M. Traditional Medicines in Africa: An Appraisal of Ten Potent African Medicinal Plants. Evidence-Based Compl Alt Med. 2013; 2013(2013):14-15
2. Dalziel JM. The Useful Plants of West Tropical Africa.Crown Agents Ed. Royal Botanic Gardens.Kew Richmond, United Kingdom; 1973. p. 166 – 179
3. Bredenkamp CL. Combretaceae: In: O.A Leistnered, Seed plants of southern Africa: Families and Genera *Strelitzia*. National Botanical Institute. Pretoria. 2000; 10: 228 – 229
4. Liben L. Combretaceae. Flore du Cameroun. Volume 25. Muséum National d'Histoire Naturelle, Paris, France; 1983. 97 p.
5. Watt JM, Breyer-Brandwijk MG. The Medicinal and poisonous plants of southern and eastern Africa, 2nd ed., E & S Livingstone, Ltd., Edinburgh, London; 1962.
6. Bongers F, Parren MPE, Traoré D. Forest Climbing Plants of West Africa: Diversity, Ecology and Management. CABI, Wallingford, United Kingdom; 2005. 273 p.
7. Burkill HM. The useful plants of West Tropical Africa.2nd Edition. Volume 1, Families A–D. Royal Botanic Gardens, Kew, Richmond, United Kingdom; 1985. 960p
8. Oliver-Bever B. Medicinal Plants in Tropical Africa. University Press Cambridge; 1986. p. 117 – 168.
9. Bouquet AJ. Natural products as an alternative remedy.2 .4th Ed. Royal Botanic Gardens. Kew; 1969. P. 166-179
10. Okwuasa C, Nwobode E, Chilaka K. The anti-ulcer activities of leaf extracts of *Combretumracemosum* (family:Combracaceae). J. Biomed Mestigation. 2006; 4(1): 9 – 14.
11. Okwuasa CN, Achukwu PUO, Azubikend V, Abah NCX. Protective effect of the leaf *Combretum racemosum* Beauv (Combretaceae) on cyclophospahamide induced pancytopaenia and liver injury. J. Pharmacol. 2012; 6: 30 – 34
12. Schepetkin IA, Kouakou K, Yapi A, Kirpotina LN, Jutila MA, Quinn MT. Immunomodulatory and hemagglutinating activities of acidic polysaccharides isolated from *combretum racemosum*. Intl Immunopharmacol. 2013; 15(3): 628 – 637

13. Francine NM, Charaf EIK, Jeanine F, Guy B, Pierre D, Jose LN, Stephanie P. Vascular effects and antioxidant activity of two *Combretum* species from Democratic Republic of Congo. J. Ethnopharmacol. 2012; 42: 194 – 200
14. Misra HP, Fridovich I. The role of superoxide anion in the antioxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1980; 247: 3170-3175
15. Luck H. In: methods in enzymatic analysis. 2nded. New York: Bergmeyer; 1974.
16. Subhangkar N, Himadri SP, Nishith RB, Bodhisattwa C. *In vitro* evaluation of antioxidant activity of *Leucas plukenetti* (Roth) Spreng. Asian j. Plant Sci. Res. 2012; 2(3): 254-262.
17. Buege JA, Aust SD. Microsomal Lipid peroxidation. Methods in Enzymology. Fleischer S, Packer L (editors). Academic Press New York; 1978. p. 52: 302 – 310.
18. Reddy KP, Subhani SM, Khan PA, Kumar KB. Effect of light and benzyl adenine and dark-treated graving rice (Oryza sativa) leaves –changes in peroxidases activity. Plant Cell Physiol. 1995; 26: 987-994.
19. Joanny Menvielle- Bourg F. Super oxide dismutase (SOD), a powerful antioxidants is now available orally. Phytotherapie 2005; 3: 1 – 4.
20. Faraci FM, Didion SP. Vascular protection: superoxide dismutase isoforms in the vessel wall. Arterioscler Thromb Vasc Biol. 2004; 24(8):1367-73
21. Seguí J, Gironella M, Sans M, Granell S, Gil F, Gimeno M, Coronel P, Piqué JM, Panés J. "Superoxide dismutase ameliorates TNBS-induced colitis by reducing oxidative stress, adhesion molecule expression, and leukocyte recruitment into the inflamed intestine". J. Leukoc. Biol. 2004; 76 (3): 537–44.
22. Maier CM, Chan PH. Role of superoxide dismutases in oxidative damage and neurodegenerative disorders. Neuroscientist. 2002; 8(4):323-34
23. Vouldoukis I, Lacan D, Kamate C, Coste P, Calenda A, Mazier D, Conti M, Dugas B. Antioxidant and anti-inflammatory properties of a *Cucumis melo* LC. extract rich in superoxide dismutase activity. *J Ethnopharmacol*. 2004; 94(1):67-75.
24. Muth CM, Glenz Y, Klaus M, et al. Influence of an orally effective SOD on hyperbaric oxygen-related cell damage. *Free Radic Res*. 2004 ;38(9):927-32.



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