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## PHYTOCHEMICAL PROFILING, TOXICOLOGICAL STUDIES AND EFFECTS OF COMBRETUM RACEMOSUM ON POSTPARTUM LACTATING FEMALE ALBINO WISTAR RATS

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### ABSTRACT

Combretum racemosum leaves are used in ethnomedicine to treat haemorrhoids, toothache, to promote lactation and weight loss after childbirth. This study investigated the phytochemical composition and effects of ethanol leaf extract of Combretum racemosum (ELECR) on postpartum female rats. Gas chromatography-mass spectrometry was used to analyse the phytochemical composition. Rats were divided into five groups: Group I (normal control: rats not treated with ELECR and not lactating), Group II (negative control: rats not treated with ELECR but lactating), while groups III-V received oral doses of ELECR at 400, 600 and 800 mg/kg body weight, respectively, once daily for 14 days. After ELECR treatment, rats were sacrificed on the 15<sup>th</sup> day after overnight fasting and blood collected by cardiac puncture for biochemical and hormonal analysis (oestrogen, progesterone and follicle stimulating hormone). GC-MS analysis revealed bioactive compounds. The ELECR-treated groups significantly reduced (p<0.05) body weight, alanine transaminase, aspartate transaminase, alkaline phosphatase, urea, creatinine, total cholesterol, triacylglycerol, low-density lipoprotein cholesterol, and malondialdehyde, while the levels of glutathione, catalase, superoxide dismutase, oestrogen, follicle stimulating hormone, red blood cell count, packed cell volume and haemoglobin increased significantly compared to the negative control (p<0.05). ELECR treatment did not alter the histomorphological architecture of the liver, uterus, pituitary and hypothalamus. This study revealed that ELECR is not toxic but reduced the body weight and increased hormone levels that promote lactation. It also showed that ELECR has hepatoprotective and lipid-lowering effects which justifies its use in folkore medicine as a tonic for postpartum weight loss with galactagogue effects.

**KEYWORDS**: Phytochemicals, toxicity profile, postpartum weight loss, *Combretum racemosum*.

## INTRODUCTION

All over the world, regardless of cultural background or ethnicity, the use of different parts of plants, be it leaves, roots, stem bark, fruits or flowers, to cure various health problems is gaining attention. According to researchers, this can be attributed to the availability of various bioactive compounds in these plant parts with potent pharmacological capabilities.<sup>[1-3]</sup> Most third world countries in Africa and Asia today rely on easily affordable and available medicinal plants to meet their health needs as modern medicine is expensive and mostly not readily available.<sup>[4-6]</sup> Evidence-based research has shown that debilitating diseases such as stroke, cancer and obesity can be treated with medicinal plants and their bioactive phytoconstituents,<sup>[7,8]</sup> resulting in more than 80% of the world's population relying on these plants to meet their health needs.<sup>[9,10]</sup> Conventional drugs for the daily treatment of asthma (ephedrine), artemisinin-based antimalarials for the treatment of Plasmodium infections and aspirin for the relief of pain

and inflammation are all novel drugs synthesized from various plants.<sup>[7,9,11]</sup> Currently, medicinal plants are used in the treatment of postpartum obesity which is elevated body mass index greater than 30 kg/m<sup>2</sup> following childbirth and to promote lactation because they are readily available, effective, cheap and have few side effects.<sup>[9]</sup>

Postpartum weight gain resulting from increased accumulation of steroids needed to maintain pregnancy, leads to low breast milk production and difficulties in initiating lactation and this remain a major challenge for new mothers who wish to exclusively breastfeed their newborn.<sup>[12]</sup> While postpartum obesity predisposes the young mother to cardiovascular diseases such as hypertension and myocardial infarction, diabetes mellitus and depression, comprehensive breastfeeding in the first six months of life is important for optimal growth and development of the child as well as for immune protection against various deadly childhood diseases,

according to Kalantari et al.<sup>[33]</sup> Breast milk is the primary source of essential nutrients for the child and therefore protects it from diseases that affect the child in early life, such as pneumonia, tetanus, gastroenteritis and sepsis,<sup>[13,14]</sup> and reduces the risk of neonatal death. Previous studies have shown that women who exclusively breastfed their newborns have a lower risk of developing ovarian cancer and type II diabetes mellitus.<sup>[15]</sup>

The hormones: estrogen, prolactin and oxytocin play an essential role in the process of lactogenesis to produce breast milk.<sup>[16,17]</sup> While estrogen is important for the growth and proliferation of breast tissue, prolactin regulates epithelial growth and the proliferation of mammary glands.<sup>[17]</sup> Tactile stimulation of the nipple through suckling leads to the release of oxytocin from the hypothalamus and posterior pituitary, which triggers contraction of the myoepithelial cells surrounding the ducts and alveoli, ultimately leading to milk let-down and ejection.<sup>[16,18]</sup> Smoking, type II diabetes mellitus, obesity, retained products of conception such as the placenta and postpartum hemorrhage are among the various factors that can negatively affect lactation.<sup>[19,20]</sup> Postpartum depression, mood swings, maternal attitudes towards breastfeeding and early introduction of artificial milk can alter neuroendocrine function by affecting prolactin and oxytocin levels, further inhibiting milk production.<sup>[21]</sup> Also, anxiety neurosis is a crucial problem that occurs in the postnatal period and many mothers seek medication to treat their negative thoughts, anxiety and cosmetic obsessions about losing body shape.<sup>[22]</sup> Herbal galactagogues such as Silvbum marianum and Moringa oleifera, which are rich in phytochemicals such as tannins and flavonoids, have been used to promote lactation in postpartum women.<sup>[23,24]</sup> Previous studies have shown that Combretum racemosum leaves are used to treat urinary tract infections, trypanosomiasis and plasmodiasis.<sup>[25,26]</sup>

Combretum racemosum (C. racemosum), commonly called uboli in Southeast Nigeria, ebi-odo among the Urhobos and Christmas rose in pidgin English belongs to the Combretaceae family. It bears a bright and attractive bunch of crimson flowers known as the Christmas rose. Previous studies have shown that uboli is rich in bioactive compounds with varying pharmacological potential. The leaf has been used over the years as an ingredient in the preparation of local soups and porridge and in African ethnomedicine to cure various ailments such as tooth ache, inflammation and bacterial infection.<sup>[27]</sup> The leaves of C. racemosum are used in folklore medicine not only as a remedy for stomach ulcers and diarrhoea, but also as a soothing agent for haemorrhoids, toothache and male infertility.<sup>[27]</sup> In addition, recent studies have shown that several plants belonging to the medicinal family Combretaceae have anti-inflammatory, antibacterial and vasodilatorv effects.<sup>[6,28,29]</sup> However, little or no work has been done on the galactogenic and weight-reducing effects of C.

*racemosum*. There is also paucity of data on how the leaf extract of *C. racemosum* modulates other postpartum biochemical parameters, such as hormonal balance, which may play some role in returning the new mother to her pre-pregnancy cosmetic state. Therefore, the aim of this study was to characterize *C. racemosum* phytochemically and to investigate its effect on lactation, weight loss and other selected biochemical parameters in female albino rats.

## MATERIALS AND METHODS

#### Collection of plant materials and identification

The leaves of Combretum recemosum were collected in Ahiara: Agu na Eze in Ahiazu Mbaise Local Government Area of Imo State, Nigeria (5.5385° N, 7.2437° E) on 18th August, 2024. The plant was identified by Dr. Udoka Emmanuel of the Department of Natural Forestry, College of Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, and the herbarium number MOUAU/CVM/VPP/HERB/18/004 was assigned and the plant deposited in the herbarium at Michael Okpara University of Agriculture, Umudike.

### Extraction of plant materials

The plant extract was prepared according to the method used by Orieke et al.<sup>[30]</sup> Freshly collected leaves of *Combretum racemosum* were properly washed and airdried in shade for about 14 days and then ground into coarse powder using a locally made grinding machine. Extraction was done by mixing 100 g of powdered material with 250 ml of 96% ethanol for 48 hours at 27°C (room temperature) with continuous shaking while the resultant mixture was filtered using Whatman Grade 1 Qualitative Filter Paper (150mm Circles 1001-150) and the filtrate was stored in a water bath at 40°C. The sample was stored in a refrigerator at 4 °C until use.

Gas chromatography-mass spectrometry (GC-MS) analysis of *Combretum racemosum* leaf extract

GC-MS analysis of the leaf extract was performed using a BUCK M910 gas chromatograph equipped with an HP-5MS section (30 m length  $\times$  250  $\mu$ m width  $\times$  0.25  $\mu$ m thickness of the film). Spectroscopic identification by GC-MS included an electron ionization frame using high-energy electrons (70 eV). Unadulterated helium gas (99.995%) was used as the transport gas with a flow rate of 1 mL/min. The underlying temperature was set to 50 -150 °C, with an expansion rate of 3 °C/min and a holding time of about 10 minutes. Finally, the temperature was increased to 300 °C at 10 °C/min. One microliter of the prepared 1% concentrates diluted with specific solvents was infused without spitting. The relative amount of compounds present in each of the concentrates was expressed as a function of the upper region generated in the chromatogram. Detection of the C. racemosum leaf extract constituents was performed by comparing the retention index of the mass spectral fragmentation patterns with those found in the National Institute Standard and Technology (NIST) database.<sup>[7]</sup>

#### Housing of animals

A total of sixty adult albino wistar rats (45 females and 15 males) were obtained from the Laboratory Animal Production Unit of the Department of Biochemistry, Abia State University, Uturu, for the study. The animals were housed in aluminum cages under standard conditions (12/12 hr light/dark cycle) at a temperature of  $25\pm2^{\circ}$ C. They were fed with standard grower pellets (Grand Cereals Ltd, Abia State) and had unrestricted access to clean drinking water for acclimatization for seven days before the experiment commenced. The studies were conducted in accordance with internationally recognised standards for the use of animals in research. Ethical approval was granted by the Abia State University Ethics Committee under approval number ABSU/REC/BMR/129.

#### Experimental design

The study was conducted in two phases. In the first phase, it was ensured that the animals became pregnant for the purposes of the experiment. For this purpose, the animals were divided into 15 groups of two female and one male rat each for a period of 28 days for breeding and gestation.<sup>[31]</sup> Litters were delivered about 21 days following copulation and breastfeeding commenced. In the second phase of the experiment, 20 lactating female albino wistar rats and 5 normal female albino wistar rats were isolated and housed in different cages. The experiment was conducted with 20 lactating female rats and 5 non-lactating female rats, which were divided into 5 groups of 5 rats each and treated as follows: Group I (normal control: rats not treated with ELECR and not lactating), Group II (negative control: rats not treated with ELECR but lactating), while ELECR groups were treated as follows: III= 400, IV= 600 and V= 800 mg/kgbody weight. The rats in all groups had free access to food and water ad-libitum. Oral administration of the extract to the rats lasted 14 days and was treated once daily. At the end, the rats were sacrificed by cervical dislocation and blood samples were collected in EDTA and normal bottles for hematologic and biochemical analyses, respectively. The tissue samples of the liver, uterus and brain (pituitary gland and hypothalamus) of the animals were also collected in plain bottles containing 10% formalin and used to prepare tissue homogenates for histopathological studies.

## **Biochemical analysis**

Liver, kidney and lipid profile (total cholesterol, high density lipoprotein, triacylglycerol, low density lipoprotein and very low density lipoprotein) parameters were determined using a spectrophotometer and Randox test kits (Randox Laboratories Limited, Antrim, UK). The haematological parameters (red blood cell count, packed cell volume, haemoglobin, white blood cell count, platelets, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, neutrophils, lymphocytes, monocytes, eosinophils and basophils) were evaluated according to the methodology established by Bain et al.<sup>[34]</sup> For the assessment of antioxidant biomarkers (glutathione peroxidase, superoxide dismutase, catalase, reduced glutathione, malondialdehyde), the protocol described by Kanu et al,<sup>[35]</sup> was used. Hormone levels, including follicle stimulating hormone, progesterone, prolactin and estradiol, in the serum samples were quantified using chemiluminescence immunoassay (CLIA) techniques according to the standard procedures of AUTOBIO diagnostics CO, LTD, Zhengzhou, China.

### Histological analysis

Histopathological examination was performed according to the method described by Loha et al.<sup>[36]</sup> The tissues of liver, uterus, pituitary gland and hypothalamus were preserved in 10% formalin. The tissues were then cut into 5  $\mu$ m slices, treated with paraffin wax and stained with hematoxylin-eosin dye. The stained sections were then examined under a light microscope by a qualified histopathologist.

### Data analysis

The data obtained from the study were analyzed using the Statistical Package for the Social Science (SPSS) version 22.0. Group comparisons were performed using the analysis of variance (ANOVA) test. Significant differences between the controls and the experimental groups were determined using the LSD (least significant difference) test. All data were expressed as mean  $\pm$ standard deviation. P-values of less than 0.05 were considered statistically significant.

# RESULTS AND DISCUSSION



Figure 1: GC-MS spectra of Combretum racemosum ethanol leaf extract.





Figure 2: Histology of the uterus, liver, pituitary and hypothalamus of *Combretum racemosum* ethanol leaf extract-treated lactating female albino wistar rats.

Haematoxylin and eosin staining (H&E), magnification  $\times 100$ . In uterus, LP= Lamina proprid, UG= uterine gland and UW= Uterine wall. In liver, CV= central vein, SS= sinusoids and H= hepatocytes. In Pituitary, AC= acidophil, B= basophils, P= pituicytes, CH= chromophores, HB= herring

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bodies, A= axon. In hypothalamus, N= neurons, A= axon and BV= blood vessel. Normal control represents group 1, negative control represents group II while 400, 600 and 800 mg/kg ELECR represent Groups III, IV and V respectively

Figure 3: Effects of *Combretum racemosum* ethanol leaf extract on renal function parameters of lactating female albino wistar rats.

Values are Mean  $\pm$  standard deviation, n=5. \* indicates a significant different (P<0.05). Cl<sup>-</sup>, Chloride ion; Na<sup>+</sup>, Sodium ion; K<sup>+</sup>, Potassium ion; HCO<sub>3</sub><sup>-</sup>, Bicarbonate. Normal control represents group I, negative control represents group II while 400, 600 and 800 mg/kg ELECR represent Groups III, IV and V respectively.



Figure 4: Effects of *Combretum racemosum* ethanol leaf extract on Serum antioxidant activities of lactating female albino wistar rats.

Values are Mean  $\pm$  standard deviation, n=5. \* indicates a significant different (P<0.05). GPx, Glutathione Peroxidase, GSH; Gluthatione, SOD; Superoxide Dismutase, CAT; Catalase, MDA; Malondialdehyde. Normal control represents group I, negative control represents group II while 400, 600 and 800 mg/kg ELECR represent Groups III, IV and V respectively.



Figure 5: Effects of *Combretum racemosum* ethanol leaf extract on reproductive hormone profile of lactating female albino wistar rats.

Values are Mean  $\pm$  standard deviation, n=5, \* indicates a significant different (P<0.05). FSH, Follicle stimulating hormone. Normal control represents group I, negative control represents group II while 400, 600 and 800 mg/kg ELECR represent Groups III, IV and V respectively.

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S/N	рт	Chamical compound	Molecular	MW	%	
5/11	N1	Chemical compound	formular	(g/mol)	Composition	
1	6.30	1-Propanesulfonyl chloride, 3-chloro-	$C_3H_6Cl_2O_2S$	177.05	0.44	
2	6.37	Benzene, 1,2,3-trimethyl-	C <sub>9</sub> H <sub>12</sub>	120.19	1.24	
3	6.50	Decane	C <sub>10</sub> H <sub>22</sub>	142.29	1.44	
4	6.85	Benzene, 1,4-dichloro-	$C_6H_4Cl_2$	147.00	1.34	
5	6.06	2-(Chloromethyl)-5-ethyl-1,3,4-		146 57	1.22	
5	0.90	oxadiazole	$C_5 \Pi_7 C \Pi N_2 O$	140.37	1.22	
6	7.20	Benzene, 1-methyl-3-(1-methylethyl)-	$C_{10}H_{14}$	134.22	1.32	
7	7.94	Oxalic acid, isobutyl nonyl ester	$C_{15}H_{28}O_4$	272.38	1.28	
8	8.16	Gamma terpinene	$C_{10}H_{16}$	136.23	1.50	
9	8.25	Dodecane	$C_{12}H_{26}$	170.33	0.94	
10	8.69	Undecane, 3,7-dimethyl-	C <sub>13</sub> H <sub>28</sub>	184.36	0.59	
11	8.96	Dodecane, 2,6,11-trimethyl-	C <sub>15</sub> H <sub>32</sub>	212.42	3.22	
12	9.12	Carbonic acid, nonyl vinyl ester	$C_{12}H_{22}O_3$	214.30	0.87	
14	9.18	Hexane, 3,3-dimethyl-	C <sub>8</sub> H <sub>18</sub>	114.23	1.30	
15	9.27	Oxalic acid, 2-ethylhexyl isohexyl ester	$C_{16}H_{30}O_4$	286.41	1.45	
16	9.34	Tridecane	$C_{13}H_{28}$	184.36	1.60	

Table 1: Chemical constituents of Combretum racemosum leaf extract identified via GC-MS.

17	9.44	Linalool	$C_{10}H_{18}O$	154.25	1.75
18	9.69	Octane, 2,3,3-trimethyl-	C <sub>11</sub> H <sub>24</sub>	156.31	0.78
19	9.81	Nonane, 3-methyl-	$C_{10}H_{22}$	142.28	1.32
20	9.92	Undecane	C <sub>11</sub> H <sub>24</sub>	156.31	0.32
21	10.03	2,6-Dimethyldecane	$C_{12}H_{26}$	170.33	0.77
22	10.85	Hydroxylamine, O-decyl-	$C_{10}H_{23}NO$	173.29	0.25
23	11.65	Terpinen-4-ol	C <sub>10</sub> H <sub>18</sub> O	154.25	0.44
24	11.77	Azulene	C <sub>10</sub> H <sub>8</sub>	128.17	0.30
25	12.02	5-Dodecene, (Z)-	$C_{12}H_{24}$	168.32	0.40
26	14.94	Benzocycloheptatriene	$C_{11}H_{10}$	142.19	0.26
28	16.85	Heptane, 2,6-dimethyl-	C <sub>9</sub> H <sub>20</sub>	128.25	0.12
29	17.18	Alpha copaene	C <sub>15</sub> H <sub>24</sub>	204.36	0.87
30	17.64	1,3-Pentadiene, (Z)-	C <sub>5</sub> H <sub>8</sub>	68.11	2.29
31	18.08 1H-Cycloprop[e]azulene, 1a,2,3,4,4   a,5,6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a   .beta7b.alpha.)]- .beta7b.alpha.)]-		$C_{15}H_{24}$	204.35	0.29
32	18.74	Santolina triene	$C_{10}H_{16}$	136.23	0.43
33	19.25	Humulene	C <sub>15</sub> H <sub>24</sub>	204.35	1.23
34	19.36	(E)betaFamesene	C <sub>15</sub> H <sub>24</sub>	204.35	2.75
35	19.87	1-(3,3-Dimethylbutyn-1-yl)-2,2- dimethylcyclopropene	C <sub>11</sub> H <sub>16</sub>	148.24	0.50
36	20.34	(E,Z)alphaFarnesene	C15H24	204.35	2.22
37	20.48	.alphaMuurolene	C15H24	204.35	0.26
38	20.69	.betaBisabolene	C15H24	204.35	6.94
39	20.98	2,4-Di-tert-butylphenol	C <sub>14</sub> H <sub>22</sub> O	206.32	2.67
40	21.07	Cyclohexene, 3-(1,5-dimethyl-4- hexenyl)-6-methylene-, [S-(R*,S*)]-	C <sub>15</sub> H <sub>24</sub>	204.35	5.55
41	22.53	Caryophyllene oxide	C <sub>15</sub> H <sub>24</sub> O	220.35	0.44
42	22.85	10-Methylnonadecane	$C_{20}H_{42}$	282.55	0.22
43	22.92	Guaiol	C <sub>15</sub> H <sub>26</sub> O	222.37	0.71
44	23.59	Apiol	C <sub>15</sub> H <sub>26</sub> O	222.37	0.13
45	24.29	Aromandendrene	C15H24	204.35	1.04
46	27.25	1-Octadecene	C <sub>18</sub> H <sub>36</sub>	252.48	1.43
47	30.01	Di-sec-butyl phthalate	$C_{16}H_{22}O_4$	278.34	0.59
48	30.28	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.47	2.46
49	30.35	5-Eicosene, (E)-	$C_{20}H_{40}$	280.53	0.09
50	30.56	Acetoxyacetic acid, tridec-2-ynyl ester	$C_{17}H_{28}O_4$	296.40	0.11
51	31.30	1-Methylcycloheptanol	C <sub>8</sub> H <sub>16</sub> O	128.21	0.16
52	31.62	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308.49	0.95
53	31.66	9-Octadecenoic acid, ethyl ester	$C_{20}H_{38}O_2$	310.51	0.88

RT, Retention Time, MW; Molecular Weight

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'	Table	2:	Effects	of	Combretum	racemosum	ethanol	leaf	extract	on	body	weight	changes	of	lactating	female
	albinc	) wis	star rats.													

Groups	Treatments	Initial body weight (g)	Final body weight (g)	Weight gain (g)	Percentage weight gain
Ι	Normal control	130.00±5.77	$160.00 \pm 2.89$	$30.00 \pm 2.89^{\circ}$	$23.37 \pm 3.27^{b}$
II	Lactating rats (negative control)	150.67±6.36	184.33±5.81	33.67±0.67 <sup>c</sup>	22.46±1.36 <sup>b</sup>
III	Lactating rats + 400 mg/kg ELECR	149.33±5.81	167.67±5.36	$18.33 \pm 0.88^{b}$	$12.34{\pm}0.98^{a}$
IV	Lactating rats + 600 mg/kg ELECR	149.67±6.12	160.33±2.91	10.67±3.38 <sup>a</sup>	$7.32\pm2.48^{a}$
V	Lactating rats + 800 mg/kg ELECR	150.67±7.86	157.00±6.56	6.33±1.45 <sup>a</sup>	4.32±1.17 <sup>a</sup>

Values are Mean  $\pm$  standard deviation, n=5, mean across the column with different alphabetical superscript indicates a significant different (P<0.05). ELECR: *Combretum racemosum* ethanol leaf extract.

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Treatments	Normal control	Lactating rats (negative control)	Lactating rats + 400 mg/kg ELECR	Lactating rats + 600 mg/kg ELECR	Lactating rats + 800 mg/kg ELECR
RBC ( $x10^{6}/mm^{3}$ )	$7.49 \pm 0.11^{d}$	$5.06 \pm 0.04^{a}$	$6.70\pm0.20^{\circ}$	$6.26 \pm 0.17^{bc}$	$6.22 \pm 0.16^{\circ}$
PCV (%)	42.37±1.32 <sup>b</sup>	$33.27 \pm 0.64^{a}$	39.73±2.13 <sup>b</sup>	39.50±0.44 <sup>b</sup>	37.90±1.47 <sup>b</sup>
Hb (g/dl)	15.27±0.57 <sup>b</sup>	12.40±0.06 <sup>a</sup>	14.93±0.55 <sup>b</sup>	14.60±0.31 <sup>b</sup>	14.17±0.63 <sup>b</sup>
WBC $(x10^{3}/mm^{3})$	9.99±0.23 <sup>a</sup>	14.73±1.31 <sup>b</sup>	$11.71 \pm 0.74^{ab}$	12.73±1.05 <sup>ab</sup>	14.62±0.79 <sup>b</sup>
PLT $(x10^{3}/mm^{3})$	490.33±2.33 <sup>ab</sup>	518.33±5.61 <sup>b</sup>	$474.00 \pm 2.65^{a}$	$461.67 \pm 6.69^{a}$	570.00±23.03°
MCV (fl)	56.61±2.60 <sup>a</sup>	$65.74 \pm 1.03^{\circ}$	59.19±1.39 <sup>ab</sup>	63.16±1.27 <sup>bc</sup>	$60.90 \pm 1.00^{abc}$
MCH (pg)	20.40±1.06 <sup>a</sup>	24.51±0.10 <sup>c</sup>	22.27±0.21 <sup>ab</sup>	23.35±0.63 <sup>bc</sup>	$22.76 \pm 0.60^{bc}$
MCHC (g/dl)	36.02±0.21 <sup>a</sup>	37.30±0.73 <sup>a</sup>	37.65±0.72 <sup>a</sup>	$36.95 \pm 0.44^{a}$	37.37±0.45 <sup>a</sup>
Neutrophils (%)	40.67±0.67 <sup>b</sup>	$35.67 \pm 0.67^{a}$	40.00±0.58 <sup>b</sup>	$40.33 \pm 0.88^{b}$	41.33±0.67 <sup>b</sup>
Lymphocytes (%)	54.33±1.20 <sup>a</sup>	$58.33 \pm 0.67^{b}$	54.33±0.33 <sup>a</sup>	$55.00 \pm 1.15^{a}$	54.33±0.88 <sup>a</sup>
Monocytes (%)	2.67±0.33 <sup>a</sup>	3.33±0.33 <sup>a</sup>	$3.00\pm0.00^{a}$	2.33±0.33 <sup>a</sup>	2.33±0.33 <sup>a</sup>
Eosinophils (%)	2.33±0.33 <sup>a</sup>	2.67±0.33 <sup>a</sup>	2.67±0.33 <sup>a</sup>	2.33±0.33 <sup>a</sup>	$2.00 \pm 0.00^{a}$
Basophils (%)	$0.00{\pm}0.00^{a}$	$0.00{\pm}0.00^{a}$	$0.00{\pm}0.00^{a}$	$0.00 \pm 0.00^{a}$	$0.00 \pm 0.00^{a}$

Table 3: Effects of Combretum racemosum ethanol leaf extract on ha	aematological parameters of lactating female
albino wistar rats.	

Values are Mean  $\pm$  standard deviation, n=5: mean across the row with different alphabetical superscript indicates a significant different (P<0.05). RBC, Red Blood Cells; PCV, Packed Cell Volume; HB, Haemoglobin; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Concentration; WBC, White Blood Cell. Normal control represents group I, negative control represents group II while 400, 600 and 800 mg/kg ELECR represent Groups IV, IV and V respectively.

Table 4: Effects of Combretum racemosum	ethanol leaf extract	on lipid profile parameter	rs of lactating female
albino wistar rats.			

Treatments	Total cholesterol (mg/dl)	HDL-C (mg/dl)	TAG (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Normal Control	$92.45 \pm 1.17^{b}$	61.95±0.84 <sup>b</sup>	74.01±3.27 <sup>a</sup>	15.70±0.59 <sup>b</sup>	$14.80 \pm 0.65^{a}$
Lactating rats (negative control)	114.66±1.11 <sup>c</sup>	60.89±0.21 <sup>ab</sup>	$84.31 \pm 1.90^{b}$	36.91±1.27 <sup>c</sup>	16.86±0.38 <sup>b</sup>
Lactating rats + 400 mg/kg ELECR	91.20±1.09 <sup>b</sup>	$61.61 \pm 0.75^{ab}$	$78.44 \pm 1.11^{ab}$	13.90±1.64 <sup>b</sup>	15.69±0.22 <sup>ab</sup>
Lactating rats + 600 mg/kg ELECR	$80.78 {\pm} 0.38^{a}$	59.99±0.56 <sup>ab</sup>	78.53±3.83 <sup>ab</sup>	$5.08 \pm 0.19^{a}$	15.71±0.77 <sup>ab</sup>
Lactating rats + 800 mg/kg ELECR	$83.88{\pm}2.20^{a}$	59.69±0.51 <sup>a</sup>	77.63±1.53 <sup>ab</sup>	8.67±1.57 <sup>a</sup>	15.53±0.31 <sup>ab</sup>

Values are Mean  $\pm$  standard deviation, n=5, mean across the column with different alphabetical superscript indicates a significant different (P<0.05). TC, Total Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; Triacylglycerol, LDL-C, Low-Density Lipoprotein Cholesterol; VLDL-C, Very Low-Density Lipoprotein Cholesterol. Normal control represents group I, negative control represents group II while 400, 600 and 800 mg/kg ELECR represent Groups III, IV and V respectively.

Table 5: Effects of Combretun	n racemosum e	ethanol leaf e	xtract on liver	function	parameters of	lactating	female
albino wistar rats.							

Treatments	Normal control	Lactating rats (negative Control)	Lactating rats + 400 mg/kg ELECR	Lactating rats + 600 mg/kg ELECR	Lactating rats + 800 mg/kg ELECR	
Total protein (g/dl)	$8.29 \pm 0.10^{b}$	$4.65 \pm 0.27^{a}$	8.06±0.14 <sup>b</sup>	7.92±0.03 <sup>b</sup>	$8.07 \pm 0.08^{b}$	
Albumin (g/dl)	$4.84 \pm 0.16^{\circ}$	$2.22 \pm 0.12^{a}$	$4.27 \pm 0.14^{b}$	$4.24 \pm 0.05^{b}$	$4.16 \pm 0.04^{b}$	
Globulin (g/dl)	3.45±0.11 <sup>b</sup>	2.43±0.17 <sup>a</sup>	$3.78 \pm 0.06^{\circ}$	3.68±0.02 <sup>bc</sup>	3.91±0.04 <sup>c</sup>	
AST (u/l)	41.67±1.67 <sup>a</sup>	93.00±1.73 <sup>c</sup>	60.33±1.45 <sup>b</sup>	59.33±3.18 <sup>b</sup>	57.33±3.71 <sup>b</sup>	
ALT (u/l)	$32.67 \pm 1.76^{a}$	53.33±1.45 <sup>c</sup>	39.67±1.45 <sup>b</sup>	44.33±1.76 <sup>b</sup>	$40.33 \pm 0.88^{b}$	
ALP (u/l)	$70.67 \pm 1.20^{a}$	115.67±2.19 <sup>c</sup>	$78.67 \pm 3.53^{ab}$	$77.00 \pm 1.00^{ab}$	$80.67 \pm 3.84^{b}$	
Total Bilirubin (mg/dl)	$0.45 \pm 0.03^{a}$	$1.26 \pm 0.04^{\circ}$	0.50±0.03 <sup>a</sup>	$0.64 \pm 0.03^{b}$	$0.64 \pm 0.07^{b}$	

Values are Mean  $\pm$  standard deviation, n=5: mean across the row with different alphabetical superscript indicates a significant different (P<0.05). AST, Aspartate Aminotransferase; ALT, Alanine Transaminase; ALP, Alkaline Phosphatase. Normal control represents group I, negative control represents group II while 400, 600 and 800 mg/kg ELECR represent Groups III, IV and V respectively.

Gestation refers to the period from conception to the time of birth. The duration of gestation varies from animal to animal and during this time the female animal undergoes many physiological changes aimed at maintaining the pregnancy to ensure a positive outcome. The postpartum period remains a very critical phase in the life of the mother and the newborn, as most infant and maternal deaths occur during this time, especially in developing countries.<sup>[27]</sup> Excessive weight gain, likely due to the increased steroid hormones needed to maintain pregnancy, and the accumulation of fat due to lack of exercise and sedentary lifestyle caused by pregnancy, as well as difficulties in initiating and maintaining lactation, are among the greatest challenges women face after the birth of their children. In traditional medicine, plants are being researched that have the potential to help postpartum women with weight loss, initiation of lactation, and regeneration of blood lost during childbirth.<sup>[12,37]</sup> Currently, the use of various plant parts such as leaves, roots, stem bark and fruits as postpartum medicine is well established and practiced in Africa due to their cultural acceptability, compatibility with human physiology and limited side effects. In addition, the phytochemicals contained in these herbs are currently used as major ingredients for the synthesis of new drugs.<sup>[8,10,38]</sup> The aim of this study was to characterize C. racemosum phytochemically and investigate its effects on selected biochemical parameters in lactating albino rats.

Gas chromatography-mass spectrometry (GC-MS) analysis is a widely accepted method for the and quantification identification of various phytoconstituents in a plant complex mixture or other biological matrices.<sup>[10,39]</sup> GC-MS analysis revealed fiftythree chemical compounds of which fourteen are bioactive compounds including  $\delta$ -terpinene, apiol, guaiol, humulene,  $\delta$ -terpinene,  $\alpha$ -copaene, linadool, therpinen-4-ol, azulene, dodecane,  $\beta$ -famesene,  $\beta$ bisabolene, caryophylene oxide and hexadecanoic acid ethyl ester (Table 1, Figure 1). Previous studies reported the antioxidant and antimicrobial properties of gammaterpinene, azulene and dodecane (Ramalho et al,<sup>[40]</sup> Bakun et al,<sup>[41]</sup> and Padma et al,<sup>[42]</sup>). Also a previous study reported that the bioactive compound betabisabolene exhibits anticancer and antitumour effects as well as synergistic antimicrobial activity against certain bacteria (Tarmo<sup>[43]</sup>), while hexadecanoic acid ethyl ester has hypocholesterolemic, nematicidal, pesticidal, and antioxidant effects.[44]

Prolonged and repeated administration of a xenobiotic over a period of 14 to 90 days may result in some adverse effects, generally classified as subacute toxicity.<sup>[45]</sup> Subacute toxicity studies serve as a guideline

for the number of days over which a substance should not be used in order not to cause toxicity. They provide an estimate of the safety margin of exogenous chemical compounds. All animals in this study gained weight after birth, regardless of the dose administered, indicating that ELECR is not toxic. However, after parturition, animals treated with 400, 600 and 800 mg/kg doses showed a significant (p< 0.05) decrease in weight gain compared to animals in groups I and II that did not receive ELECR (Table 2). These results provide scientific evidence that *C. racemosum* tonic when taken by mothers after childbirth helps to lose the weight gained during pregnancy.

Blood is the primary transport medium for toxic substances and any adverse effect on blood parameters serves as an indicator of a person's health status.<sup>[46]</sup> Blood disorders that manifest when toxins are ingested include thrombocytopenia, leukopenia, lymphocytosis, bone marrow depression, infections and all forms of anaemia.<sup>[8,10,47]</sup> Assessment of haematological parameters is also a viable tool for monitoring a patient's treatment and recovery. Administration of ELECR at a dose of 400, 600 and 800 mg/kg improved the erythropoietic process by significantly increasing (p<0.05) the levels of red blood cell, packed cell volume and haemoglobin when compared with the group II animals which were lactating (postpartum) but did not receive ELECR (Table 3). This does not only corroborate the non-toxicity of the extract but lends credence to the use or ELECR in restoring the haemoglobin of individuals following blood loss and the anaemic state that frequently accompanies child birth. Non-significant changes (p>0.05) in white blood cells (WBC) differentials, including basophils, eosinophils and monocytes (Table 3) was noted, implying that the extract did not adversely affect the process of leukopoiesis. Our results are similar to those of Miaffo et al,<sup>[37]</sup> where the administration of the aqueous extract of Combretum molle significantly increased the values of RBC, HB and PCV, while the changes in the values of WBC differentials were not significant.

The lipid profile is a diagnostic tool to detect abnormalities that can be attributed to a disturbed lipid metabolism. Dyslipidemia and its associated health problems diabetes mellitus. such as cancer. atherosclerosis, hypertension and pulmonary embolism are detected by determining the lipid profile.<sup>[32]</sup> In addition, postpartum obesity predisposes the new mother to cerebrovascular disease, myocardial infarction, stroke, lethargy and decreased self-esteem.<sup>[48]</sup> The animals in groups III, IV and V (Lactating animals administered different doses of ELECR) showed a reduction p<0.05 in total cholesterol, triacylglycerol, low density lipoprotein

cholesterol and very low density lipoprotein cholesterol levels compared to the animals in group II, which served as a negative control (Table 4). The values for HDL-C in the normal control animals are also comparable with the values of the animals treated with the ELECR (Table 4). The result of the lipid profile in this experiment confirms the previously reported data that animals receiving our extract showed a lower percentage weight gain compared to the control rats. The hypolipidemic effect of this extract could be due to the presence of hexadecanoic acid ethyl ester.<sup>[44]</sup> Miaffo et al.<sup>[37]</sup> also reported similar anticardioprotective hyperlipidemic and effects of Combretum molle.

The liver is the most important organ for the biotransformation and detoxification of drugs and plays the most important role in the metabolism of macromolecules in the body. Liver pathologies such as alcoholic and non-alcoholic fatty liver disease, viral hepatitis, acetaminophen-induced hepatotoxicity, hepatitis and cirrhosis lead to hepatocyte damage and the associated leakage and increase in liver biomarkers such as alanine transaminase, aspartate transaminase, Alkaline phosphatase, liver protein and bilirubin.<sup>[49]</sup> These biomarkers are well-established indicators of liver disease. Treatment of the experimental animals with extract doses of 400, 600 and 800 mg/kg resulted in a significant (p<0.05) reduction in liver enzymes: ALT, AST and ALP as well as total bilirubin when the treated groups were compared to lactating animals that did not receive the extract. However, groups III, IV and V showed a significant increase (p<0.05) in total protein content, albumin and globulin when compared to the negative control (Table 5). These results are similar to those of the normal control animals that did not give birth, both in terms of biomarker values and histological findings. The histology of the group I animals (normal control) is not different from that of the ELECR-treated animals (Figure 2), indicating that ELECR is not toxic. Okwuosa et al,<sup>[50]</sup> reported a significant decrease in AST, ALT and ALP levels when animals that had cyclophosphamide-induced liver injury were treated daily with crude methanol leaf extract of C. racemosum for 14 days. Serum electrolytes, urea and creatinine levels remain the most important indicator of renal status.<sup>[51,52]</sup> Elevated urea, creatinine and potassium levels are indicative of kidney damage and renal dysfunction.<sup>[9]</sup> Our results show a statistically significant decrease (P < 0.05) in urea and creatinine concentrations in animals treated with different doses of the ELECR compared to the negative control animals (Figure 3). This indicates that the ELECR is not toxic but rather reno-protective.

One of the body's defence mechanisms against free radical damage and oxidative stress caused by elevated levels of reactive oxygen species (ROS) is the production of various substances known as antioxidants.<sup>[6,53,54]</sup> Cellular damage caused by ROS leads to pathological conditions. A number of exogenous substances used in

the treatment of oxidative stress and complications are cytotoxic, prompting the reliance on herbal products to scavenge free radicals.<sup>[6,55,56]</sup> Administration of ELECR significantly increased (P < 0.05) the levels of glutathione peroxidase, superoxide dismutase, catalase and reduced glutathione in the treated animals compared to the negative control group. However, malondialdehyde levels decreased significantly (P< 0.05) when the ELECR-treated animals were compared with the negative control group (Figure 4). The reduction in MDA levels and increase in GSH, CAT and SOD found in our study have been shown to protect against free radicals and oxidative stress.<sup>[57]</sup> The presence of bioactive compounds such as azulene, dodecane and gammaterpinene with strong antioxidant capabilities could be responsible for the protection against oxidative stress observed in this extract.<sup>[40,41,42]</sup> Previous studies on the different Combretum species (Combretum. molle, *Combretum indicum, Combretum albidum*) have also shown their antioxidant properties.<sup>[58-61]</sup>

Oestrogen and prolactin are known to be the linchpin of milk production after the birth of a child. While oestrogen promotes breast tissue proliferation and growth, prolactin regulates milk production and mammary gland growth.<sup>[16,17]</sup> Administration of the aqueous leaf extract of Combretum racemosum significantly (P< 0.05) increased oestrogen levels and follicle stimulating hormone (FSH) compared to the negative control. Treatment with the extract did not significantly alter prolactin levels (Figure 5). This suggests that ELECR can increase breast milk production. Treatment with the extract also did not adversely alter the histology of the hypothalamus and anterior pituitary (Figure 2). The fact that there was no change in uterine histomorphology in the ELECR-treated rats compared to the negative control group suggests that ELECR may also play a positive role in uterine involution and healthy weight in the ELECR-treated postpartum rats.

## CONCLUSION

This study clearly demonstrated the non-toxic and protective tendency of Combretum racemosum leaf extract after 14 days of treatment. Our study showed that Combretum racemosum ethanol leaf extract has hepatoprotective, renoprotective, anti-hyperlipidemic and antioxidant potential and is safe for therapeutic uses. The phytochemical profile of the plant extract contains fourteen bioactive compounds with well-established pharmacological activities. The study also demonstrates that although the extract does not directly increase lactation, it may achieve this by promoting breast tissue growth and supporting milk flow by increasing estrogen level, as our results show. This study therefore supports the use of Combretum racemosum ethanol leaf extract in new mothers due to its weight-regulating and lactationpromoting effects, as well as its protection against oxidative stress and improvement of erythropoiesis. Further human studies should be conducted to address issues such as postpartum obesity and ascertain if the galactogogue effect of this extract seen in experimental animals is applicable to humans following childbirth.

#### **Conflict of interest**

Authors declare that they have no competing interests.

#### **Authors' Declaration**

The authors declare that the work showcased in this article is original and that they are responsible for any claims pertaining to the content of this article.

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