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EVALUATION OF ANTIDEPRESSANT EFFECTS OF ETHANOL LEAF EXTRACT OF *Combretum micranthum* G. Don (Combretaceae) IN MICE

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ABSTRACT

Combretum micranthum (Combretaceae) leaf is used against depression in Africa traditional medicines, but there is no scientific basis for its use. Hence, the antidepressant effect of ethanol leaf extract of *Combretum micranthum* was investigated using forced swim, tail suspension, yohimbine-induced lethality and reserpine-induced diarrhea tests at doses of 100, 200 and 400 mg/kg per oral in mice. The extract at all the doses used, significantly ($p<0.05$) shortened the immobility time in forced swim and tail suspension tests, as well as significantly ($p<0.05$) reduced the number of mean feacal droppings in reserpine-induced diarrhea suggesting antidepressant effect of the extract. The extract significantly ($p<0.05$) potentiated the sub-effective doses of fluoxetine and imipramine, suggesting that the extract may be used as an adjunct to the prototype drugs fluoxetine and imipramine. This study therefore concluded that the extract may possess antidepressant effect and may be used alone or with prototype drugs.

KEYWORDS: ***Combretum micranthum* leaf extract; reserpine-induced depression; tail suspension test; forced swim test**

INTRODUCTION

Depression is a devastating life-threatening mental disorder that affects 20% of the population world over, with female to male ratio being about 5:2 [1]. It is one of the top five diseases among the global listing of diseases, which is estimated to be the second leading cause of disability by the year 2020 [2]. Depression is characterized by mood changes, sleep or appetite disturbances, low self-esteem, fatigue or loss of energy, loss of interest in pleasurable stimuli, suicidal ideation, psychomotor retardation or agitation, poor concentration and melancholic [1, 3, 4].

Depression occurs as a result of a functional deficit in the brain monoamine transmitters (epinephrine, serotonin, and dopamine) [1, 5] as the underlying pathophysiological basis of depression [6]. Thus, almost all the established antidepressants target monoamine system [7, 8], but have in attendance a variety of undesirable effect such as sleep disturbances, sexual dysfunction, cardiac toxicity, weight gain and hypoplaxia [9-11].

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The undesirable side effects of the existing antidepressants have no doubt necessitated the search for safer antidepressant herbal remedies, which is on the increase [12]. This is evident from the increasing interest in the development of safe and efficacious alternative therapy for this mental illness [13].

To this effect, many medicinal plants used in traditional medicine as antidepressants include *Hoodia gordonii [6], Vigna unguiculata [14] and Adansonia digitata [15].* These medicinal plants extracts have been scientifically validated, while others such as *Combretum micranthum* used as an antidepressant agent in ethnomedicine [16] is yet to be scientifically evaluated.

Combretum micranthum (Combretaceae) is a bushy shrub or creeper which can grow up to the height of 20 m. It is dominant in sub-Saharan Africa, where the leaf is consumed as herbal bush tea [17-19]. In traditional medicine, the decoction of the leaf is used as anti-inflammatory and remedy for indigestion, constipation or nausea [20], wound and sore healing [21-23], malaria, cough and bronchitis [22, 23, 24].

Previous research findings have reported the anti-inflammatory [25], antibacterial [20], antidiabetic [26], and anti-malaria [27, 28] activities of *Combretum micranthum*.

Earlier phytochemical investigation has shown the presence of flavonoids such as vitexin, orientin and their derivatives, isovitexin and homoorientin, myricetin-3-*O*-glucoside, and myricetin-3-*O*-rutinoside; alkaloids such as stachydrine, hydroxyl-stachydrine, and choline; flavan alkaloids such as kinkéloids A, B, C and D and sugar alcohols such as *m*-inositol and sorbitol [29-32].

Considering the ethnomedicinal report of the use of *Combretum micranthum* as an antidepressant in the literature [16], this study was designed to investigate its antidepressant effects using a mouse model of depression.

MATERIALS AND METHODS

Plant Collection and Authentication

The aerial parts of *Combretum micranthum* were collected at the Crown Estate of Igbinedion University Okada, Edo state in February 2018. It was collected and authenticated by Mr. G. A. Ademoriyo of the Herbarium Unit, Department of Botany, Obafemi Awolowo University, Ile-Ife and herbarium voucher number IFE- 17707 was obtained.

Preparation of Plant Materials

The plant materials were air-dried at room temperature for two weeks and pulverized with a mechanical grinder. Three hundred grams of the powder was extracted with 70% ethanol for 72 hrs. The filtrate was freeze dried to yield 27.86 g (9.3%) of the extract and coded ELCM.

Drugs

The following drugs were used in this study: imipramine hydrochloride and fluoxetine hydrochloride (Sigma Aldrich, St. Louis, MO, USA), diazepam (Roche, Basel, Switzerland) and physiological saline (Unique Pharmaceutical Limited, Lagos, Nigeria). All drugs were dissolved in normal saline while ELCM was dissolved in 3% DMSO in normal saline. All drugs and ELCM were freshly prepared on each day of the experiment.

Animals

Adult albino mice of both sexes (18–25 g) were obtained from the Central Animal House of the Igbinedion University Okada (IUO), Edo State. The animals were maintained on standard animal pellets and water *ad libitum*. Experimental procedures were approved by the Igbinedion University Animal Ethical Committee vide the approval number IUO/ETHICS/22/011 which is in adherence to the United State National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research [33].

Pharmacological Experiments

General experimental design

Mice were randomized into 5 groups containing 5 mice each. **Group I** (Vehicle): animals in this group received normal saline p.o (10 mL/ kg); **Groups II – IV** (Treatment groups): animals in these groups received 100, 200 and 400 mg/kg, p.o of ELCM and **Group V** animals received the reference drug.

Forced-Swim Test (FST)

Mice were observed in FST as previously carried out elsewhere [34]. Briefly, each mouse was forced to swim in a cylindrical glass jar (25 x 12 x 25 cm) containing fresh water up to 15 cm height and maintained at 25^{o}C. The duration of immobility was taken for 4 minutes, out of the 6 minutes of swimming, with the initial 2 minutes of vigorous struggling discarded. The immobility is considered as when the mouse remained floating, making only the movement of its limbs essential to keep its head above the water. In a pre-test session 24 hours before the test, each mouse was placed individually into the cylinder and allowed to swim for 15

minutes. Imipramine (20 mg/kg, p.o.) was used as the positive control drug.

Tail Suspension Test (TST)

Mice were observed in TST as previously carried out [35]. On the edge of a table, 50 cm above the floor, mice were suspended with adhesive tapes, approximately 1 cm from the tip of the tail of each mouse. The period of immobility was recorded for 4 minutes, out of the 6 minutes of observation, with the first 2 minutes of observation discarded. Each mouse was considered immobile when the mouse hanged passively, without showing any movement. Fluoxetine (20 mg/kg, i.p.) was used as a positive control drug.

Yohimbine-induced Lethality Test

Five groups of mice (n=5) were randomly assigned as Group I: mice were orally administered 3% Tween 80 in normal saline (10 mL/kg); Group II-V: mice received ELCM at doses of 100, 200 and 400 mg/kg respectively, while Group V mice were given a positive control drug imipramine (25 mg/kg i.p.), 30 minutes before intraperitoneal injection of yohimbine (35 mg/kg). The number of death after 24 hrs treatment with yohimbine was recorded [36].

Reserpine-induced Diarrhea

Six groups of mice (Group 1–6) were reserpinized by the administration of reserpine (2.5 mg/kg, i. p.) 1 h after the respective drug administration. Group (1) received distilled water (10 mL/kg), group (2–5) received ELCM (100, 200 and 400 mg/kg, p.o.) respectively while group (6) received Imipramine (25 mg/kg). The effect of the acute administration of ELCM and prototype drug, imipramine on reserpine induced diarrhea was observed. Mice were observed for the presence of diarrhea at 1, 2, 3 and 4 h after reserpine injection [37].

Combined Effects of ELCM and Conventional Antidepressants on TST

In another experiment, the effect of the combined administration of the lowest dose of ECLM (100 mg/kg) with sub-effective doses of antidepressants fluoxetine (5 mg/kg) and imipramine (0.1 mg/kg in the TST was determined. To this end, mice received extract or vehicle and immediately after, the antidepressant or vehicle was administered. Sixty minutes later, the TST or open-field test was carried out [38]. The sub-effective doses of fluoxetine and imipramine used with ELCM was selected from literature [38].

Open Field Test

The open field (OFT) test was carried out on the dark grey floor of an observation cage made of a wooden box (100 cm x 100 cm x 30 cm) subdivided into 16 equal parts. One hour after oral treatment with the ELCM (100, 200 and 400 mg/kg) and 30 minutes after intraperitoneal injection with diazepam (1 mg/kg), each mouse was gently placed inside the open field and observed for the number of squares crossed with the four paws and the number of rearing for 5 minutes [39].

Statistical Analysis

All data are presented as mean ± SEM. The results were analyzed by One way analysis of variance (ANOVA) followed by Dunnett's post hoc test to determine significant effect using GraphPad InStat® Biostatistics software. The level of significance for all tests was set at $p<0.05$ compared to the vehicle group.

RESULTS

Effect of ELCM on Forced Swimming (FST) Test in Mice

ELCM at 100, 200 and 400 mg/kg, per oral and positive control drug imipramine, significantly ($p<0.05$) shortened the immobility time in FST when compared to the vehicle-treated control group. The extract at all the doses used showed a stronger anti-immobility effect compared to the positive control drug imipramine. The result is presented in Figure 1.

Effect of ELCM on Tail Suspension (TST) Test in Mice

The ELCM at all the doses of 100, 200 and 400 mg/kg, per oral and positive control drug fluoxetine (20 mg/kg, p.o.), significantly ($p<0.05$) shortened the immobility time in TST, when compared to the vehicle-treated control group. The effect of ELCM at all the doses used is comparable to the positive control drug fluoxetine. The results are presented in Figure 2.

Effect of ELCM on Yohimbine-induced Lethality Test in Mice

The extract at all the doses used did not significantly potentiate yohimbine-induced lethality test when compared to the vehicle-treated control mice. However, there was a significant ($p<0.05$) potentiation of yohimbine toxicity in the imipramine group when compared to the control treated group. The results are presented in Table 1.

Effect of ELCM on Reserpine-induced Diarrhea in Mice

The extract at all the doses used significantly ($p<0.05$) reduced the number of feacal droppings when compared to the vehicle-treated control group. The anti-diarrhea potential of ELCM was comparable to that of imipramine. The results are presented in Table 2.

Effect of Combined Treatment of Sub-effective Doses of Conventional Antidepressants with ELCM in TST

The extract significantly ($p<0.05$) potentiated the sub-effective doses of fluoxetine (Figure 3 Panel A) and imipramine (Figure 3 Panel B) when compared to the vehicle-treated control group. However, sub-effective doses of fluoxetine and imipramine did not show any significant ($p>0.05$) effect when compared to the vehicle-treated control group. The results are presented in Figure 3.

Effect of ELCM on Spontaneous Locomotor Behaviour in Mice

ELCM at 100, 200 and 400 mg/kg did not show any significant effects on locomotor activity in mice. Also, there was no dose-dependent increase in locomotor activity by ELCM as assessed by the OFT (Table 1). However, the standard sedative drug, diazepam (1 mg/kg) significantly ($p<0.05$) reduced rearing behavior in mice compared to the vehicle-treated control group. The results are presented in Figure 4.

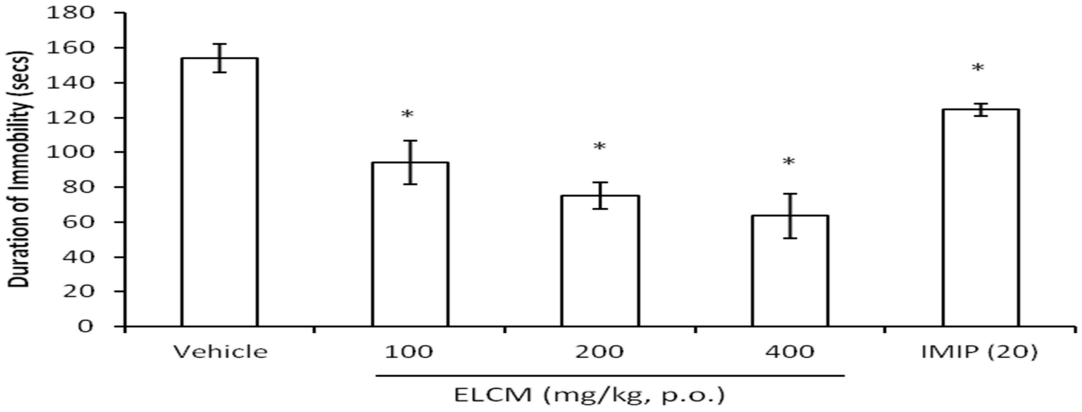

Figure 1: Effect of ethanol leaf extract of *C. micranthum* on FST test in mice

Vehicle; 3% DMSO in normal saline, ELCM; Ethanol leaf extract of *C. micranthum*, IMIP; imipramine. Each bar represents Mean ± SEM, n=5. *p<0.05 compared to the vehicle [$F_{(4, 20)}$=14.935, p<0.05] (ANOVA, Dunnett's post hoc analysis).

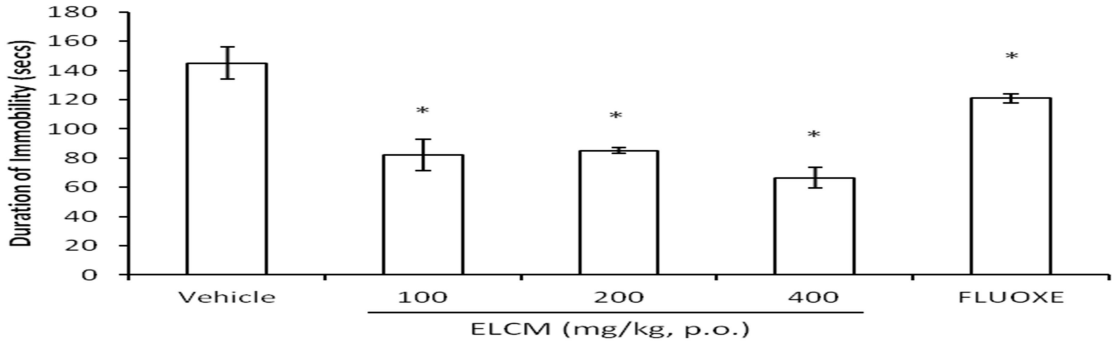

Figure 2: Effect of ethanol leaf extract of *C. micranthus* on TST test in mice

Vehicle; 3% DMSO in normal saline, ELCM; Ethanol leaf extract of *Combretum micranthum*, IMIP; imipramine. Each bar represents Mean ± SEM, n=5. *p<0.05 compared to the vehicle. [$F_{(4, 25)}$=17.127, p = 0.05] (ANOVA, Dunnett's post hoc analysis).

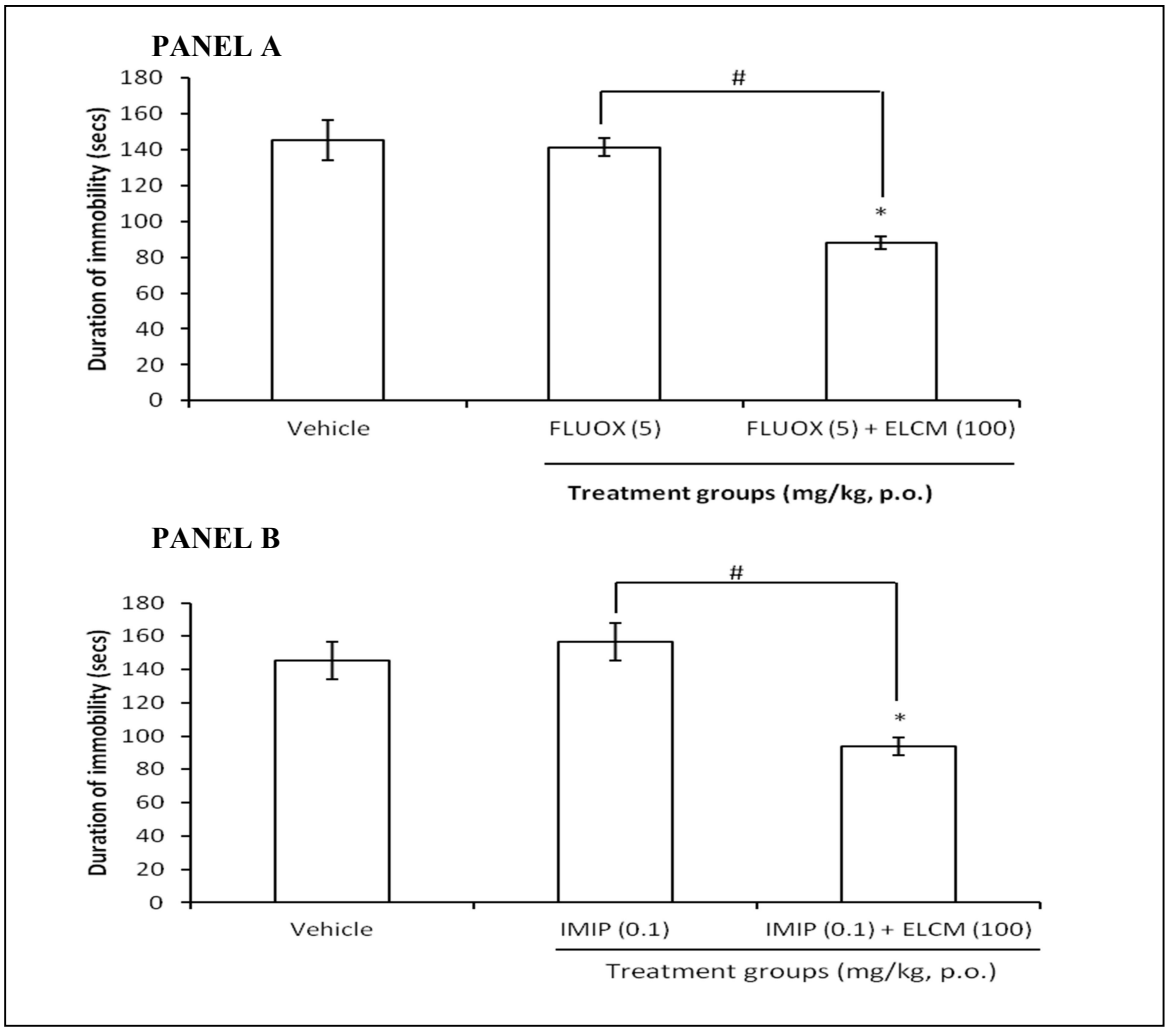

Figure 3: Effect of ELCM on sub-effective dose of fluoxetine (Panel A) and imipramine (Panel B) in mice

Vehicle; 3% DMSO in normal saline, IMIP; Imipramine, ELCM; Ethanol leaf extract of *Combretum micranthum*. Each bar represents Mean ± SEM, n=5. *$p<0.05$ and #$p<0.05$ compared to the vehicle and imipramine respectively. (ANOVA, Dunnett's post hoc analysis)

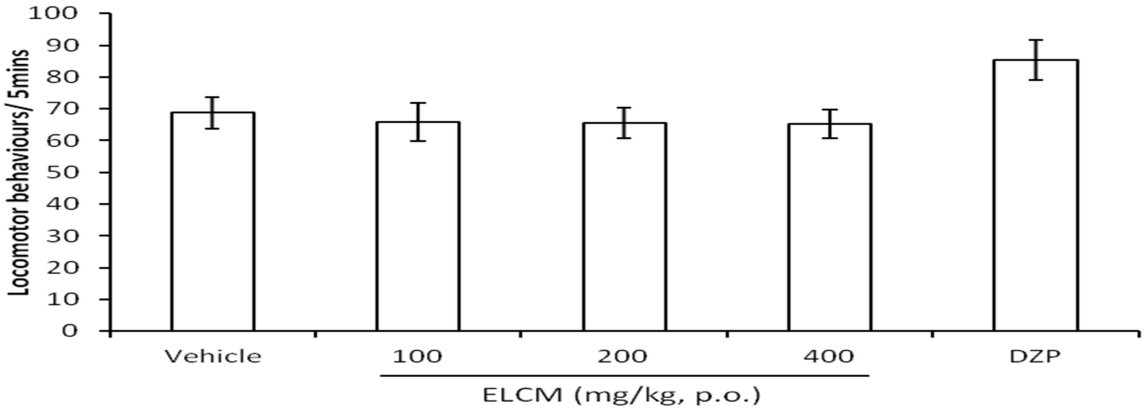

Figure 4: Effect of ethanol leaf extract of *C. micranthus* on locomotor in mice

Vehicle; 3% DMSO in normal saline, ELCM; Ethanol leaf extract of *Combretum micranthum*, DZP; diazepam (1 mg/kg, i.p.). Each bar represents Mean ± SEM, n=5

Table 1: Effect of ELCM on yohimbine-induced lethality test in mice

Treatment groups	Dose (mg/kg)	Number of death	% Mortality
Vehicle	10 mL/kg	0/5	0
ELCM	100	0/5	0
	200	0/5	0
	400	0/5	0
IMIP	25	3/5	60

Vehicle; 3% DMSO in normal saline, ELCM; Ethanol leaf extract of *Combretum micranthum*, IMIP; imipramine. Each value represent mean ± SEM (n=5). *p<0.05 compared to the vehicle treated control group.

Table 2: Effect of ELCM on reserpine-induced diarrhea in mice

Treatment groups	Dose (mg/kg)	60 mins	120 mins	180 mins	240 mins
Vehicle	10 mL/kg	3.60 ± 0.93	2.60 ± 0.40	1.80 ± 0.37	2.20 ± 0.37
ELCM	100	1.20 ± 0.37*	1.20 ± 0.37*	0.60 ± 0.24*	0.60 ± 0.40*
	200	0.80 ± 0.37*	0.40 ± 0.24*	0.20 ± 0.20*	0.60 ± 0.40*
	400	0.60 ± 0.24*	0.20 ± 0.20*	0.60 ± 0.24*	0.60 ± 0.40*
IMIP	25	0.20 ± 0.20*	0.60 ± 0.24*	0.60 ± 0.24*	0.60 ± 0.24*

Vehicle; 3% DMSO in normal saline, ELCM; Ethanol leaf extract of *Combretum micranthum*, IMIP; imipramine. Each value represent mean ± SEM (n=5). *p<0.05 compared to the vehicle treated control group

DISCUSSION

This study investigated the antidepressant-like effect of ethanol leaf extract of *Combretum micranthum* (ELCM) using mouse models of depression. The findings showed that ELCM may have antidepressant-like effect at doses that did not stimulate the experimental animals suggesting that the observed antidepressant-like effect was not due to psychostimulant effect.

Previous research finding has estimated the oral LD_{50} of the leaf extract of *Combretum micranthum* to be greater than or equal to 5000 mg/kg and suggested its safety [40]. Doses of 100, 200 and 400 mg/kg earlier used in other study were used for its antidepressant evaluation in this study because antidepressant drugs are used over a long period of time [15, 40].

Mice exhibited a state of hopelessness in forced swim (FST) test, indexed by their immobility time when forced to swim in an inescapable cylinder which correlates to negative mood [41]. This immobility time in FST has been reported to be decreased by synthetic antidepressants [41] and medicinal plant extracts [14, 42]. The reduction in immobility time following acute ingestion of ELCM to mice in FST suggests an antidepressant-like effect. This finding adds to the existing literature of medicinal plants suggested to possess antidepressant effects by reducing immobility time in FST [14, 41].

The tail suspension (TST) test has become one of the most widely employed mouse models of depression, used to screen the acute antidepressant-like effect of medicinal agents [43]. It is based on the premise that mice become immobile when hang by the tail in short-term inescapable stress, which reflects a state of despair [43]. The reversal, therefore, of the immobility posture is an index of antidepressant-like effect [14, 15]. The reversal of the immobility time in TST by ELCM suggests an antidepressant-like effect. This finding is in consonance with earlier findings of medicinal agents that decreased immobility time in TST and was suggested to possess antidepressant-like effects [15, 44].

Acute doses of selective serotonin reuptake inhibitors such as fluoxetine do not exhibit antidepressant-like effects on the FST [45-47] hence acute administration of imipramine which has been shown by several studies to be effective in FST was used as positive control drug [14] in this study. Since the neurochemical pathways mediating antidepressant effect differs in FST and TST [48].

TST was used to study the combined effect of ELCM and the conventional antidepressants because of its strong correlation to the antidepressant effects in man and its distinguishing feature from other psychotropic drugs, such as anxiolytics and antipsychotics [15, 49]. In addition, the lowest dose of ELCM in this study was used to evaluate the synergistic of ELCM and the conventional antidepressants- fluoxetine and imipramine in TST.

The potentiating effect of ELCM on the sub-effective dose of the conventional Selective Serotonin Reuptake Inhibitor (SSRIs) fluoxetine may suggest additive interaction of ELCM with fluoxetine probably through the noradreno-serotonergic pathway as is known for SSRI class of drugs [50], and consequent enhancement of the antidepressant effect of fluoxetine. Thus, ELCM may be used as an adjunct to fluoxetine, which may help to reduce the adverse effects associated with the prolonged use of the effective dose of fluoxetine [12, 51]. The finding of this study is in agreement with the antidepressant effect of the combined administration of *Moringa oleifera* leaf extract with the low doses of fluoxetine, which was suggested as a veritable tool for the development of alternative medicines in the treatment of depression [12].

Subsequently, the potentiating effect of ELCM on the sub effective dose of tricyclic antidepressant (TCA) such as imipramine may as well suggest the additive interaction of ELCM and imipramine with serotonergic pathway. The interaction may probably be via the inhibition of the reuptake of serotonin [45, 46], and consequent enhancement of the antidepressant effect of imipramine. Thus, ELCM may as well be used as an adjunct to TCA with the advantage of improving the efficacy of synthetic antidepressants and decreasing their adverse effects thereby allowing the prescription of the lower doses of synthetic antidepressants [52].

Yohimbine is an α_2 adrenergic receptor antagonist which has been implicated in the antidepressant action of numerous medicinal plants [14, 15]. The non potentiating effect of ELCM on yohimbine-induced lethality test may probably rule out the involvement of α_2 adrenergic receptor pathways in the antidepressant effect of ELCM.

The combined administration of the sub-effective dose of the conventional antidepressant fluoxetine or imipramine with the lowest dose of ELCM did not alter the spontaneous locomotor effect of the mice. Thus, ruling out “False” positive synergistic effect of the combined treatments.

Previous findings have shown that reserpine is depressogenic, it irreversibly inhibits the vesicular

uptake of monoamines and leads to hypothermia, ptosis, and diarrhea as its physiological consequences [37, 53]. These reserpine syndromes have been demonstrated to be reversed by major classes of a synthetic and medicinal plant extract with antidepressant activities [37]. The reversal of diarrhea by ELCM may suggest that the extract has antidepressant potentials. This finding is in line with the previous finding of the reversal of diarrhea by *Olax subscorpioidea* and as a consequence was suggested to have an antidepressant effect [37].

Several scientific findings have suggested that flavonoids and alkaloids ameliorate depressive disorders [12, 45, 54]. For example some isolated compounds from *Combretum micranthum* such as vitexin and orientin [29-32] which have also been isolated from other members of Combretaceae family [55] have been reported to possess antidepressant effects in other studies [56-57]. The antidepressant effects of other isolated compounds from *Combretum micranthum* such as choline and inositol [29-32] have been reported from other medicinal plant studies [58, 59]. The efficacies of some herbal medicines and nutraceutical containing flavonoids and polyphenolic compounds have been proven to be similar to some of the synthetic antidepressants [12]. Subsequently, the interaction of flavonoids [60] and alkaloids [45, 61, 62] with the monoaminergic pathways to elicit antidepressant-like effects have been suggested. Since flavonoids and alkaloids are some of the isolated compounds present in the leaves of *Combretum micranthum* [31, 60], it will, therefore, not be out of place to suggest that flavonoids and alkaloids, either in additive, synergy or counter interaction with other phytoconstituents in ELCM may be responsible for the observed antidepressant effect of ELCM in this study.

CONCLUSION

This study concludes that ELCM may have an antidepressant effect which may be used alone or as an adjunct to the conventional antidepressants. However, further studies may be warranted to carry out bio-guided isolation and characterization of the bioactive principles in ELCM as well as determine the antidepressant effect of ELCM on other animal species and humans to give a more robust interpretation and validation of the antidepressant effect of this extract. Also, isobologram analysis may be carried out to clarify any synergistic effects between ELCM and conventional antidepressants.

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CONFLICT OF INTEREST

No conflict of interest declared

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