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Antidepressant Activity of Ethanol Extract and Residual Aqueous Fraction of *Carissa edulis* (Apocynaceae) Root Bark in Mice

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ABSTRACT

Carissa edulis (Vahl) (family, apocynaceae) is used traditionally for the management of headache, chest pain, gonorrhea, rheumatism, epilepsy and some mental illness. This study was aimed at evaluating the antidepressant activity of the ethanol extract of *Carissa edulis* root bark and its residual aqueous fraction. Two experimental animal models namely Forced Swim Test (FST) and Tail Suspension Test (TST); using mice as experimental animals were used. The extract in FST exhibited dose-dependent and significant ($p < 0.05$) decrease in the duration of immobility at doses of 5, 10 and 20 mg/kg (55.3 ± 5.24 s, 28.7 ± 1.39 s and 22.7 ± 1.52 s, respectively) when compared to the control group (57.1 ± 5.93 s). For the TST, the extract produced dose-dependent decrease (92.1 ± 4.66 s, 75.3 ± 4.22 s and 63.0 ± 2.47 s at 5, 10 and 20 mg/kg, respectively) however, only 20 mg/kg of the extract and imipramine (10 mg/kg), produced significant ($p < 0.05$) decrease in the duration of immobility when both were compared to the control group (94.0 ± 9.79 s). However, there was no significant activity for the residual aqueous fraction (RAF) at all the tested doses (150, 300 and 600 mg/kg), in both FST and TST; but the activity was significant ($p < 0.05$) with imipramine (10 mg/kg) as compared with the control group. The ethanol root bark extract of the plant possesses bioactive principles with antidepressant activity. Therefore, the plant part could be considered active as whole extract. This lend scientific credence to the use of the plant by traditional herbalists in mental illness.

Introduction

Depression is a state of low mood and aversion to activity that can affect a person's thoughts, behaviour, feelings and sense of well-being. It is characterized by apathy, loss of energy, retardation of thinking and activity, as well as profound feelings of gloominess, despair and suicidal ideation.[1] Depressive disorder is a prevalent psychiatric disorder, which affects 21% of the world population.[2] Although a number of synthetic drugs are being used as standard treatment for clinically depressed patients, they have adverse effects that can compromise the therapeutic treatment.[3] Some of these adverse effects are cardiac toxicity, body weight gain, and sleep disorder [2]; others include nausea, mania, tremor, dystonia, dry mouth and hypertension.[4] Hence, there is a need for alternative remedies based on natural products.[3] Medicinal plants are being considered as effective alternatives in the treatment of depression, and this practice has progressed significantly in the past decades.[5] Both developed and developing countries seem to have a great demand for these plants due to increased accessibility of natural products and in some instances the primary source of health care available to the poor in developing countries.[6]

Carissa edulis Vahl belongs to the family Apocynaceae; a native of South Africa; commonly called *Cizaki* in Hausa; *Kanboro* in Fulfulde; *Emir* in Arabic; *Muyunzo* in Luganda, *Endelkoring-neominoem* in Africana; *Agam* in Amharicand *Mlanoa-mboo* in Swahili.[7] It is a spiny shrub that may reach a height of 5 ft and an equal breadth. The bark is grey and smooth with straight woody double-pronged spine often in pairs.

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The plant has various ethnomedical uses such as in the treatment of fever, sickle cell anemia and hernia, treatment of edema, toothache, cough, ulcer, worm infestation, management of epilepsy, mental illness and cancer. The plant extracts have been previously shown to possess antidiuretic effect[8]; analgesic and antimicrobial activities,[9,10] hypoglycemic,[11] antiviral,[12] anticancer,[13, 14] anticonvulsant, anxiolytic, and sedative activities.[15, 16, 17, 18] The present research studied the antidepressant property of the ethanol extract and residual aqueous fraction of *Carissa edulis* root bark; since previous reports on this effect is lacking.

Materials and Methods

Plant materials

The root bark of *C. edulis* was collected by herbalist in July 2010, at Basawa Village, Zaria, Nigeria. The plant sample was identified and authenticated by Malam Umar Gallah, a botanist in the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University, Zaria. This was made by comparing the sample with the already deposited specimen (voucher number 601). The root bark was air-dried at room temperature under shade and closely monitored until a constant weight was obtained. The dried samples were powdered with wooden mortar and pestle. The powdered sample (500 g) was cold-macerated with 2 L of 70%$^{v}/_{v}$ ethanol in water and the filtrate was concentrated to dryness using water bath maintained at 40ºC.

Fractionation of the crude extract

Briefly, the ethanol extract was dissolved in distilled water in separating funnel and defatted with petroleum ether (PE) to get PE fraction. The aqueous portion was further partitioned with ethyl acetate (EA) where EA fraction was obtained. Then the aqueous portion was partitioned again with n-butanol (NB) to obtain NB fraction. Finally, the left over aqueous

portion was referred to as residual aqueous fraction (RAF).

Animals

One hundred and twenty Swiss Albino mice (18–24 g) were obtained from Animal House Facility, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. They were maintained under good laboratory care and fed with standard animal feeds (Feeds Masters, Ilorin, Nigeria), and were allowed free access to water. The animals were used in compliance with the National Institute of Health Guide for the Care and use of Laboratory Animals (Publication nos. 85-23, revised 1985). The Institutional Approval Number for the protocol was given as DAC/IW-OT/003-10.

Antidepressant activity method

Forced Swim Test

The method previously described[19] was employed. Mice were randomly divided into eight groups containing six mice each. Group 1 received normal saline (10 mL/kg) while group 8 received imipramine (10 mg/kg); served as negative and positive controls respectively. Groups 2, 3 and 4 were the extract-treated groups and received varying doses as follows; ethanol extract (5, 10 and 20 mg/kg) while groups 5, 6 and 7 were for the residual aqueous fraction (150, 300 and 600 mg/kg); each respectively. Administrations were done via intraperitoneal route for all the groups. Thirty minutes post-treatment, each animal was placed individually in a 5 L glass beaker, filled with water up to a height of 15 cm and was observed for duration of 6 min. A mouse was considered immobile when it floated motionless or made only those movements necessary to keep its head above the water surface. Values for the last 4 min were considered as the duration of immobility. The glass beaker was rinsed and re-filled with fresh water for each experimental cycle.

Tail Suspension Test

The method previously described[20] was adopted for this study. Mice were randomly divided into eight groups containing six mice each. Group 1 received normal saline (10 ml/kg) while group 8 received imipramine (10 mg/kg) to serve as negative and positive controls, respectively. Groups 2, 3 and 4 were the extract-treated groups and received varying doses as follows; ethanol extract (5, 10 and 20 mg/kg) while groups 5, 6 and 7 were for the residual aqueous fraction (150, 300 and 600 mg/kg); each respectively. Administrations were done via intraperitoneal route for all the groups. Thirty minutes post-treatment, mouse in each group was hung by tail on a plastic string 75 cm above the surface with the help of masking tape, and were adjudged immobile when they hung passively and completely motionless. The duration of immobility was observed for a period of 8 min and the last 6 min value was considered and recorded.

Statistical Analysis

Results were expressed as Mean ± SEM. The level of significance between means was tested by one-way ANOVA followed by Dunnett's post hoc test using SPSS version 16 and results were regarded as statistically significant at $p \leq 0.05$.

Results and Discussion

The extract in Forced Swim Test exhibited dose-dependent and significant ($p < 0.05$) decrease in the duration of immobility at doses of 5, 10 and 20 mg/kg (55.3 ± 5.24 s, 28.7 ± 1.39 s and 22.7 ± 1.52 s, respectively) when compared to the control group (57.1 ± 5.93 s). Also, for the Tail Suspension Test, the extract produced dose-dependent decrease in duration of immobility (92.1 ± 4.66 s, 75.3 ± 4.22 s and 63.0 ± 2.47 s at 5, 10 and 20 mg/kg, respectively, however, only 20 mg/kg of the extract and imipramine (10 mg/kg) produced significant ($p < 0.05$) decrease in the duration of immobility when both were compared to the control group (94.0 ± 9.79) (Tables 1 and 2).

However, no significant ($p > 0.05$) decrease in duration of immobility was observed for the residual aqueous fraction (RAF) at all the tested doses (150, 300 and 600 mg/kg), in both FST and TST, but the activity was significant ($p < 0.05$) with imipramine (10 mg/kg) as compared with the control group (Figures 1 and 2).

Previous study on the phytochemical screening and HPLC finger printing of the extract revealed the presence of saponins and tannins as the major constituents[21]. These compounds have been reported to have multiple biological effects including Central nervous system activities[22]. Hence, these constituents may be responsible or contribute to the antidepressant activity of ethanol extract of *C. edulis*.

This study evaluated the antidepressant effect of ethanol root bark extract of *Carissa edulis* and residual aqueous fraction obtained from the extract in comparison to imipramine using Forced Swim test and Tail Suspension test models. These models are the most commonly used preliminary screening tests for characterizing potential antidepressant drugs.[1] Drugs which decrease immobility time leads to increase in the motor activity of mice which inhibit depression developed due to swimming and tail suspension of mice in these tests. [1, 23] Although all antidepressant drugs were observed to reduce immobility in the FST, the test has been observed to be sensitive and relatively specific to all major classes of antidepressants like tricyclics (e.g. imipramine, amitriptyline), selective serotonin reuptake inhibitors (e.g. Sertraline) and monoamine oxidase inhibitors (e.g. Tranylcypromine)[24]. It has been practically validated that swimming is sensitive to serotonergic compounds such as fluoxetine, while climbing is sensitive to compounds like tricyclic antidepressants with selective effects on noradrenergic transmission[25]. The extract exhibited significant reduction in the immobility time in both tests, signifying its antidepressant potential, but the activity was more potent with forced swim test. Forced Swim test is quite sensitive and relatively specific to all major classes of antidepressants.[4] It is the most widely used tool for assessing antidepressant activity pre-clinically due to its ability to detect a broad spectrum of antidepressant agents [26, 27] It is a behavior test, which, in rodents, gives an indication of the clinical efficacy of various types of antidepressant drugs.[28] The immobility seen in rodents during swimming reflects behaviour despair as seen in human depression and that the antidepressant drugs are able to reduce the immobility time in mice.[6,19] Similarly, antidepressants are also reported to be active in the Tail Suspension test and could have a greater pharmacological sensitivity as compared with Forced Swim test.[29] Also, the observed decrease in the duration of immobility in this model further suggested the antidepressant activity of the ethanol extract. According to past report[20] the immobility displayed by rodents in Tail Suspension test reflects behavioral despair, characteristic of depressive disorders in humans. Numerous antidepressant compounds are postulated to act via different mechanisms involving the serotonergic, noradrenergic and/or dopaminergic systems.[30] Imipramine prevents reuptake of nor adrenaline and serotonin resulting in their increased availability in the synapse and therefore an increase in adrenergic and serotonergic neurotransmission.[31] Other studies have shown that the dopaminergic activation is also involved in struggling behavior of both the Forced Swim and Tail Suspension tests.[32, 33]

Table 1: Effect of Ethanol Root Bark Extract of *Carissa edulis* and Imipramine on Forced Swim Test in Mice

Treatment	Dose (mg/kg)	Duration of Immobility (s)
Control	10 mL/kg	57.1 ± 5.9
C. edulis	5	55.3 ± 5.2
C. edulis	10	28.7 ± 1.4*
C. edulis	20	22.7 ± 1.5*
Imipramine	10	11.7 ± 2.3*

Values are presented as Mean ± SEM, n = 6 animals per group, *C. edulis* = Ethanol root bark extract of *Carissa edulis*, Control = Distilled water. * Significant difference at $p < 0.05$ (ANOVA) followed by Dunnett's Post hoc test.

Table 2: Effect of Ethanol Root Bark Extract of *Carissa edulis* and Imipramine on Tail Suspension Test in Mice

Treatment	Dose (mg/kg)	Duration of Immobility (s)
Control	10 ml/kg	94.0 ± 9.8
C. edulis	5	92.1 ± 4.7
C. edulis	10	75.3 ± 4.2
C. edulis	20	63.0 ± 2.5*
Imipramine	10	49.4 ± 3.3*

Values are presented as Mean ± SEM, n = 6 animals per group, *C. edulis* = Ethanol root bark extract of *Carissa edulis*, Control = Distilled water. * Significant difference at $p < 0.05$ (ANOVA) followed by Dunnett's Post hoc test.

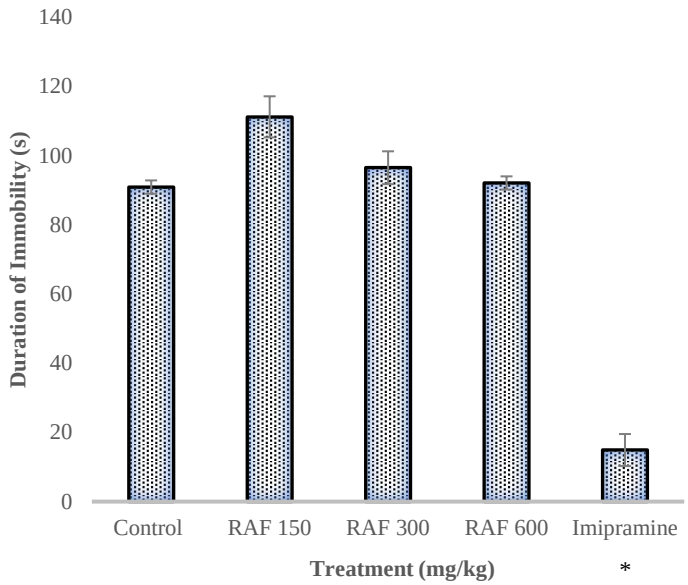

Figure 1: Effect of Residual Aqueous Fraction (RAF) of Ethanol Root Bark Extract of *Carissa edulis* and Imipramine on Forced Swim Test in Mice. Values are presented as Mean ± SEM, n = 6 animals per group, RAF = Residual Aqueous Fraction of Ethanol root bark extract of *Carissa edulis*, Control = Distilled water. * Significant difference at $p < 0.01$ (ANOVA) followed by Dunnett's Post hoc test.

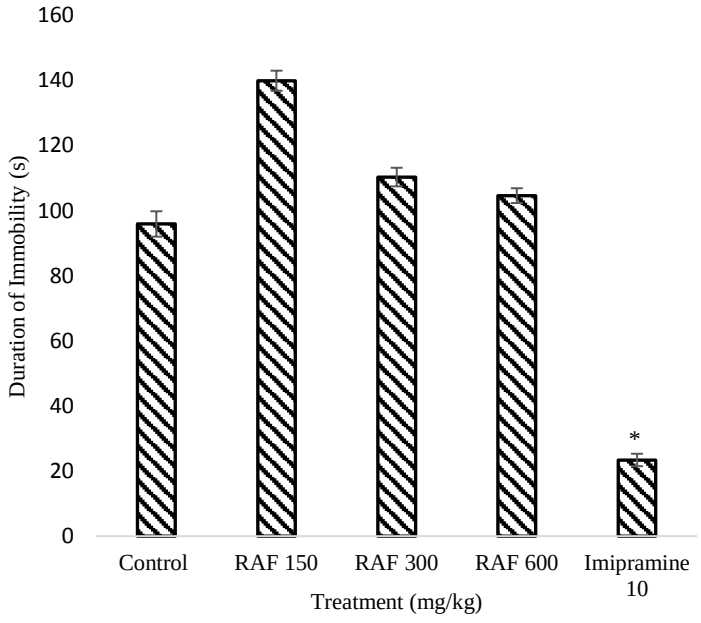

Figure 2: Effect of Residual Aqueous Fraction (RAF) of Ethanol Root Bark Extract of *Carissa edulis* and Imipramine on Tail Suspension Test in Mice. Values are presented as Mean ± SEM, n = 6 animals per group, RAF = Residual Aqueous Fraction of Ethanol root bark extract of *Carissa edulis*, Control = Distilled water. * Significant difference at $p < 0.01$ (ANOVA) followed by Dunnett's Post hoc test.

Therefore, it could be said that the activity of the ethanol extract could be via some of these mechanisms. However, there was no significant antidepressant activity with residual aqueous fraction, and hence, the fraction may lack some of the bioactive principles responsible for the antidepressant property of the ethanol extract.

Conclusion

From the current findings, it could be concluded that the ethanol root bark extract possesses antidepressant activity and thus, the research provided scientific justification to the traditional use of the plant in the treatment of mental illnesses.

Conflict of Interest

The authors state that there is no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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