



METHANOL AERIAL PARTS EXTRACT OF CARALLUMA DALZIELII (ASCLEPIADACEAE) POSSESSED IN-VIVO ANTIDEPRESSANT ACTIVITY IN MICE

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Abstract

Though *Caralluma dalzielii* N.E Br (Asclepiadaceae) is widely used traditionally for treating nervous system disorders, there is an absence of scientific reports about its pharmacological effect against depression. In this work, the potential antidepressant effects of the methanol extract of the aerial parts of *Caralluma dalzielii* were evaluated in mice using behavioral models (tail suspension and forced swimming tests) sensitive to clinically effective antidepressant agents. The extract at the doses of 10, 20 and 40 mg/kg body weight (intraperitoneally) was able to significantly ($p < 0.05$) decrease the immobility time of mice in a non-dose dependent and dose dependent manner when subjected to both tail suspension and forced swimming tests respectively and the effects were comparable to the effect produced by the standard drug imipramine (10mg/kg). It can be concluded that the present work suggested that methanol extract of the aerial parts of *Caralluma dalzielii* possessed potential antidepressant activity which can be explored for use clinically in the treatment of patients with depressive disorders.

Key-words: *Caralluma dalzielii*, depression, antidepressant activity, tail suspension test and forced swim test.

1.0 Introduction

Depression is the leading cause of disability and the second leading contributor to the global disease burden in the age category of 15 to 44 years for both sexes in 2015 (Jawaid *et al.*, 2015). It is the most prevalent mental disorder and is recognized to be symptomatically, psychologically and biologically heterogeneous (Thase & Howland, 1995) and is characterized by apathy, loss of energy, retardation of thinking & activity, profound feelings of gloominess, hopelessness, guilt, worthlessness, helplessness, despair and suicidal ideation (Santosh *et al.*, 2011). Depression persistently presents a major medical problem despite availability of antidepressants such as selective reversible inhibitors of monoamine oxidase-A enzyme (MAO-A), selective noradrenaline reuptake inhibitors (SNRIs), selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (Yu *et al.*, 2002). Based on the above considerations, it becomes very pertinent to search for new antidepressant agents that are safe, cheap, effective, readily available, with fast onset of action and minimal side effects. Various plants are being used in complementary and alternative medicines for management of mood disorders (Santosh *et al.*, 2011).

Caralluma dalzielii N.E Br (Asclepiadaceae) is cactus-like in shape with smooth, light green and quadrangular stems with a coarsely crenate to undulate margins between the faces, with very smelly deep purple flowers that consist of 5 triangular purple petals and five sessile stems fused to the rim of a white 5 lobed stigma. It grows better in West Africa and Sudan (de Kock & Meve, 2007), but distributed across the Sahel and can be up to 1m in height (Plowes, 2008). Several scientific studies have demonstrated the anti-diabetic (Tanko *et al.*, 2013), phytochemical constituents, acute toxicity, anti-inflammatory and analgesic (Umar *et al.*, 2013) activities of the plant.

2.0 Materials & methods

2.1 Animals

Fifty (50) mice (16-25g) of both sexes were procured from the animal facility, department of pharmacology, Bauchi State University Gadau. They were kept in standard wire meshed cages in a well-ventilated room, fed on standard animal feeds with free access to water and maintained under standard laboratory conditions of temperature and light prior to study (Magaji & Malami, 2018).

2.2 Drugs & chemicals

Imipramine (Asson, batch no. 1608010) and chemicals used include methanol (Sigma Aldrich), normal saline and distilled water. All drugs and chemicals were locally procured.

2.3 Equipment

These include water bath, hot air oven, beaker, weighing balance, motor and pestle, spatula, whatman's filter paper no1, masking tape, syringe and needle of various sizes, video recording device, stopwatch, measuring cylinder, tail suspension test apparatus and forced swim test apparatus.

2.4 Plant collection, authentication & extraction

Fresh *Caralluma dalzielii* aerial parts were obtained from Zaria town in Kaduna state-Nigeria with the help of herbalist. The plant material was identified and authenticated by Namadi Sunusi, Department of Biological Sciences, Ahmadu Bello University Zaria, Kaduna state-Nigeria. The sample was compared with an already deposited specimen and voucher number (ABU0280) was given for reference.

Fresh aerial parts of *Caralluma dalzielii* were shade dried at room temperature to a constant weight. The dried plant material was packed and grounded in to fine powdered material using motor and pestle. About 108grams of the fine powdered material was extracted in 4L of methanol absolute (100%) using cold maceration method for 3 days with regular shaking (morning and evening). The extract was filtered with whatman's filter paper No 1, concentrated the filtrate and evaporated to dryness using electric oven at the temperature of $45^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ and finally air dried. The extract was weighed and kept in an air tight container marked MECD until used.

2.5 Preliminary phytochemical analysis

The phytochemical analysis was carried out as described by Sofowora (1984).

2.6 Acute toxicity studies (LD₅₀ determination)

Median lethal dose (LD₅₀) was determined using Lorke's method (1983).

2.7 Antidepressant studies

2.7.1 Tail suspension test (TST)

The method described by Steru *et al.* (1985) was adopted. Thirty (30) mice were randomly divided into five groups of six (6) mice each (n=6). Group I was treated with normal saline, group II received imipramine at 10mg/kg dose, group III, IV & V were treated with the extract at the doses of 40, 20 and 10 mg/kg body weight respectively. Thirty (30) minutes post treatment, all the mice were suspended individually about 50cm above the floor with the help of adhesive tape placed approximately 1cm from the tip of their tails. Immobility time was recorded during the entire six (6) minutes period. Each animal was considered immobile when hanged passively motionless. A decrease in the immobility time was considered antidepressant like effect.

2.7.2 Forced swim test (FST)

The method described by Porsolt & Bertin (1977) was adopted. Thirty (30) mice were randomly divided into five (5) groups of six (6) mice each (n=6). Group I was treated with normal saline at 10ml/kg dose, group II received imipramine at 10mg/kg dose, while groups III, IV & V were treated with the extract at the doses of 40, 20 and 10mg/kg body weight respectively. Thirty (30) minutes post-treatment, all the mice were forced to swim individually in an open cylindrical container of 15cm diameter, 30cm height and a depth of 15cm containing water at 25°C. Immobility time was recorded during the entire six (6) minute period. When the mice ceased struggling and remained floating motionless in the water but only keeping their heads above water by making the necessary movements, they are said to be immobile. A decrease in the immobility time was considered antidepressant like effect.

2.8 Data analysis

Data were analyzed using SPSS statistical software version 20. Results were expressed as Mean \pm SEM. Analysis for difference between means were carried out using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Values of $p < 0.5$ were considered statistically significant.

3.0 Results

3.1 Percentage yield of the extract

The percentage yield obtained was 7%.

3.2 Median lethal dose (LD₅₀) value

The intra-peritoneal (ip) median lethal dose (LD₅₀) of the methanol extract of *caralluma dalzielii* aerial parts in mice was approximated to be 140mg/kg body weight.

3.3 Phytochemical constituents

The preliminary phytochemical screening of the extract revealed the presence of flavonoids, glycosides, saponins and alkaloids (Table 1).

Table 1: Phytochemical constituents of methanol extract of *Caralluma dalzielii* aerial parts

Constituent	Inference
Flavonoids	+
Tannins	-
Saponins	+
Alkaloids	+
Glycosides	+

Key: + = Present, - = Absent

3.4 Tail suspension test (TST)

At the doses of 40 and 20mg/kg body weight the extract significantly reduced the immobility time compared to the control group (table 2).

Table 2: Effects of MECD on duration of immobility in the tail suspension test (TST)

Treatment	Dose (mg/kg)	Immobility time (sec)
N\S	10ml/kg	213±11.8
IMP	10	54±34.5*
MECD	40	102±23.5*
MECD	20	88±15.0*
MECD	10	156±9.1

Values presented as Mean ± SEM, n=6, *= significantly different from control at p< 0.5 using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test. N/S = normal saline, IMP = imipramine, MECD = methanol extract of *Caralluma dalzielii* aerial parts, *=significant difference

3.5 Forced Swim Test (FST).

At the doses of 40 and 20mg/kg body weight the extract significantly reduced the immobility time compared to the control (table 3).

Table 3: Effects of MECD on duration of immobility in forced swim test (FST)

Treatment	Dose (mg/kg)	Immobility Time (Sec)
N\S	10ml/kg	201±16.0
IMP	10	104± 25.0*
MECD	40	109±15.09*
MECD	20	117±13.7*
MECD	10	119± 28.0

Values presented as Mean ± SEM, n = 6, * = significantly different from control at P< 0.5 using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test. N/S = normal saline, IMP = imipramine, MECD = methanol extract of *Caralluma dalzielii* aerial parts.

4.0 Discussion

It becomes very pertinent to find effective remedies so as to address the problem of lots of morbidities associated with the high incidence of depression in the community (Santosh *et al.*, 2011). Despite availability of several antidepressant agents, the issues of side effects and cost limit their use and hence produce poor prognosis. This calls for an immediate need to search for safe, cheap and effective alternative medicines to content with the disorder. Though *Caralluma dalzielii* is widely used traditionally for treating nervous system disorders, there is an absence of scientific reports about the evaluation of its pharmacological effects. In this work, it was demonstrated that the administration of different doses of the methanol extract of aerial parts of *Caralluma dalzielii* in mice was able to induced antidepressant-like effects.

Median lethal dose (LD₅₀) value of a substance is a measure of the substance's acute toxicity. It gives additional information about the margin of safety of a substance. The median lethal dose (LD₅₀) of the MECD was approximated to be 140 mg/kg body weight which suggests that the extract is highly toxic according to Lorke (1983) and this contradicts the work of Tanko *et al.* (2012) who found the LD₅₀ of the same plant to be 2,154 mg/kg body weight.

The antidepressant effects of the methanol extract of the aerial parts of *Caralluma dalzielii* was assessed using the two (2) most widely used animal (behavioral) models for antidepressant screening (tail suspension and forced swimming tests) which are quite sensitive and relatively specific to all major classes of antidepressants (Santosh *et al.*, 2011). The administered doses of the extract (40 & 20mg/kg, ip) produced significant (p< 0.05) antidepressant-like effects in both tail suspension and forced swimming tests with comparable efficacies to imipramine (10mg/kg, ip). The MECD produced better antidepressant-like activity in tail suspension test as compared to the forced swim test. This could owe to the less stressful nature of the tail suspension test than the forced swimming test (Thierry *et al.*, 1986). The observed activity is most probably due to the presence of flavonoids, alkaloids or saponins or both which have been implicated in literature to have antidepressant activity (Roloff *et al.*, 2009). Previous studies have established that enhancement of 5-HT and catecholamine neurotransmission in the brain is mainly responsible for the antidepressant-like effects in forced swimming and tail suspension tests (Borsini & Meli, 1988) and alkaloids have been found to act as reversible inhibitors of monoamine oxidase enzyme (AbdelFatah *et al.*, 1997) thereby enhancing the brain concentration of the biogenic amines (dopamine, serotonin, histamine, norepinephrine and epinephrine). There were studies also recently that have shown the possibility that flavonoids may mediate their antidepressant-like effects by interacting with adrenergic and serotonergic systems (Santosh *et al.*, 2011). These actions produce the antidepressant-like effects.

In this study, the pattern through which MECD reduced the immobility time in both TST and FST was similar to that produced by imipramine used in this study; hence, the mechanism of antidepressant action exhibited by our extract (MECD) could be linked to the enhancement of neurotransmission via mono-aminergic pathway.

Previous researches suggested that the neurogenesis that may be inhibited by depression in the hippocampus due to decrease level of brain derived neurotrophic factor (BDNF) can be promoted by antidepressants (Sapolsky, 2000; Rajkowska *et al.*, 1999). This can open a new research gate to explore the potential of MECD to promote neurogenesis that has been hijacked by depression and other neurological disorders.

5.0 Conclusion and future prospective

The present work suggested that MECD possesses potential antidepressant effects which support its traditional use and could be of therapeutic interest for use in the treatment of patients with depressive disorders.

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