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Sedative and Anti-Stress Potentials of Methanol Root Bark Extract of *Milicia Excelsa* (Moraceae) in Mice

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ABSTRACT

Milicia excelsa (Moraceae) root is consumed as tonic to reinvigorate the body after stressful events in African medicine, but there is scant documentation of its use. Hence, this study examined the anti-stress and sedative-hypnotic property of methanol root bark extract of *Milicia excelsa* (MRBE) via oral route in mice. The anti-stress effect of MRBE was investigated using acute restraint (ARS) paradigm and Unpredictable Chronic Mild Stress (UCMS) model, while the sedative-hypnotic effect was assessed using pentobarbital-induced hypnosis. The anxiety-, and depressive-like behaviours in mice were assessed following ARS, while the depressive behaviour was assesses following UCMS. The oral administration of graded doses of MRBE (75-300 mg/kg), demonstrated significant ($p<0.05$) lowering of the anxiety indices compared to stressed control. The MRBE (75-300 mg/kg) in ARS and 75 mg/kg in UCMS showed significant ($p<0.05$) reduction in the duration of immobility in tail suspension paradigm, indicative of anti-depressive-like property. The MRBE (75-150 mg/kg) demonstrated significant ($p<0.05$) reduction in the latency to sleep and elongated the sleeping time suggesting sedative-hypnotic effect. It is therefore concluded that MRBE may have anti-stress and sedative effects. Thus, providing scientific basis for its anti-stress effect in ethnomedicine.

Keywords: *Milicia excelsa* root, acute restraint test, unpredictable chronic mild stress, anxiety, depression

Introduction

Physical stress remains an integral and inevitable phenomenon in human life (Adeniyi *et al.,* 2023). This could in part be due to the heightened physical and psychological pressure of our nowadays lifestyle (Belloni *et al.,* 2022). These have hitherto contributed to the emergence of stress-related disorders including but not limited to depression and anxiety (Aiyelero *et al.,* 2023; Bansal *et al.,* 2023). Stress involves the interactions of neurotransmitters present in the brain and its modulation by peripheral factors (Mora *et al.,* 2012). Severe stressful conditions

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alter the body's homeostasis by negatively impacting on the various neurotransmitters in the brain regions responsible for stress regulation (Mora *et al.,* 2012) leading to anxiety, depression and sleep disorders, among other neurobehavioural disorders (Ram *et al.,* 2008; Kao *et al.,* 2024).

The hypothalamic-pituitary-adrenal (HPA) axis is another brain pathway that participates in the regulation of the bodily response to stress. This pathway can also be disrupted by stress culminating in hormonal imbalance such as an increase in corticosterone level which is an important prognosis of responses to chronic stress (Oken *et al.,* 2015; Adebesin *et al.,* 2017).

Several synthetic drugs are currently available for the management of brain disorders such as stress, anxiety, depression and insomnia (Feizi e*t al.,* 2019; Kenda *et al.,* 2022; Jensen *et al.,* 2024). These agents have their various adverse effect such as sexual dysfunction, addiction, hypertension, obesity, diabetes mellitus, suicidal ideation among others are reported in literature (Feizi e*t al.,* 2019; Jensen *et al.,* 2024).

The undesirable effects associated with the use of anti-stress and sedative-hypnotics have limited their use and warrant the development of new agents to treat these neuro-behavioural disorders, especially from medicinal plants with ethnomedicinal relevance.

Medicinal plants remain popular across the globe due to their low cost, easy accessibility and lower undesirable effects relative to modern medicine (Jensen *et al.,* 2024). Many have their biological activities documented while many remained to be scientifically investigated (Jensen *et al.,* 2024). One of such plants is *Milicia excelsa*, whose root bark is yet to be scientifically assessed for its antistress, antianxiety and sedative-hypnotic potentials upon the reports of its leaf and stem bark use in the management of the aforementioned neuro-behavioutral disorders (Akinpelu *et al.,* 2020a; Sofidiya *et al.,* 2021; Aiyelero *et al.,* 2023).

Milicia excelsa synonymously referred to as *Cholorophora excelsa* belongs to the Moraceae family (Mulberry family) (Ofori, 2007). *Milicia excelsa* is variously called African teak in English, 'Iroko' in Yoruba, 'Oje' in Igbo and 'Loko' in Hausa. It is a large deciduous tree which grows naturally in the humid area of West Africa to a height of 30 m (Ofori, 2007).

The root and stem bark are used as sedative (Ibrahim *et al.,* 2007), anti-rheumatism (Ndah *et al.,* 2013), anti-cough, anti-tuberculosis and for treating sexual dysfunction (Betti *et al.,* 2002) and male sterility (Ofori *et al.,* 2007).

Previous pharmacological experiments have demonstrated that the root bark may possess antidiarrhea (Adebayo *et al.,* 2019) and anticancer (Oke-Altuntas *et al.,* 2016) properties while some of its metabolites have proved useful against pain and inflammation (Ouete *et al.,* 2013). Other pharmacological effects such as anxiolytic (Sofidiya *et al.,* 2021), antistress (Akinpelu *et al.,* 2019; Aiyelero *et al.,* 2023) have been attributed to the leaf and stem bark extracts of the plant.

Qualitative phytochemical screening revealed the presence of flavonoids, phenols, tannins and alkaloid from the root bark extract of *Milicia excelsa* (Adebayo *et al.,* 2019).

This research was conceptualized to scientifically assess the stem bark extract for anti-stress and sedative-hypnotic potentials using mouse behavioural models.

2.0 Materials and Method

Plant identification, collection and authentication

The root bark of *Milicia excelsa* was collected from Bode-Osi in Olaoluwa Local Government of the State of Osun, Nigeria. It was authenticated by Mr. S. O. Popoola of the Forest Research Institute of Nigeria, Ibadan where the Voucher Number; FHI-109661 was obtained.

Preparation and extraction of the plant materials

The extraction procedures used was as earlier documented by Adebayo *et al.* (2019). Briefly, *Milicia excelsa* root bark was ground into fine powder and 300 g of the powder was extracted with 0.5 L of 80% methanol using soxhlet extraction apparatus. The filtrate gotten was concentrated *in vacuo* and freeze-dried to yield

13.1 g (4.37%) crude methanol extract coded MRBE.

Animals

The mice used in this behavioural investigation were male adult mice. These mice were bred in the Central Animal House of the Igbinedion University, Okada (IUO), Edo State. They were kept under natural 12-hour light/12-hour dark cycles. They had unrestricted freedom to clean drinkable water and standard commercial diet (Guinea feeds brand, Bendel Feeds Nigeria). The experimental protocols used in this experimental quest were approved by the Animal Ethical Committee of the Igbinedion University, Okada (IUO/ETHICS/22/012) which was not at variance with the National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (NIH, 1985).

Drugs

Diazepam (DZP) (Roche, Basel, Switzerland), imipramine hydrochloride (IMIP), Tween 80 (Sigma Aldrich, St. Louis Missouri, USA) and normal saline (Fidson Healthcare Plc, Ogun State, Nigeria). The extract was initially dissolved with 3% Tween 80 and marked up with physiological saline to 50 mg/ml solution.

Experimental procedures for acute restraint stress (ARS)

Thirty adult male mice were allotted into 6 groups containing 5 mice each (n=5). Group-I (unstressed group): received physiological saline (10 mL/kg) - ARS; Group-II (stressed control): received physiological (10 mL/kg) + ARS; Group III: received MRBE (75 mg/kg) + ARS; Group IV: received MRBE (150 mg/kg) + ARS; Groups V: received MRBE (300 mg/kg) + ARS; Group-VI (reference drug): received imipramine (15 mg/kg, i.p.) + ARS for tail suspension test (TST) or received diazepam (2 mg/kg, i.p.) + ARS for elevated plus maze (EPM) behavioural assays. One hour after oral treatments in groups I-V and 30 minutes following respective intraperitoneal treatments in group VI. Mice in all groups except Group I (unstressed group) were exposed to ARS in a 4 cm long cylindrical and transparent 50 ml tube with a lid cover restricting both turning and movement for a duration of 2 hrs. On the expiration of 2 hours restraint in the tube, mice were singly removed from the tube and transferred into the home cage for 20 minutes before behavioural assessment on either EPM or TST as earlier done by Aiyelero *et al.* (2023).

Assessment of anxiety-like behaviour following ARS on EPM in mice

The anxiety-like behaviours of mice were assessed following ARS on EPM as earlier reported (Aiyelero *et al.,* 2023). Immediately after the expiration of 20 minutes post ARS, each mouse was singly and gently without agitation put on one of the open arms of EPM and allowed free exploration of the EPM for time course of 5 minutes. While the mouse was exploring the EPM, the open arm entries (OAE) into and time of stay (OAD) on the open arms were noted and recorded. The index of open-arm avoidance scores (Akinpelu *et al.,* 2019, Aiyelero *et al.,* 2023), interpreted as anxiety status were calculated for each mouse using the mathematical expression below:

Anxiety status = (100 – [% OAE + % OAD]/2

Assessment of depressive-like behaviour following ARS on in mice

The depressive-like behaviors of mice were assessed following ARS on TST as earlier reported (Vazhayil *et al.,* 2017; Aiyelero *et al.,* 2023). Immediately after the expiration of 20 minutes post ARS, mice were in turn and singly suspended on the edge of a table 50 cm high from the floor with adhesive paper tape placed approximately 1 cm from the tail tip. The duration of immobility measured in seconds for the last 4 minutes in a 6 minutes period was recorded with the use of a stop watch. Mouse was adjudged immobile when it hung motionless with the head facing the floor.

Unpredictable Chronic Mild Stress (UCMS) procedure

Thirty mice were allotted into 6 groups (n=6) and treated as done above for ARS. Sixty minutes after oral ingestion of MRBE (75, 150 and 300 mg/kg) in groups II-V and half an hour after imipramine (10 mg/kg, i.p) injection, mice in group II-VI were subjected to UCMS for 2 weeks using different stressors as earlier carried out by Adebesin *et al.* (2017). Twenty-four hours

expiration after the completion of last stress session, tail suspension test was assessed.

Assessment of depressive-like behaviour following UCMS in mice

The TST used to assess the depressive-like behavior of mice following UCMS was as earlier described in TST evaluation following ARS in mice on the 15th day as first written under UCMS protocol (Adebesin *et al.,* 2017).

Assessment of sedative effect of the extract in pentobarbitone-induced sleeping time

Twenty-five adult male albino mice were grouped into 5 experimental groups (n=5 per group). Group-I (control): mice were orally administered physiological saline (10 mL/kg). Group-II (reference drug control): mice were orally treated with DZP (2 mg/kg), while groups Group-III-V (MRBE treated groups) were orally administered MRBE (75, 150 and 300 mg/kg). One hour following oral treatments of mice in groups I, III-V mice and half an hour following intraperitoneal injection of diazepam in group II mice. Mice were singly injected intraperitoneally pentobarbitone sodium (40 mg/kg). The time duration in seconds between injection of pentobarbitone sodium and loss of righting reflex was considered and recorded as latency to sleep and the time duration in seconds between the loss of righting reflex and its regain was recorded as sleeping time (Akinpelu *et al.,* 2020).

Statistical analysis

Each bar connotes Mean ± SEM, n=5. Statistical analysis was carried out using One-way ANOVA, followed by Dunnett's post hoc analysis for the behavioural assessments $^{*}p<0.05$ and $^{\#}p<0.05$ when comparison was made to the control (unstressed) and control stressed respectively.

Results

Effect of the extract on anxiety-like behaviours induced by ARS on EPM

Significant ($p<0.05$) reductions in the percentages open arm activities (open arm entries and duration of stay in the open arm) as well as a significant ($p<0.05$) increase in anxiety indices in stressed control in comparison to control unstressed mice. However, a significant ($p<0.05$) increase in percentage open arm activities as well as significant ($p<0.05$) reduction in anxiety indices were observed in all MRBE (75-300 mg/kg) treated mice when comparison was made to the stressed control mice (Figure 1A). In a similar manner, MRBE (75-300 mg/kg) showed significant ($p<0.05$) increase in percentage time spent in the open arm when compared to the stressed control mice (Figure 1B). A significant ($p<0.05$) mitigation in anxiety indices in MRBE and diazepam treated mice was observed when comparison was made to the stressed control mice (Figure 1C).

Effect of the extract on depressive-like behaviours induced by ARS on TST

A significant ($p<0.05$) prolongation in time spent immobile was observed in stressed control mice when comparison was made to the unstressed control mice following ARS in mice. However, MRBE (75-300 mg/kg) showed significant ($p<0.05$) and dose-dependent reduction in immobility time when comparison was made to the stressed control. Similarly, fluoxetine (5 mg/kg) also significantly ($p<0.05$) reduced the immobility time when comparison was made to the stressed control mice [Figure 2].

Effect of the extract on depressive-like behaviours induced by UCMS on TST

A significant ($p<0.05$) prolongation in the immobility time was observed in stressed control when comparison was made to the unstressed control. The induced elongation in time spent immobile observed in stressed control was significantly ($p<0.05$) reduced by MRBE at the lowest tested dose of 75 mg/kg and by imipramine at 10 mg/kg [Figure 3].

Effect of the extract on pentobarbital-induced sleeping time

The MRBE (75, 150 and 300 mg/kg) and DZP (2 mg/kg) showed significant ($p<0.05$) reduction in sleep onset [Figure 4A] and extended the sleep duration [Figure 4B] compared to the control. The sleep potentiating effect of MRBE is comparable to the prototype sedative agent diazepam.

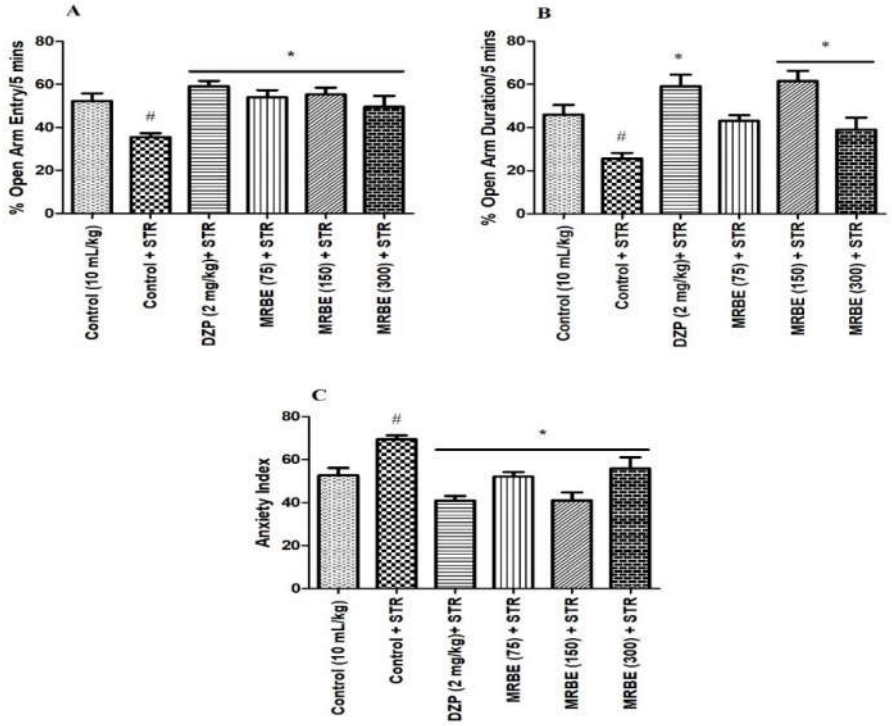

Figure 1: Potential of crude extract of *Milicia excelsa* on percentage frequency of open arm entry (A), percentage frequency of open arm duration and anxiety index (C) in experimental mice.

*Each bar connotes mean ± standard error of mean (SEM), n=5. #p<0.05 and *p<0.05 compared to the control (unstressed) and control stressed respectively.*

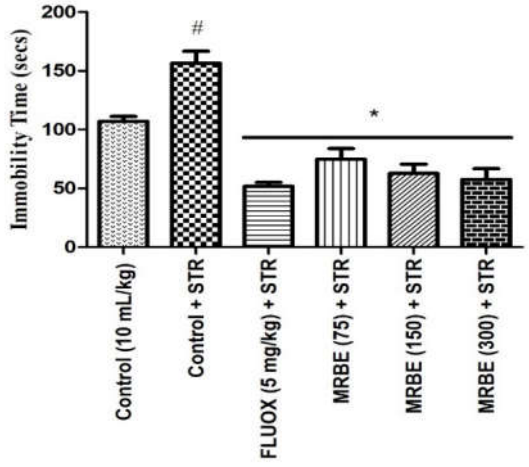

Figure 2: Potential of crude extract of *Milicia excelsa* on immobility time in tail suspension test following ARS in mice.

*Each bar connotes mean ± SEM, n=5. #p<0.05 and *p<0.05 when comparison was made to the control (unstressed) and control (stressed) respectively.*

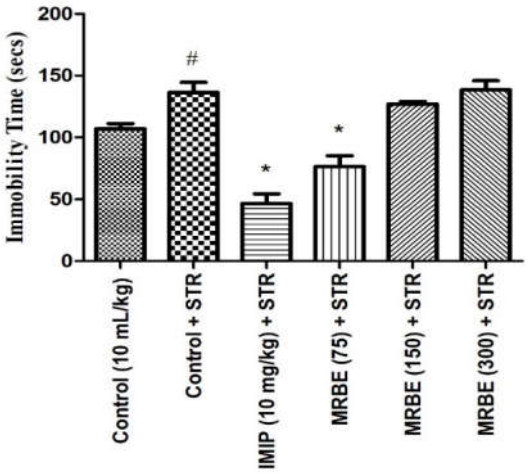

Figure 3: Potential of crude extract of *Milicia excelsa* on immobility time in tail suspension test following UCMS in mice.

*Each bar connotes mean ± SEM, n=5. #p<0.05 and *p<0.05 compared to the control (unstressed) and control stressed respectively.*

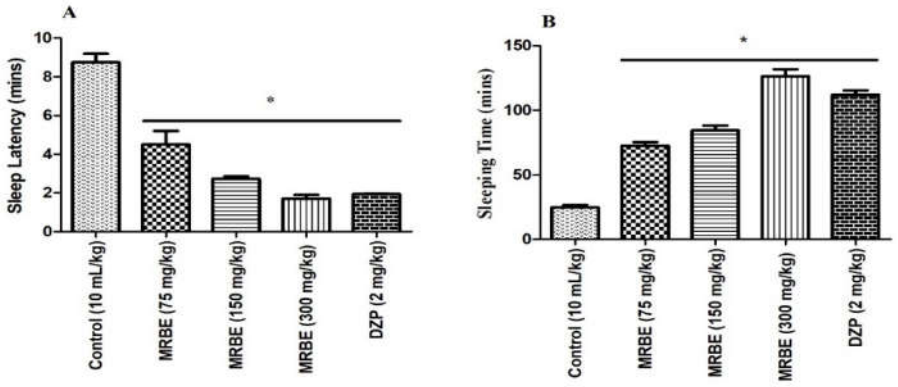

Figure 4: Potential of methanol root bark extract of *Milicia excelsa* on sleep onset (A) and sleep duration (B) in mice.

*Each bar connotes Mean ± SEM, n=5. *p<0.05 when comparison was made to the control.*

Discussion

The findings of this behavioural assessment provide scientific rationale for the utilization of MRBE in the mitigation of stress-related disorders such as anxiety, depression and insomnia in traditional medicine.

Earlier reports have determined the acute oral toxicity of MRBE to be equal to or greater than 5000 mg/kg in mice (Adebayo *et al.,* 2019). Hence, low doses of 75, 150 and 300 mg/kg were used in this behavioural assessments.

The acute restraint stress (ARS) behavioural paradigm has previously been employed to study the molecular basis for stress-induced pathological conditions (Spinieli *et al.,* 2024). Specifically, it has been used as a reliable experimental model for studying anxiety-, and depressive-like behaviours in rodent models of elevated plus maze (EPM) and tail suspension paradigm (TST) (Akinpelu *et al.,* 2019; Akanmu *et al.,* 2024)

The reduction in the EPM indices of open arm activities (open arm entry and duration) leading to an increase in anxiety indices of mice suggest the induction of anxiety in stressed mice (stress induced anxiety). The mitigation of stress induced anxiety by MRBE is indicative of the antistress potential of MRBE in mice which might be acting via GABAergic pathway (Aiyelero *et al,* 2023; Akanmu *et al.,* 2024). This suggestion is in concordance with medicinal plant such as *Stachytarpheta cayennensis* whose anxiety potential has earlier been scientifically corroborated in ARS in mice (Akanmu *et al.,* 2024).

The induction of depression-like behavior in TST following ARS as earlier been reported in several behavioural studies involving animals (Vazhayil *et al.,* 2017; Aiyelero *et al.,* 2023) and the mitigating potentials of medicinal plants/agents have also been reported (Akinpelu *et al.,* 2019; Aiyelero *et al.,* 2023). The amelioration of depressive-like state by MRBE following ARS indicates that the extract may possess antidepressant-like potentials. The involvement of noradrenergic and/or serotonergic neurotransmission may therefore, be proposed as its probable mechanism of antistress action since earlier studies have reported the suppression of noradrenaline and serotonin levels in different brain compartments of rodents and their differentiation in several studies on depression following ARS (Vazhayil *et al.,* 2017).

Our finding is in conformity with the earlier reported medicinal agents such as *Milicia excelsa* stem bark and *Clerodendrum serratum* leaf ameliorated ARS induced depression in rodents (Vazhayil *et al.,* 2017; Aiyelero *et al.,* 2023).

The suitability and clinical relevancy of UCMS as an animal stress model that mimics the

behavioural characteristics and neurochemical interplay seen in depressed patients have earlier been documented (Oken *et al.,* 2015; Adebesin *et al.,* 2017). This is simply because, exposing animals to mild stressors in an unpredictable fashion is a replica of how individuals experience stress on a daily basis throughout their life-time (Adebesin *et al.,* 2017). The reversal of the depressive-like effect induced by UCMS by the extract suggests that the extract may possess antidepressant-like effects, since the immobility which characterizes the TST is a resemblance of loss of stamina or energy as evident in sufferers of major depression (Adebesin *et al.,* 2017). Our finding is in conformity with the earlier reported medicinal agents such as methyl jasmonate and riluzole ameliorated UCMS induced depression in rodents (Adebesin *et al.,* 2017; Bensa *et al.,* 2023).

The pentobarbital-induced hypnosis test has earlier been employed in screening the sedative-hypnotic properties of medicinal agents, particularly for medicinal agents whose effects are mediated via GABAergic system (Akinpelu *et al.,* 2020a; Gong *et al.,* 2025).

The shortening of the sleep onset and simultaneous prolongation of the time spent sleeping by the extract suggest that MRBE may have sedative-hypnotic potential. The sleep potentiating effect of MRBE may therefore be beneficial for sleep induction and sleep maintenance. This finding is in tune with several medicinal plants such as *Menyanthes trifoliata* leaf and *Milicia excelsa* stem bark whose sleep potentiating effects have been scientifically demonstrated (Akinpelu *et al.,* 2020a; Gong *et al.,* 2025).

Conclusion

In conclusion, the study demonstrated that methanol root bark extract may possess antistress and sedative-hypnotic properties. However, further studies may be carried to examine its effects on other stress-induced disorders. Further studies may also be warranted to delineate neural mechanism of its antistress potential.

Conflict of Interest

We declare none among ourselves

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