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# Antinociceptive evaluation of *Motandra guineensis* (Thonn) A. DC. (Apocynaceae) aerial part in mice.

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Abstract

Motandra guineensis (Thonn) A. DC. (Apocynaceae) aerial parts are used in ethnomedicine to treat stomachaches, toothaches, and Motandra guineensis (Thonn) A. DC. (Apocynaceae) aerial parts are used in ethnomedicine to treat stomachaches, toothaches, eye infections, and other pain-related illnesses in eastern Nigeria. However, the plant has not been scientifically evaluated for its traditional uses. Therefore, this study examined the antinociceptive activity of the crude ethanol extract of the aerial parts of Motandra guineensis (MG). The antinociceptive effect of the MA crude extract was assessed in mice using the acetic acid-induced writhing test, hot plate test, and formalin assay at oral doses of 50, 100, and 200 mg/kg (n=6). An oral acute toxicity test was conducted on the mice. Phytochemical screening was performed using standard methods.
In the acetic acid-induced writhing model, the extracts showed significant and dose-dependent antinociceptive effects. In the formalin test, all doses of the extract showed a significant ($P < 0.05$) effect during both phases. The results of the hot plate test were significant ($P < 0.05$) at 60, 90, 120, and 150 min across all doses. The extract did not produce any toxicity at doses of up to 5000 mg/kg b.w. Alkaloids, tannins, flavonoids, glycosides, terpenoids, and saponins were observed in the extract. These results support the traditional use of M. guineensis in treating various diseases associated with pain.

**Keywords:** *Motandra guineensis,* antinociceptive, phytochemistry screening, acute toxicity.

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## Introduction

Pain is defined by the International Association for the Study of Pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (Leppert and Buss, 2012). It is a disabling condition that is the only symptom for the diagnosis of several diseases in many cases (Srebro *et al*., 2022). To relieve pain, conventional analgesics such as opiates and non-steroidal anti-inflammatory drugs (NSAIDs) are used. However, their long-term administration may induce several adverse effects, such as gastrointestinal ulcers, hepatotoxicity, bleeding, renal disorders, and immunosuppression (Leandro *et al*., 2020). Therefore, the development of more effective and safe analgesic drugs is needed. Over the years, secondary metabolites from plants have contributed significantly to the improvement of important therapeutic drugs used in modern medicine, and one of the most essential analgesic drugs employed in clinical practice today is the morphine alkaloid (Estella *et al*., 2022).

Despite this advancement, many traditional medicinal plants used in pain management have not been scientifically evaluated to provide evidence of their efficacy and the phytochemical constituents responsible for their activities. One such plant is *Motandra guineensis. Motandra guineensis* (Thonn) A. DC. is a species of the Apocynaceae family, commonly called dogbane (Oiseoghaede *et al*., 2024). It is called “Ntinabo”, “Agba doje” and “Bodekadun” by the Igbo, Yoruba, and Hausa tribes of Nigeria, respectively. The stems were hollow, with dark green paired follicle fruits and decussate leaves. It is mostly found in secondary forests in Nigeria and other African countries, including Ghana, Togo, and Liberia. The leaf sap of MG is applied to the eyes to treat eye infections, as a mouthwash or massage to the gums to treat toothache, or instilled in the nose in case of fainting, headache, or calm insanity. In Nigeria, boiled leaf extract is used as an enema to alleviate stomach aches in women who have just given birth. It is also used to treat malaria and urinary tract infections (Burkill 1985; Oiseoghaede *et al*. 2024).

The antiproliferative potential of ethanol leaf extract against human melanoma and ovarian cancer cells (Oiseoghaede *et al*., 2024) and the depressant activity of the aerial part of the central nervous system have been reported (Sofidiya *et al*., 2022). The present study aimed to evaluate the antinociceptive activity of the ethanol extract of MG aerial parts using different models of pain in mice.

## Materials and Methods

*Plant materials*

The aerial part (leaves and stem) of *Motandra guineensis* was collected from Obollo in Enugu State, Nigeria, in June 2022 and authenticated by Mr. Ossai of the International Centre for Ethnomedicine Development (Intercede), Nsukka, Enugu State. The herbarium specimen was deposited in the Department of Pharmacognosy, Faculty of Pharmaceutical Science, Enugu State University of Science and Technology (ESUT), Nigeria. A Voucher specimen (ESUT/PCG/067) was deposited in the herbarium. The leaves were cleaned, air dried for 14 days, and crushed into a coarse powder using a Christy and Norillis' 8 Laboratory Milling Machine.

*Extraction of plant material*

The crude extract of the aerial part of MG was prepared by macerating 1640 g of dried powdered samples in 5 litres of absolute ethanol at room temperature for 72 h with constant shaking. The extract was then filtered using a double-layered muslin cloth and evaporated in vacuo at 40 °C using a rotary evaporator (Buchi, UK). The solution was then concentrated in a water bath at 45 °C. The concentrated extract was then placed inside a desiccator for absolute evaporation of ethanol. The dark green crude extract was stored in a bottle and refrigerated until use.

*Preliminary phytochemical screening*

Preliminary phytochemical analysis was carried out on the crude extract using standard methods (Okoh *et al*., 2024).

*Experimental Animals*

One hundred (100) adult Swiss albino male mice (16 -32 g) were used for this study. They were purchased from a private firm (Korede Farm Ltd., Ikeja, Lagos). They were acclimatized for two weeks and maintained under well-ventilated conditions with a 12-hour light cycle in the Animal House of the Department of Pharmacology, ESUT. The animals were allowed free access to standard mouse cubes (Livestock Feed PLC, Ikeja, Lagos, Nigeria) and water *ad libitum* until 12 h before the experiment. The weights of the animals were measured before administration of the extract. The experimental procedures used in this study conformed to the National Research Council's Guide for the Care and Use of Laboratory Animals, and an ethical approval number: ESUT/AEC/0636/AP498 was issued.

*Chemicals and drugs*

The following drugs and chemicals were used: aspirin (acetylsalicylic acid), morphine, acetic acid, distilled water, formalin, chloroform, absolute ethanol, ammonia, Dragendorff’s reagent, and ferric chloride. The Plant extract and aspirin were suspended in distilled water and administered orally. Acetic acid and formalin were diluted in distilled water (0.6%v/v and 1%v/v, respectively) and injected intraperitoneally.

*Pharmacological tests*

*Acute toxicity*

The acute toxicity of *M. guineensis* aerial crude extract was determined according to the procedure described by Lorke (1983). This method has two phases: phase one and phase two. In phase one, nine albino male mice (20–30 g) were used. Nine animals were divided into three groups of three animals each. Each group was administered different doses (10, 100, and 1000 mg/kg) of the test substance. In phase two, nine animals were equally divided into three groups of three animals each. Each group of animals was administered different doses (1600, 3200, and 5000 mg/kg) of *M. guineensis* crude extract. Mice were fasted for 12 h before the administration of the extract. The control group received water. The treated animals were then monitored for 24 h for toxicity signs and symptoms or any abnormalities. The observation was continued once a day for the next 7 days for manifestations of delayed toxicity (Lorkes, 1983).

*Anti-nociceptive studies*

*Acetic acid writhing test*

The analgesic activity of *M. guineensis* extract was evaluated on acetic acid-induced writhing, as described by Zakaria *et al.* (2009). The mice were divided into five groups of six animals each. The animals were fasted for 24 h before the experiment. Animals in groups 1, 2, and 3 were pre-treated with 50, 100, and 200 mg/kg of the crude extract, respectively, while group 4 received aspirin 100 mg/kg (a standard drug), and group 5 (negative control) received distilled water. One hour before intraperitoneal injection of 0.1 ml of 0.6 % (v/v) acetic acid. After intraperitoneal injection of acetic acid, the number of writhes during the following 30 min was counted. The percentage inhibition of writhing was calculated using the following formula:

$$\%\ inhibition\ =$$

$$Mean\ number\ of\ Writhing\ (Control) - Mean\ number\ of\ Writhing\ (Test) / Mean\ number\ of\ Writhing\ (Control)\ x\ 100$$

*Formalin licking test*

This test was performed using the method described by Hunskaar and Hole (1987) and as reported by (Young *et al*. (2005) and Sarker and Chowdhury (2023). The mice were divided into five groups of six animals each. The animals were fasted for 24 hours before the experiment. Animals in groups 1, 2, and 3 were pretreated with 50, 100, and 200 mg/kg of MG crude extract, while group 4 received morphine (100 mg/kg), a standard drug. Control mice (group 5) received distilled water. One hour before subcutaneous injection of 20 µL of 1 % (v/v) formalin into the right hind paw of the mice. The time (in seconds) spent licking and biting the injected paw was taken as an indication of the pain response. Responses were measured for 5 min after formalin injection (i.e. first phase) and 15-30 min after formalin injection (second phase). The percentage inhibitions of the two phases were calculated using the following formula:

$$\%\ inhibition\ =$$

$$Reaction\ time\ (Control) - Reaction\ time\ (Treatment) / Reaction\ time\ (Control)\ x\ 100$$

Mice used in this experiment were initially screened by placing the animals in turn on a thermostatic hot plate set at 55±1 ◦C, and animals that failed to lick the hind paw or jump (nociceptive responses) within 10 seconds were discarded. Eligible animals were divided into five groups of six mice each, and the pretreatment reaction time for each mouse was determined. Groups 1, 2, and 3 received 50, 100, and 200 mg/kg of the body weight of the plant extract, respectively, orally. Group 4 received distilled water (vehicle), while group 5 received the standard drug morphine 100 mg/kg (o.p). Latency time (in seconds) for each mouse was determined on a hot plate during a maximum period of 30 s, at intervals of 60, 90, 120, and 150 min after administration of the vehicle, extract, or morphine. A post-treatment cutoff time of 30 s was used (Gupta *et al*., 2005). The amount of protection against thermal pain stimulus of each treatment group was calculated for different times using the following formula:

$$\%\ inhibition\ =$$

$$Mean\ of\ Posttreatment - Mean\ of\ Pretreatment / Mean\ of\ Pretreatment\ \ x\ 100$$

*Statistical Analysis*

The results are presented as the mean ± S.E.M. Statistical significance between groups was calculated using analysis of variance (ANOVA), followed by Tukey's or Bonferroni's test. *P < 0.05 and was considered significant. Statistical analysis was performed using the Instant Statistical Package (GraphPad Prism Software, Inc., San Diego, CA, USA).

**RESULTS**

*Extractive yield*

The extractive yield from 1640 g of powdered *Motandra guineensis* aerial parts in absolute ethanol was 110 g (6.71%, w/w).

*Acute toxicity*

There were no signs of toxicity or mortality following oral administration of the extract at doses up to 5000 mg/kg.

*Effect of the extract on acetic acid-induced writhing test*

The extract significantly ($P < 0.05$) and dose-dependently reduced abdominal writhing in mice compared to the negative control (**Figure 1**). The percentage protection was 44.13, 67.09, and 90.05% at doses of 50, 100, and 200 mg/kg, respectively.

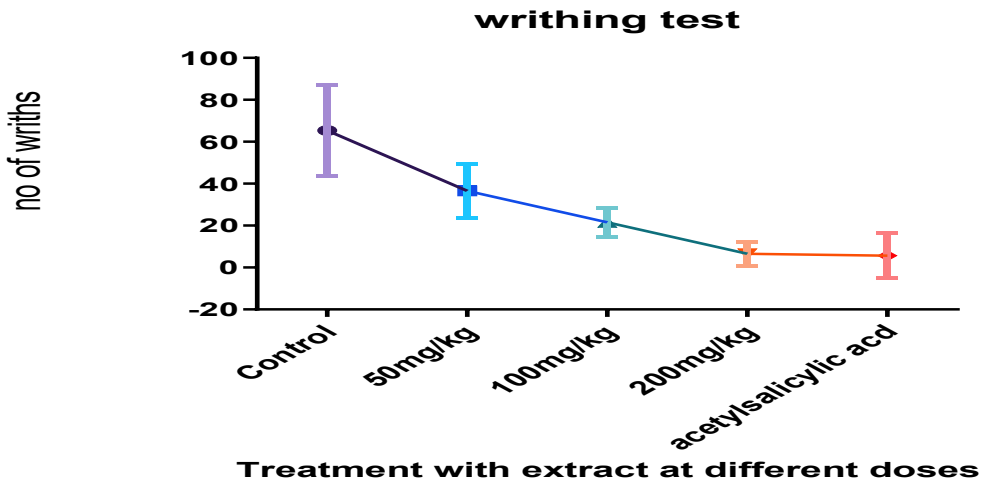


**Figure 1:** Effect of the extract on acetic acid-induced writhing response in mice

*Effect of the extract on formalin-induced pain*

The extract exhibited significant ($P < 0.05$) anti-nociceptive effects at all doses in both the early and late phases of the experiment compared to the control (**Figure 2)**. In the early phase of the experiment, percentage inhibition of the licking was 64.64, 53.74 and 84.78 %, while in the late phase, the percentage inhibition was 63.26, 71.34 and 93.38% at doses 50, 100, and 200 mg/kg, respectively. There was no significant difference ($P < 0.05$) between the dose of 200 mg/kg and the standard drug in both phases.

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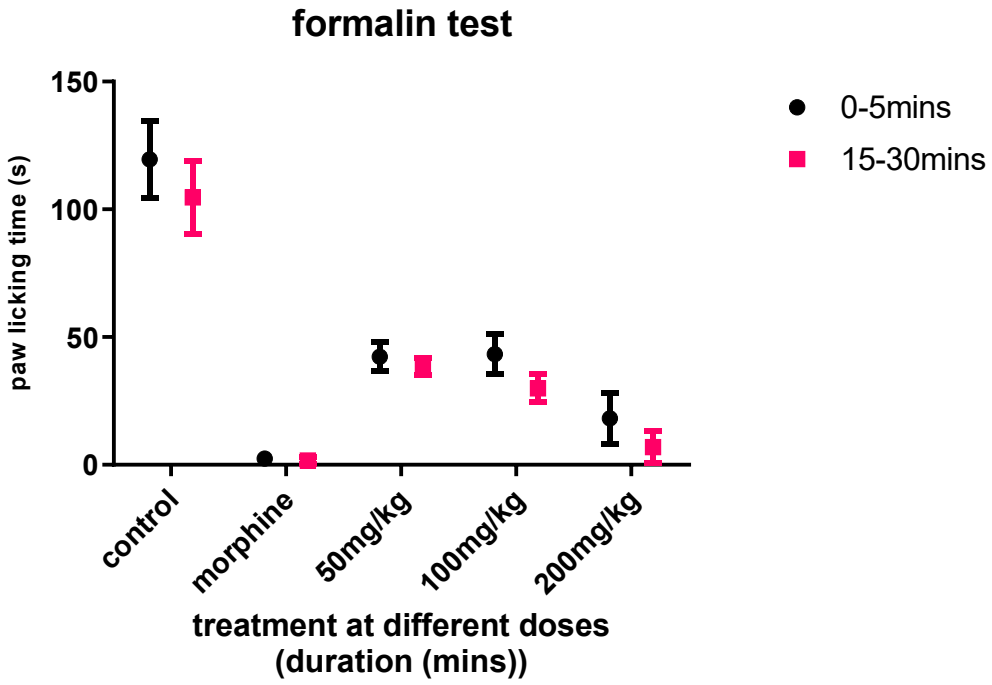


**Figure 2:** Anti-nociceptive effect of the extract on the formalin-induced pain response in mice

*Effect of the extract on hot plate thermal pain*

The extract increased the latency time for nociception in mice above that of the control throughout the observation period (Figure 3). The percentage inhibition at 60, 90, 120 and 150 minutes were 31.52, 25.21, 16.84 and 10.24 % (50 mg/kg), 45.28, 40.92, 30.95 and 23.94 % (100 mg/kg) and 75.77, 71.96, 61.11, and 46.26 % (200 mg/kg), respectively. Maximum latency time was recorded at 200 mg/kg and 60 min, with the highest percentage inhibition (75.69%). Morphine showed the highest percentage of inhibition when compared to the extract.71.96, 61.11 and 46.26 % (200 mg/kg) respectively. Maximum latency time was recorded by 200 mg/kg and at 60 minutes with the highest percentage inhibition (75.69%). Morphine had the highest percentage inhibition when compared to the extract.

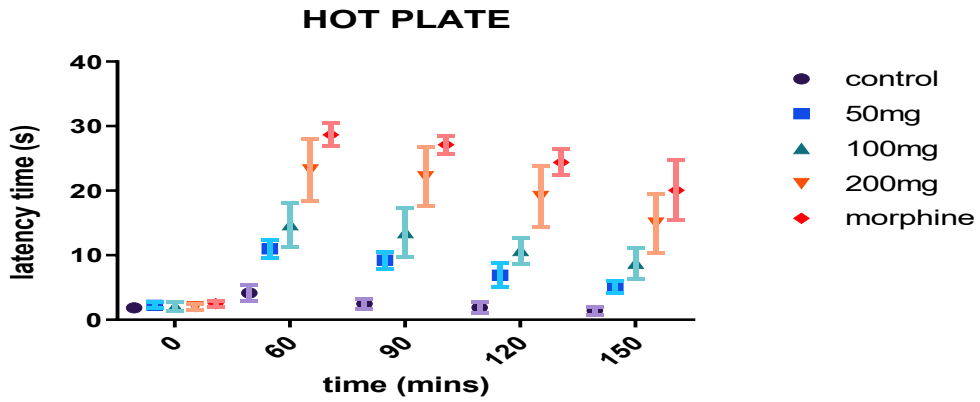


**Figure 3:** Anti-nociceptive effect of the extract on the hot plate test.

*Phytochemical analysis*

Preliminary phytochemical screening revealed the presence of alkaloids, phenols, glycosides, tannins, steroids, and absence of anthraquinones in the extract (Table 1).

**Table 1**: Preliminary phytochemical screening of *Motandra guineensis* crude extract

| Constituents | Crude Extract |
|---|---|
| Saponins | + |
| Alkaloids | + |
| Phenols | + |
| Flavonoid | + |
| Glycoside | + |
| Tannins | + |
| Terpenoids | + |
| Anthraquinones | – |
| Steroids | + |

Key: + = present, - = absent

**Discussion**

Plants are a valuable source of new molecules and are considered an alternative strategy in the search for new drugs. Many investigations on the medicinal uses of plants have been carried out (Momoh *et al*., 2016). Motandra guineensis is widely used in folk medicine in tropical Africa, including Nigeria, and to the best of our knowledge, no report on the anti-nociceptive activity of this plant has been recorded in the literature.

The study of plant species that have traditionally been used for pain relief should still be considered a logical research strategy in the search for new analgesic drugs (Rengasamy *et al*., 2021). Therefore, the current study was carried out to evaluate the anti-nociceptive effect of *M. guineensis* employing acetic acid-induced writhing, hot plate test, and formalin-induced paw licking. These models made it possible to investigate the peripheral as well as the centrally mediated effect of the extract. Acetic acid-induced abdominal constriction is a visceral pain model used as a screening tool for assessing the antinociceptive or anti-inflammatory activity of a new analgesic agent. In this model, acetic acid causes an analgesic response that involves the intraperitoneal release of several mediators, including neurotransmitters and neuromodulators, kinins, histamine, acetylcholine, substance P, and prostaglandins (Garcia-Mayorga *et al*., 2023). These mediators increase vascular permeability, reduce the threshold of nociception, and stimulate the nervous terminal of nociceptive fibers (Alamgeer *et al.*, 2020). In this model, crude *M. guineensis* extract inhibited nociception in a dose-dependent manner. This indicates that the anti-nociceptive action of the extract could be attributed to the reduction in peripheral nociception by the inhibition of prostaglandin release. Subcutaneous injection of formalin in mice induced a biphasic nociceptive response. The early phase (first 5 min) is thought to be caused predominantly by C-fibre activation due to the peripheral stimulus caused by formalin. A second burst of licking behaviour occurs after 15 to 30 min and seems to be related to the inflammatory response elicited by formalin (Sofidiya *et al*., 2022). The crude extract demonstrated antinociceptive activity by blocking both phases of the formalin response. However, the effects of the extract were more pronounced in the early phase. Manifestation in the early phase may be due to a reduction in neurogenic activity. The effects of the extract on both phases suggest peripheral and central mechanisms (Ofeimum *et al*., 2022). The hot plate method was used to study centrally acting analgesics. The test involves spinal and supraspinal pathways and m-opiate receptor agonism in

the regulation (CNS modulation) of the nociceptive response (Gupta *et al*., 2005). The results showed a dose-dependent antinociceptive response. The antinociceptive response in the hot plate model was not as pronounced as that observed in the acetic acid-induced model.

Acute toxicity tests are often performed to determine the dose of materials that can produce harmful effects on a group of test organisms within a short-term duration under controlled conditions. The result of the acute oral toxicity of the extract indicated an LD50 greater than 5000 mg/kg, although some animals exhibited loss of appetite and general weakness, which may be due to the action of the plant extract on the central nervous system (Sofidiya *et al.*, 2022).

The pharmacological activities of medicinal plants are usually attributed to their secondary metabolites present in them. Preliminary phytochemical analysis of *the* aerial parts *of M. guineensis* revealed the presence of phenols, tannins, glycosides, steroids, terpenoids, alkaloids, reducing sugars, and saponins. Studies have suggested that plant materials that contain tannins, alkaloids, flavonoids, and phenolic acids possess analgesic and anti-inflammatory effects in experimental animals (Estella *et al.*, 2022). Phenols have been reported to exert analgesic effects primarily by targeting prostaglandins (Uthayaraghavi, 2022). There are also reports on the antinociceptive activity of tannins (Younis *et al.*, 2022). Alkaloids, terpenoids, and steroids have been reported to inhibit the synthesis of prostaglandin synthase, which stimulates the production of PGE2 (Chen, 2011). Steroids, such as corticosteroids, are commonly used adjuvant analgesics and play an important role in neuropathic and bone pain treatment (Pereira *et al.*, 2015).

Terpenoids found in *Elaeagnus angustifolia* fruit have been reported to be responsible for their anti-inflammatory and antinociceptive activities (Imraish *et al.*, 2024). Georgian et al. also reported the therapeutic and medicinal uses of terpenoids found in plants (Cox-Georgian *et al*., 2019). Therefore, the presence of these phytochemicals in the crude extract of MG may be responsible for the high inhibition of pain.

In conclusion, the results of this study provide evidence for the safety and antinociceptive effect of *Motandra guineensis* aerial parts.

**Declaration of conflicting interest**

The authors declared no conflict of interest

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