NJB, Volume 36 (1) 59-68, June, 2023

ANTI-PSYCHOTIC EFFECTS OF LEAF EXTRACTS OF *PALISOTA HIRSUTA* (Thunb.) K. Schum. (Commelinaceae) AND *COSTUS AFER* Ker-Gawl (Zingiberaceae) ON KETAMINE-INDUCED PSYCHOTIC MICE

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Received 12th August, 2023; accepted 20th September, 2023

ABSTRACT

This study assessed the anti-psychotic activity of the methanol leaf extracts of *Palisota hirsuta* (Thunb.) K. Schum. (Commelinaceae) and *Costus afer* Ker-Gawl (Zingiberaceae) in Ketamine-induced psychotic mice. Antipsychotic activity of the two plant extracts (*Palisota hirsuta* and *Costus afer*) at different doses were assessed using the Ketamine animal model of psychosis. Locomotor and stereotype behaviours were studied in an open-field and transparent chambers, while the force swim test was investigated in a cylindrical glass containing water. From the locomotion test, both doses of the plant extracts tested for activity showed a significant reduction in hyperactivity. *Palisota hirsuta* and *Costus afer* (500 mg/kg) antagonised the stereotypy effect induced by Ketamine in the mice. Furthermore, the two doses of *Palisota hirsuta* (250 and 500 mg/kg) and *Costus afer* at 500 mg/kg had high anti-depressant activity in that the immobility time recorded during the force swim test was reduced significantly. *Palisota hirsuta* extract had a good anti-psychotic effect at both the higher (500 mg/kg) and lower doses (250 mg/kg), whereas *Costus afer* extract had a greater anti-psychotic effect at a higher dose of 500 mg/kg than at a lower dose and these results are similar to those of the positive control drug (Haloperidol).

Key words: *Costus afer; Palisota hirsuta;* anti-psychotic; hyperactivity; immobility

https://dx.doi.org/10.4314/njbot.v36i1.5

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INTRODUCTION

Psychosis is a major debilitating, complex and costly illness that affects 1% of the world's population (Sharma *et al*., 2016) and it has become highly prevalent among the populace due to the ambitious lifestyle, urbanisation, and stressful environment (Yadav *et al*., 2015). The cognitive, positive and negative symptoms are the three main symptoms of psychosis (Parle and Kadian, 2013). Positive symptoms have to do with the loss of contact with reality and consist of hallucinations, delusions and positive formal thought disorder. The negative symptoms have to do with the absence of normal behaviours such as perception of reality, coping with the stressors of life, appropriate coping mechanism, being unable to work and interact with people, while the cognitive symptoms manifest as deficit in attention, learning, memory, concentration and executive functions. Visual hallucinations are the most common psychotic experiences (Gureje *et al.*, 2010).

There is a rise in the incidence of psychiatric disorder and anti-psychotics are known to possess serious side-effects such as weight gain, movement effects like tremors and sedation. Thus, it is necessary to search for a drug with lesser or no side-effect. Also, the increasing cost of pharmaceutical drugs has resulted in the choice of herbal drugs over pharmaceutical drugs; herbal drugs are readily accessible and affordable. *Palisota hirsuta* (Family Commelinaceae) is known locally as Ikpereaturu (Igbo). The decoction of the leafy stems and the whole plant are used in baths for oedema and for urethral discharge, respectively (Sarpong *et al.*, 2017). The roots and leaves are traditionally used to treat infertility; the root alone is used in the management of anaemia, dysentery and rheumatism (Mshana *et al.*, 2000).

Costus afer Ker-Gawl (Family Zingiberaceae) is commonly known as Ginger lily, Spiral ginger and Bush cane; it is locally known as Irekeomede (Yoruba) and Okpeke (Igbo). It is commonly found in humid and monstrous forests and riverside (Ekpo *et al.* 2008). It is highly valued for its anti-diabetic, anti-inflammatory and antiarthritic properties in South-East and South-West Nigeria (Soetan, 2008; Soladoye *et al.*, 2008). The young and tender leaves when chewed are believed to give strength to the weak and dehydrating patient (Anyiam *et al*., 2020).

Herb sellers and herbalists claimed that *Costus afer* Ker Gawl has been used to treat psychosis in an ethno-botanical survey carried out by Sonibare *et al.* (2008). Therefore, it is necessarry to investigate and validate the plant for true anti-psychotic activity. Also, *Palisota hirsuta* was selected for this experiment after its mistaken identity for *Costus afer.* In this study, the locomotion and stereotypy models were used to assess the inhibitory effect of the plant extracts on the positive symptoms of psychosis produced by Ketamine in the experimental animals, while the force swim test model was used to assess the inhibitory effect of the plant extracts at varying doses on the negative symptoms of psychosis in the Ketamine-induced mice.

MATERIALS AND METHODS

Preparation of plant crude extracts

The leaves of *Palisota hirsuta* (PH) were collected in April 2021, from Isore farm, Ipara Remo, Ogun State, while *Costus afer* (CA) leaves were collected from Eleyele, Ibadan. *Palisota hirsuta* plant was identified and authenticated at the Forest Herbarium Ibadan (FHI) by Mr. Adeyemo and the voucher specimen was deposited with voucher number FHI 113303. *Costus afer* was identified and authenticated at the University of Ibadan Herbarium by Mr D. P. O. Esimekhuai, where voucher specimen was deposited with voucher number UIH-23090. The leaves of the two plants were air-dried at room temperature under the shade for two weeks after which they were crisp-dried in a regulated oven at 40°C for 5 min prior to pulverisation. Extraction of the pulverised samples of *Palisota hirsuta* (250 g) and *Costus afer* (350 g) was done separately by maceration in 2.5 L of 100% methanol for 72 hours. The mixture was stirred from time to time using a glass rod to enhance extraction. The extraction procedure was repeated for 48 and 24 hours, respectively, for maximum extraction using the same solvent. The filtrates were pooled and evaporated *in vacuo* using a rotary evaporator and the final drying was done using a regulated water bath at 40ºC. The crude extracts were stored in sterile air-tight bottles.

Experimental Animals

Swiss albino male mice (20 – 25 g) were obtained from the Central Animal House, University of Ibadan. The animals were housed in plastic cages at room temperature and were fed with balanced rodent pellets and water *ad libitum*. The animals were acclimatised for one week before use for the experiments. The animals used in this assay were grouped into six with six animals in each group as follows: Group 1: Ketamine only (30 mg/kg), Group 2: Ketamine + PH extract (250 mg/kg), Group 3: Ketamine + PH extract (500 mg/kg), Group 4: Ketamine + CA extract (250 mg/kg), Group 5: Ketamine + CA extract (500 mg/kg), Group 6: Ketamine + Haloperidol (0.2 mg/kg). The experiment was conducted in accordance with the directions of Guide for the Care and Use of Laboratory Animals. All applicable international, national and/ or institutional guidelines for the care and use of animals were followed. In addition, the experiments were performed in accordance with the University of Ibadan – Animal Care and Use Research Ethics Committee with approval number UI-ACUREC/008-0822/15.

Drug Administration

Haloperidol (0.2 mg/kg) and the methanol extracts (250-500 mg/kg) were administered to the animals using oral cannula according to their weight. They were returned into their plastic containers and left for one hour. Thereafter, Ketamine (30 mg/kg) was administered intraperitoneally to each animal in all the groups based on their weight. This process of administration of the extracts, Haloperidol and Ketamine to the animals was repeated for 10 days consecutively.

Behavioural Assays
Hyper-locomotion and stereotypy were performed based on previously described methods (Arowona *et al*., 2014; Chatterjee *et al.,* 2015). Force swim test was performed according to the method described by Ben-Azu *et al*. (2016) and Sonibare *et al*. (2020).

Locomotion Test
Movements of the mice across the lines in the open field chambers were observed for 5 minutes and recorded using a digital camera. The number of lines crossed was counted and recorded. The process was repeated for every group with intermittent cleaning of the locomotion chambers using 70% alcohol, in order to prevent the olfactory effect left by the previous animal in the chamber.

Stereotypy Behaviour
Immediately after 5 minutes of locomotion session, the animals were introduced into a transparent container having scanty wood shavings. Each mouse was examined for stereotypy behaviour induced by Ketamine for 45 minutes. The following stereotypy behaviours were recorded: 0 (no movement), 1 (head movement), 2 (intermittent sniffing action), 3 (intense licking) and 4 (chewing action). Similarly, there was an intermittent cleaning of the transparent chambers using 70% alcohol.

Force Swim Test
After the animals have been pre-treated for 10 days, the force swim test was then performed 24 hours after the last treatment day. The mice were placed individually in the centre of a transparent glass cylinder, which contained water at room temperature to a depth of 16 cm. Each mouse was placed in the cylinder and swimming activity was recorded for 5 min after 1 min of acclimatisation. Duration of immobility was observed and recorded. The mice were considered immobile when they were floating motionless in water.

Statistical Analysis
Stereotype scores were calculated manually. Dunnet's multiple comparing test was used for the statistical analysis of the results obtained and a p-value of 0.05 showed the plant extracts were significant.

RESULTS

Hyperactivity
The two doses (250 – 500 mg/kg) of *Palisota hirsuta* methanol leaf extract reduced the number of lines crossed by the animals, thereby inhibiting the hyperactivity of the mice significantly at ***$p < 0.05$ as shown in Figure 1. Both 500 mg/kg and 250 mg/kg doses of *Costus afer* leaf extract considerably reduced the number of lines crossed as against animals in Ketamine group (*$p < 0.05$) as shown in Figure 2. This finding is comparable to that of Haloperidol with ** $p < 0.05$ significance.

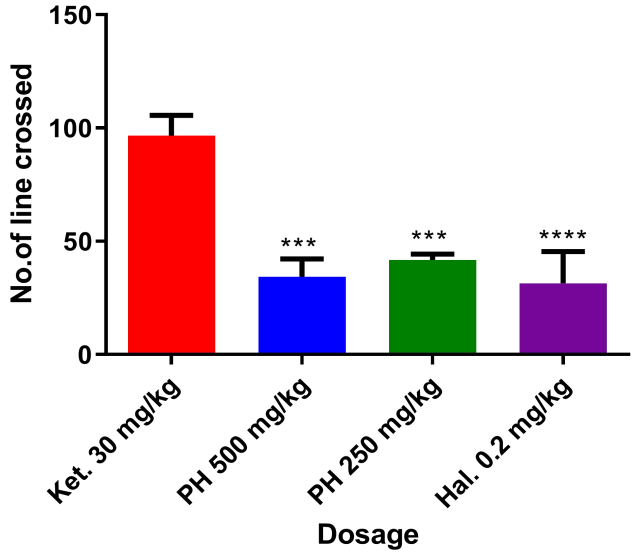

Figure 1: Locomotion result of the effect of methanol crude extract of *Palisota hirsuta* on Ketamine-induced psychosis in mice. Data were mean ± SEM (n = 6). ***p = 0.001, ****p = 0.0001 compared with negative control group (one-way ANOVA followed by Dunnett's multiple comparison post-hoc test). Keys: Ket: Ketamine, PH: *Palisota hirsuta,* Hal: Haloperidol

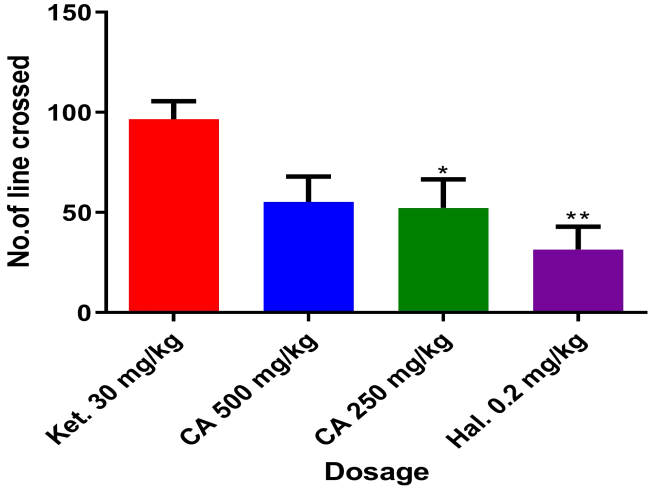

Figure 2: Locomotion result of the effect of methanol crude extract of *Costus afer* on Ketamine-induced psychosis in mice. Data were mean ± SEM (n = 6). *p = 0.04; ** p = 0.003 compared with negative control group (one-way ANOVA followed by Dunnett's multiple comparison post -hoc test). Keys: Ket: Ketamine, CA: *Costus afer,* Hal: Haloperidol

Stereotypy Behaviour

Palisota hirsute has a calming effect at a dose of 500 mg/kg at 45 min of the experiment, which was different from the group that received only Ketamine (Figure 3). *Costus afer* at a dose of 500 mg/kg reduced the animals' stereotypy behaviour at 30 min and 45 min, although the impact was not significant (Figure 4).

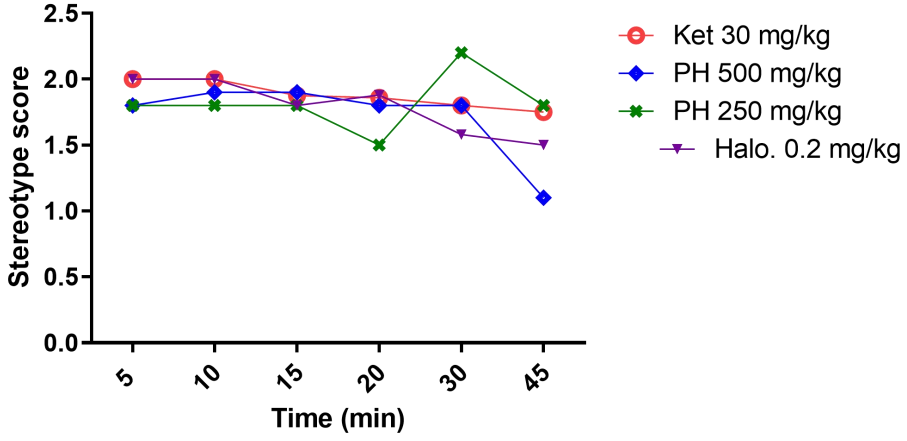

Figure 3: Stereotypy behaviour result of the effect of *Palisota hirsuta* methanol leaf extract 5 min post-Ketamine treatment in a transparent observation chamber. Data were mean ± SEM (n = 6), compared with negative control group (Ket 30 mg/kg) (one-way ANOVA followed by a Dunnett's multiple comparison post hoc.

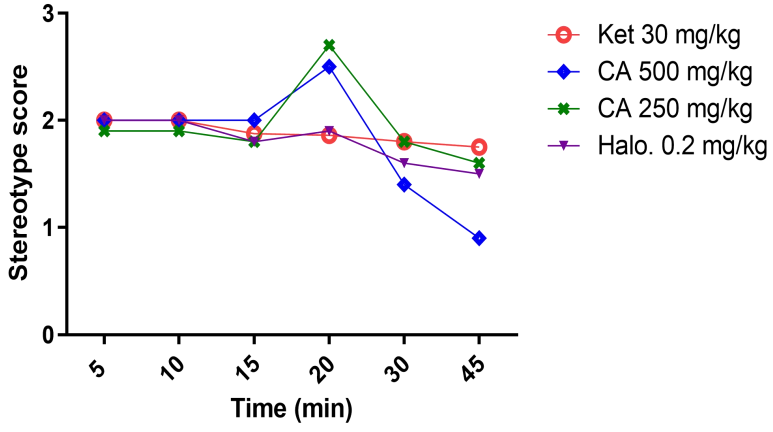

Figure 4: Stereotypy behaviour result of the effect of *Costus afer* methanol leaf extract in mice, 5 min post-Ketamine treatment in a transparent observation chamber. Data were mean ± SEM (n = 6), compared with negative control group (Ket 30 mg/kg) (one-way ANOVA followed by a Dunnett's multiple comparison post-hoc.

Forced Swim Test

The immobility duration observed throughout the experiment was greatly reduced in both doses of *Palisota hirsuta* (250 mg/kg and 500 mg/kg): however, only the 250 mg/kg dose of *P. hirsuta* exhibited a significant anti-depressant effect at *p < 0.05 (Figure 5). Furthermore, *Costus afer* at a dose of 500 mg/kg did not have a depressive effect on the animal. This result was similar to that obtained from the Haloperidol group as shown in Figure 6.

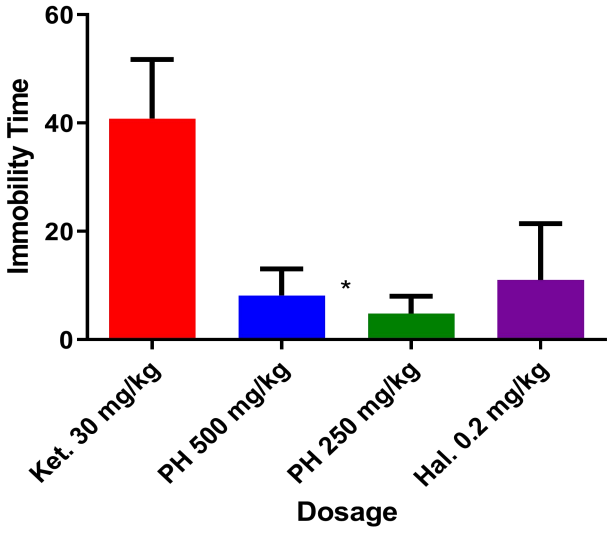

Figure 5: Effect of *Palisota hirsuta* methanol leaf extract on Ketamine-enhanced immobility in mice. *P = 0.04, compared with negative control group.

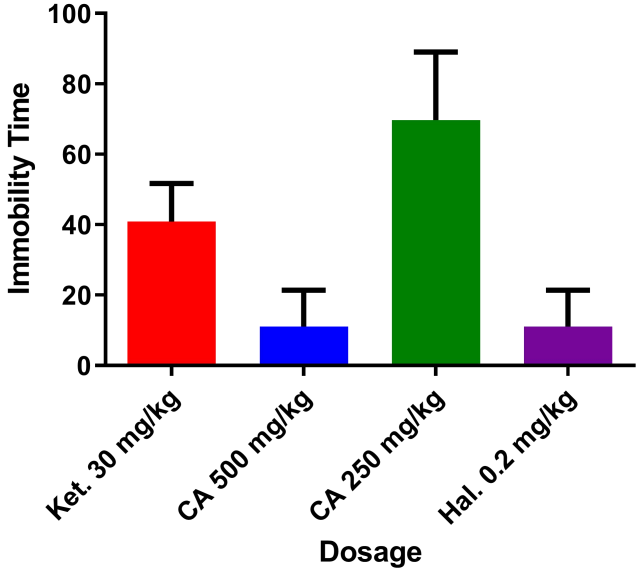

Figure 6: Effect of *Costus afer* methanol leaf extract on Ketamine-enhanced immobility in mice.

DISCUSSION

Medicinal plants are rich in secondary metabolites, which have the ability to interact with the central nervous system (CNS) to produce effects that can be beneficial in the management of psychosis. These secondary metabolites are known to have minimal adverse effects compared to the conventional anti-psychotic drugs, which have numerous disturbing adverse effects (Oritsetimenyin and Lydia, 2021). Following the administration of Ketamine in experimental animals, hyperactivity, stereotypy behaviours, deficits in pre-pulse inhibition (Jentsch and Roth, 1999), social interaction and memory (Becker *et al.*, 2004), which model the positive, negative and cognitive symptoms of schizophrenia, respectively, are reported to be induced via a mechanism that involves the antagonism of the N-methyl-D-aspartate (NMDA) receptor in the animals. According to Oranje *et al.* (2009), in addition to antagonism on the NMDA receptor, Ketamine is known to be a dopamine (D2) receptor agonist. Ketamine inhibits the release of gamma amino butyric acid (GABA) by inhibiting the NMDA receptor present on the GABAergic efferent neurons in the brain. GABA, an inhibitory neurotransmitter, helps in controlling the release of dopamine. Ketamine administration leads to reduction in the release of GABA, thus leading to an increase in dopamine release that stimulates stereotype behaviours and hyperlocomotion, which are positive symptoms of psychosis (Lorrain *et al.,* 2003; Hons *et al.*, 2010). The methanol crude extracts of *Palisota hirsuta* and *Costus afer* significantly reduced the number of lines crossed and induced by Ketamine at different doses. This implies that both extracts may act by increasing the release of GABA, thereby preventing increase in dopamine release.

Stereotypy is a known model for checking positive symptoms of psychosis induced by Ketamine in animals (Vijeepallam *et al.,* 2016; Arowona *et al.*, 2022), showing repetitive head movement, intense licking, chewing and intermittent sniffing. The animals of the negative control group (Ketamine only) especially maintained this stereotype behaviour up till 45 min of experiment as seen in the stereotypy score (Figure 3). *Palisota hirsuta* gave a steady calming effect at a dose of 500 mg/kg from 10 min to 45 min of the experiment. In addition, *Costus afer* at a dose of 500 mg/kg was able to reduce the repetitive behaviour induced by Ketamine at 30 min and 45 min of the experiment. The stereotypy test result showed that both leaves of *Palisota hirsuta* and *Costus afer* (500 mg/kg) demonstrated anti-psychotic activity by reducing the repetitive behaviour produced by Ketamine. Ketamine, an antagonist of NMDA receptor, has been reported to enhance negative symptoms of psychosis such as depression in animals (Patil *et al.*, 2007). Studies have shown that long-term use of Ketamine increases the immobility rate in mice in force swim test (Chatterjee *et al.*, 2011; Sonibare *et al.*, 2020). This study showed that mice administered with Ketamine only were depressant and with no hope to survive. However, *Palisota hirsuta* leaves at a dose of 250 mg/kg gave significant anti-depressant activity and *Costus afer* leaves at 500 mg/kg, although not significant, did not possess depressant property. The immobility result showed that the leaves of both plants possess anti-depressant activity.

Haloperidol, being a typical anti-psychotic drug, has a mechanism of action, which involves the blocking of the post-synaptic dopamine receptors in the mesolimbic system of the brain. This antagonism effect of Haloperidol on the dopamine receptor explains its activity against Ketamine-induced psychosis. Although Haloperidol was meant to give a much lowering anti-psychotic effect, this was not so in this experiment and this might be attributed to the fact that a very low dose of Haloperidol (0.2 mg/ kg) was used instead of a higher dose. Hence the use of higher dose more than 0.2 mg/kg is suggested in future research.

The antagonism of Ketamine-induced hyper-locomotion, stereotype behaviours as well as immobility of the mice in force swim test by PH and CA leaf extracts suggest the anti-psychotic property of the plants. This observation is supported by earlier reports in which *Cissampelos owariensis* (Arowona *et al.*, 2022)*, Terminalia macroptera* (Ior *et al.,* 2021), *Philenoptera cyanescens* (Sonibare *et al.*, 2020) and *Terminalia ivorensis* (Ben-Azu *et al.,* 2016) inhibited Ketamine-induced behavioural models in mice.

CONCLUSION

This study showed that methanol leaf-extract of *Palisota hirsuta* has anti-psychotic effect at both higher and lower doses. *Costus afer* leaf extract at a higher dose of 500 mg/kg showed more anti-psychotic effect when compared to the lower dose. The anti-psychotic property of the leaf extracts of both plants justifies their traditional use in the treatment of mental patients in southwest Nigeria.

ACKNOWLEDGEMENT

The authors acknowledge the Department of Pharmacognosy, University of Ibadan, for giving them the opportunity to carry out the research.

AUTHORS' CONTRIBUTION

Author AOI carried out the laboratory work, analysed, interpreted the findings from the results and wrote the draft manuscript; ITA co-supervised the study, assisted in the laboratory work, analysis and result interpretation. MAS took part in research concept and design, supervised and reviewed the draft manuscript. All authors read and approved the final manuscript.

COMPLIANCE WITH ETHICAL STANDARDS

Ethical statement

The authors declare that the experiments were performed in compliance with the protocol and approval by the Ethics Committee of the University of Ibadan – Animal Use and Care Research Ethics with approval number UI-ACUREC/008-0822/15.

Conflict of Interest: The authors declare that they have no competing interests.

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