Toxicological evaluation, antioxidant and *in vivo* antipsychotic activities of *Acanthospermum hispidum* DC. and *Clerodendrum capitatum* (Willd.) Schumach. & Thonn. leaf extracts

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ABSTRACT

Background: Nature provides substances for the treatment of diseases and ailments including psychosis. *Acanthospermum hispidum* and *Clerodendrum capitatum* have been reported in ethnobotany for the management of psychosis and Central Nervous System Disorder.

Objective: This study evaluated the leaf extracts of *Acanthospermum hispidum* (AH) and *Clerodendrum capitatum* (CC) for their toxicity, antioxidant and antipsychotic activities.

Methods: The methods used were brine shrimp lethality assay for the cytotoxicity and acute toxicity test was conducted using Lorke's method. The antioxidant activity was measured using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), while total phenolic content (TPC) was determined using Folin-Ciocalteu reagent. Antipsychotic property was evaluated in mice using antagonism of ketamine-induced stereotype behaviours and inhibition of hyperlocomotion.

Results: Extract of CC was non-toxic (LC_{50}: 936.3±56.7 µg/mL), while that of AH was relatively toxic (LC_{50}: 402.3±9.1 µg/mL). Acute toxicity (LD_{50}) of >5000.0 and 2154.1 mg/kg b.w. were obtained for CC and AH, respectively. *Acanthospermum hispidum* had higher, free radical scavenging activity (IC50: 33.9±1.6 µg/mL) and TPC (240.3±4.6 µg GAE/g) than CC (IC50: 219.0±12.0 µg/mL; TPC: 117.9±1.3 µgGAE/g). Crude extract of AH (125-500 mg/kg p.o) significantly suppressed stereotype behaviours induced by ketamine (20 mg/kg i.p) in mice as compared with CC (125-500 mg/kg p.o), suggesting the antipsychotic effect of these plant extracts. The extracts further produced a significant reduction in spontaneous motor activities of the mice.

Conclusion: Leaf extracts of *Acanthospermum hispidum* and *Clerodendrum capitatum* demonstrated cytotoxic, antioxidant and significant antipsychotic activity in mice.

Keywords: Antioxidant, Antipsychotic, *Acanthospermum hispidum*, *Clerodendrum capitatum*, Cytotoxicity.

Évaluation toxicologique, activités antioxydantes et antipsychotiques in vivo des extraits de feuilles *d'Acanthospermum hispidum* DC. et *Clerodendrum capitatum* (Willd.) Schumach . & Thonn.

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RÉSUMÉ

Contexte: La nature fournit des substances pour le traitement des maladies et des affections, notamment la psychose. L'*Acanthosperme hispidum* et le *Clerodendrum capitatum* ont été signalés en ethnobotanique pour la prise en charge de la psychose et des troubles du système nerveux central.

Objectif: Cette étude a évalué les extraits de feuilles d'*Acanthospermum hispidum* (AH) et *Clerodendrum capitatum* (CC) pour leur toxicité, leurs activités antioxydantes et antipsychotiques.

Méthodes: Les méthodes utilisées étaient un test de létalité des artémias pour la cytotoxicité et un test de toxicité aiguë a été réalisé en utilisant la méthode de Lorke. L'activité antioxydante a été mesurée à l'aide du 2, 2-diphényl-1-picrylhydrazyl (DPPH), tandis que la teneur phénolique totale (TPC) a été déterminée à l'aide du réactif Folin- Ciocalteu. La propriété antipsychotique a été évaluée chez la souris en utilisant l'antagonisme des comportements stéréotypés induits par la kétamine et l'inhibition de l'hyperlocomotion.

Résultats: L'extrait de CC était non toxique (LC_{50} : 936,3 ± 56,7 µg/mL), tandis que celui de AH était relativement toxique (LC_{50} : 402,3 ± 9,1 µg/mL). Une toxicité aiguë (LD_{50}) de > 5000,0 et 2154,1 mg/kg p.c. a été obtenue pour CC et AH, respectivement. *Acanthosperme hispidum* avait une activité de piégeage des radicaux libres (IC50 : 33,9 ± 1,6 µg/mL) et un TPC (240,3 ± 4,6 µg GAE/g) plus élevés que le CC (IC50 : 219,0 ± 12,0 µg/mL ; TPC : 117,9 ± 1,3 µ gGAE /g). L'extrait brut d'AH (125-500 mg/kg p.o) a supprimé de manière significative les comportements stéréotypés induits par la kétamine (20 mg/kg ip) chez la souris par rapport au CC (125-500 mg/kg p.o), suggérant l'effet antipsychotique de ces extraits de plantes. Les extraits ont également produit une réduction significative de l'activité motrice spontanée des souris.

Conclusion: Les extraits de feuilles d'*Acanthospermum hispidum* et de *Clerodendrum capitatum* ont démontré une activité cytotoxique, antioxydante et antipsychotique significative chez la souris.

Mots-clés: Antioxydant, Antipsychotique, *Acanthospermum hispidum*, *Clerodendrum capitatum*, Cytotoxicité.

INTRODUCTION

Mental illness is a medical condition that disrupts a person's thinking, mood, emotions, ability to relate to others and day-to-day activities while depression, obsessive compulsive disorder, anxiety, phobias and post-traumatic stress disorders are some of the less severe mental disorders.[1] Schizophrenia, multiple personality disorder and manic-depressive psychosis are more severe mental disorders that distort reality due to hallucinations and delusions. There are several mental illnesses, which could be categorized as neurosis or psychosis. Psychosis (Schizophrenia) is a heterogeneous chronic neurological disease that affects an average of 1% of the world's population.[2] The treatment for mental illness could be possible with behaviour modification, counseling, suggested therapies and drugs.[3] Antipsychotic drugs also termed neuroleptic drugs are the cornerstone of management and treatment of schizophrenia, they are effective in the treatment of hallucinations, delusions, and thought disorder.[3] These conventional antipsychotics have been reported with adverse effects such as Diabetes and weight gain, hence, the search for safer, novel, neuroleptic agents with higher efficacy. Nature provides materials for the treatment of diseases and ailments including psychosis. The antipsychotic property of some medicinal plants have been reported and documented.[4-8] The two plants selected for this study have been reported in ethnobotany for the management of psychosis and Central Nervous System Disorder.[9,10]

Several medicinal applications of *A. hispidium* include its use as treatment of jaundice, malaria, vomiting, cephalgias, head-ache, abdominal pain, convulsions, stomach ache, memory disorder, constipation, eruptive fever, snake bite, epilepsy, gonorrheal, hepato-biliary disorders, malaria, microbial infections and viral infections.[11,12] Reported biological activities of *A. hispidum* include abortifacient and teratogenic activity,[13] antiviral,[14] antimicrobial,[15] antiplasmodial,[16] antidiarrheoal,[17] antitumour,[18] antibacterial,[19] and antitrypsomal.[20]

Clerodendrum capitatum (Willd.) Schum. and Thonn. is a flowering plant from the family Lamiaceae, commonly called Glory bower. It is locally named "Abosa" in Yoruba, "Mashayi" in Hausa speaking parts of Nigeria and Gung in Sudan.[21] The roots of this plant are used traditionally in the management of male erectile dysfunction in Sudan,[22] while in Nigeria, this plant is used to treat fever, Diabetes mellitus, obesity, diarrhoea, asthma, pyreticosis, tuberculosis and hypertension.[23] In the survey conducted by Sonibare *et al*.,[9] it was documented that the dry powder of the leaves of *Clerodendrum capitatum* is mixed with black soap and used as antipsychotic agent to wash the head of a psychotic patient with alcohol. However, there is no literature report on the scientific validation of the documented folkloric use of the two plants in treating psychosis. Thus, this study seeks to evaluate the antipsychotic effect of the leaf extracts of the two plants in ketamine models of psychosis in mice. Cytotoxicity, acute toxicity and antioxidant activities were also assessed.

METHODS

Experimental animals

Swiss albino male mice (18 - 26 g) were obtained from the Central Animal House, University of Ibadan. The animals were housed in plastic cages under 12 h light/dark cycle at room temperature and were fed with balanced rodent pellets and water ad libitum. They were acclimatised for 2 weeks before use for experiments. The experiments were performed according to the rules of National Institutes of Health Guide for care and use of laboratory animals.

Plant collection and identification

The leaves of *Acanthospermum hispidum* and *Clerodendrum capitatum* were collected from Federal College of Forestry in Ibadan and Ipara Remo in Oyo and Ogun States, respectively in May 2017. The identification of the plants was done at the Forest Herbarium Ibadan (FHI), at Forestry Research Institute of Nigeria (FRIN) located at Jericho, Ibadan, Oyo State, Nigeria.

Preparation of extract

Plant samples were air-dried, pulverized and macerated in absolute ethanol. The filtrates were evaporated using the rotary evaporator (RE300DB) at 40^{o}C and the resulting crude extracts were stored in the refrigerator for further use.

Qualitative phytochemical screening

Plant samples were subjected to various phytochemical tests to identify the secondary metabolites present using standard procedure[24]. The metabolites tested for include: tannins, saponins, flavonoids, anthraquinones, cardiac glycosides, steroids, terpenes and alkaloids.

Toxicological evaluation

Brine shrimp lethality assay

The methodology of McLaughlin *et al*.,[25] was used for the hatching of brine shrimp eggs. Briefly, 300 mL of natural seawater was used in the hatching chamber after which little quantity of the brine shrimp egg was added to the covered part of the divider tank. The other part was exposed to light. The set-up was left for 48 h for complete hatching. The plant crude extracts dissolved in 2-3 drops of Tween 80 were prepared in concentration ranging from 1 - 1000 µg/mL. After 48 h of hatching, ten shrimps were counted into each vial and were placed under the light. Experiment was set up in triplicates with control using sea water only. After 24 h, the number of surviving and dead nauplii were counted and recorded with the aid of a magnifying lens. The LC_{50} values (the concentration of extract causing 50% mortality of nauplii larvae) were determined using Graph pad Prism.

Determination of acute toxicity

The method described By Lorke 24 was used to determine the LD_{50}, which is the index of acute toxicity. Male Albino mice weighing between 20 - 25 g were used for the study. This method involved an initial dose finding procedure, in which the animals were divided into three groups of three animals. Doses 10, 100 and 1000 mg/kg body weight (b.w.) were administered per oral (p.o), such that one dose is for each group. The treated animals were monitored for 24 h mortality. From the results of the above step, three different doses of the extracts; 1600, 2900 and 5000 mg/kg b.w. were chosen and administered p.o, respectively to three groups of one mouse per group. The treated animals were monitored for 24 h mortality. The LD_{50} was calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death.

Antioxidant assay

DPPH free radical scavenging activities

The free radical scavenging activity of the crude extracts were evaluated according to the methods described in literature[27,28] with slight modifications. Briefly, 1 mL methanol solution of test sample and standard (ascorbic acid and gallic acid) at different concentrations (200, 100, 50, 25, 12.5, 6.25 and 3.13 µg/mL) were mixed separately with 3 mL (0.004%) of freshly prepared 1,1-diphenyl-2-picryl-hydrazyl-hydrate (DPPH-Sigma Aldrich). In the control, 1 mL methanol replaced the test sample. The reaction mixtures were incubated at 27 °C and allowed to react for 30 min in the dark after which the absorbance was measured at 517 nm using UV-VIS spectrophotometer (Spectrum lab 752S, China) and converted into percentage of antioxidant activity. The results were recorded in triplicate. The concentration of sample required to scavenge 50% of the DPPH free radical (LC_{50}) was determined from a calibration curve by linear regression.[29] The percentage of inhibition of DPPH (%) was calculated as follows:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Estimation of total phenolic content

Total phenolic content was measured using Folin-Ciocalteu method previously reported in literature with slight modification.[30,31] Folin-Ciocalteu reagent (5 mL) diluted ten-fold, was introduced into 1 mL each aliquot of the extracts (300 µg/mL) and was allowed to stand for 3 min after which 4 mL of 7.5% Na_2CO_3 solution in distilled water was added to the mixture. Experiment was prepared in triplicate. Content of the mixture was thoroughly mixed and incubated at 27 ^{o}C for 30 min. Blank was set up with 1 mL methanol, 5 mL of Folin Ciocalteu reagent and 4 mL of 7.5% Na_2CO_3. Absorbance of mixture after 30 min of incubation was read at 765 nm using UV-VIS spectrophotometer (Spectrumlab 752S, China). A linear dose response regression curve was generated using absorbance reading of gallic acid (12.5- 0.39 µg/mL) at wavelength of 765 nm. Result of TPC was expressed as mg GAE/g of dry weight of extracts. The TPC in the plant extract was calculated using the formula below:

$$\text{TPC} = \frac{CV}{M}$$ where;

TPC is the total phenolic contents mg GAE/g of dry weight of extracts, C is the concentration of equivalent gallic acid established from calibration curve µg/mL, V is the volume of extract (mL) and M is the weight of plant extract (0.03 g).

***In vivo* antipsychotic models**

Antagonism of where ketamine-induced stereotyped Behaviours

The antipsychotic effect of the plant crude extracts was assessed using antagonism of Ketamine-induced stereotyped behavioural paradigm in mice predictive of human psychosis as previously described by Bourin *et al*,.[32] Eight groups of mice (six per group) were pre-treated orally with *Acanthospermum hispidum* or *Clerodendrum capitatum* leaf ethanol extracts (125, 250 or 500 mg/kg b.w), Risperidone (1 mg/ kg) and Distilled water 1 h before Intraperitoneal (i.p) injection of Ketamine (20 mg/kg). The mouse was placed in a transparent observation chamber (20 cm × 20 cm × 23 cm), 5 min after receiving the ketamine injection. Thereafter, stereotype behaviours were recorded for a period of 2 min at 5, 10, 15, 30, 45, and 60 min. Stereotype behaviours were scored as 0 = absence of stereotype behaviour, 1 = presence of stereotyped movements of the head, 2 = intermittent sniffing, 3 = chewing, and 4 = intense licking. Thereafter, the transparent chamber was thoroughly wiped with cotton wool soaked with 70% ethanol. Total stereotypy scores were determined manually. Behavioral tests were performed between 10 a.m. and 4 p.m.

Inhibition of hyperlocomotion induced by ketamine

The open field test was also used to screen the effect of plant crude extracts on hyperlocomotion in mice induced with ketamine (20 mg/kg) as described by Brown *et al*.,[33] with slight modification. Groups of mice (six per group) were treated orally with *Acanthospermum hispidum* or *Clerodendrum capitatum* leaf ethanol extract (125, 250 or 500 mg/kg b.w). After 1 h, each mouse received i.p injection of ketamine (20 mg/kg) and was placed immediately at the centre of an open field chamber. The total number of lines crossed and duration of ambulation(s) were recorded for 5 min using a digital camera after Ketamine administration. Ambulation is the time the mouse spent in sedentary positions without movement. The open field apparatus was immediately wiped with cotton wool soaked with 70% ethanol.

Statistical analysis

All experiments with IC50 and LC_{50} were conducted in triplicate with all values represented as mean ± SEM (standard error mean). The stereotype score was calculated manually. Thereafter, statistical analysis was carried out using Graph pad Prism, version 5.01. One-way ANOVA at p = 0.05 was used to test the significant difference of data followed by Dunnett multiple comparison test.

RESULTS

Extraction yield

The pulverized leaves of *Acanthospermum hispidum* (800 g) and *Clerodendrum capitatum* (1200 g) yielded 8.4 % and 11.7 % of extracts respectively (Table 1).

Table 1: The Percentage Yield of *Acanthospermum hispidum* and *Clerodendrum capitatum*

Plants	Weight of Powder (g)	Weight of extract (g)	% Yield (w/w)
Acanthospermum hispidum	800	66.8	8.4
Clerodendrum capitatum	1200	139.9	11.7

Phytochemical analysis

The phytochemical screening of pulverized *A. hispidum* and *C. capitatum* leaves revealed the presence of alkaloids, flavonoids, tannins, steroids and absence of terpenoids in both plants (Table 2).

Table 2: Phytochemical constituents of powdered leaf extracts of *Acanthospermum hispidum* (AH) and *Clerodendrum capitatum* (CC)

TESTS	OBSERVATIONS	INFERENCES	INTENSITIES	
			AH	CC
Alkaloids;				
Wagner	Cream precipitate	Presence of Alkaloids	+++	+
Mayer	Yellow precipitate	Presence of Alkaloids	+++	+++
Dragendorff's	Reddish-brown	Presence of Alkaloids	+++	++
Steroids	Deep green coloration	Presence of Phytosteroids	++	+
Flavonoids	Deep green coloration	Presence of Flavonoids	++	+++
Tannins/phenols	Intense colouration of blue-black or green	Presence of Tannins/Phenols	++	+
Saponins	Formation of foam to a length of 1 cm	Presence of Saponins	+++	+++
Terpenoids	Deep red colouration	Presence of Terpenoids	-	-
Glycosides	Yellow colour with white precipitate	Presence of Glycosides	++	++

+++: Instantaneous and Abundant; ++: Abundant; +: Sparingly present; -: Absent

Brine shrimp lethality assay

The minimum lethal concentration (LC_{50}) of *Acanthospermum hispidum* and *Clerodendrum capitatum* showed 402.3 and 936.3 µg/mL, respectively (Table 3).

Table 3: Brine shrimp lethality assay (BSLA) of leaf extracts of *Acanthospermum hispidum* (AH) and *Clerodendrum capitatum* (CC)

Samples	LC_{50} (µg/mL)
Acanthospermum hispidum	402.3±9.1
Clerodendrum capitatum	936.3±56.7
Cyclophosphamide	224.7±0.4

Acute toxicity study

The acute toxicity result revealed that animals treated with *Acanthospermum hispidum* leaf extract showed mortality 24 h after the experiment at the doses of 2900 and 5000 mg/kg and none of the rats treated with different dosages up to 5000 mg/kg dose of the *Clerodendrum capitatum* extract showed mortality after 24 h and 7 days of observation (Table 4).

Table 4: Determination of LD_{50} of leaf extracts of *Acanthospermum hispidum* and *Clerodendrum capitatum*

Concentration (mg/kg b. w.)	Phases	No of mice	Mortality	
			A. hispidum	*C. capitatum*
Control	1	3	0/3	0/3
10		3	0/3	0/3
100		3	0/3	0/3
1000		3	0/3	0/3
1600	2	1	0/1	0/1
2900		1	1/1	0/1
5000		1	1/1	0/1

Estimation of antioxidant property

The DPPH free radical scavenging activity revealed a minimum inhibitory concentration of 33.9±1.6 and 219.0±12.1 µg/mL for *A. hispidum* and *C. capitatum* leaves extracts, respectively. The IC_{50} value for ascorbic acid was 6.9±0.2 µg/mL. The total phenol content of both *A. hispidum* and *C. capitatum* extracts were 240.3±4.6 and 117.9±1.3 µg gallic acid equivalent of per gram extracts, respectively as presented in Table 5.

Table 5: DPPH Free radical scavenging activity and total phenolic content of leaf extracts of *Acanthospermum hispidum* and *Clerodendrum capitatum*

Samples	IC_{50} for DPPH (µg/mL)	TPC (µgGAE/g)
Acanthospermum hispidum	33.9±1.6	240.3±4.6
Clerodendrum capitatum	219.0±12.1	117.9±1.3
Ascorbic acid	6.9±0.2	-

Antipsychotic study

Antagonism of ketamine-induced stereotyped behaviours

In this study, extract of *A. hispidum* gave a tranquilizing effect at all administered doses (125 - 500 mg/kg), while the dose of 125 mg/kg for *C. capitatum* extract was consistent in reducing the repetitive behaviours observed in the animals (Figure 1 and 2).

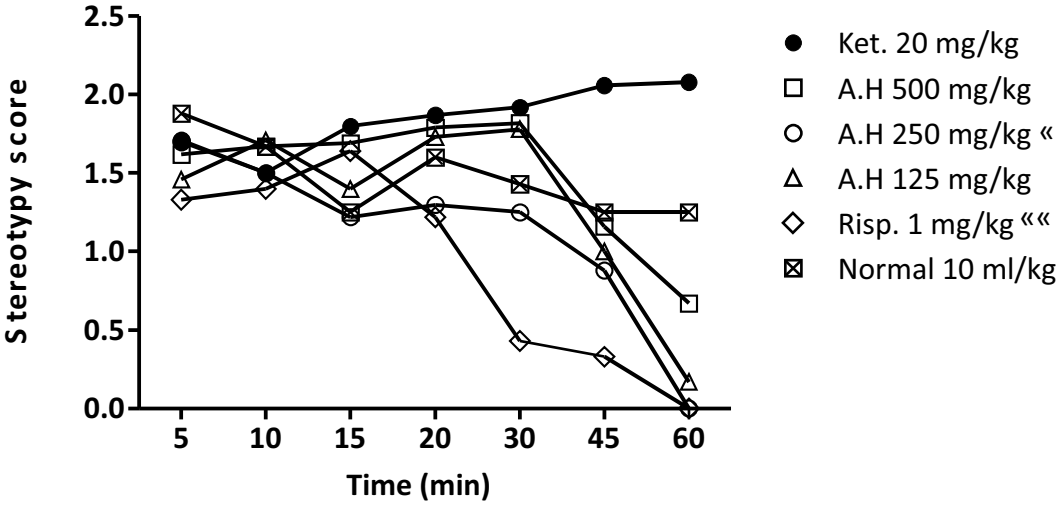

Figure 1: Effect of *Acanthospermum hispidum* crude extract and Risperidone on stereotype behaviour in mice, n = 6; one-way ANOVA followed by Dunnett post hoc test for multiple comparison, *p < 0.05, **p < 0.01

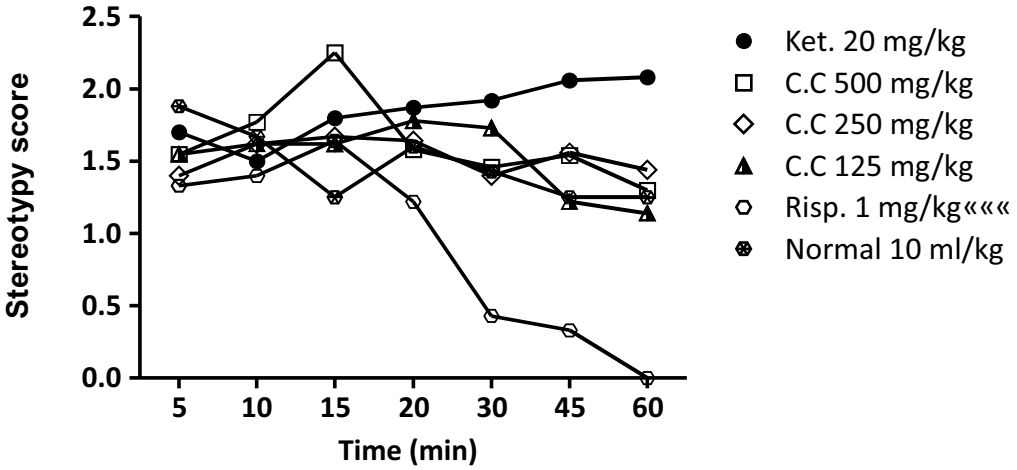

Figure 2: Effect of *Clerodendrum capitatum* crude extract and Risperidone on stereotype behaviour in mice, n = 6; one-way ANOVA followed by Dunnett post hoc test for multiple comparison, ***p < 0.001

Hyperlocomotion study

The hyperlocomotion study revealed that 125 and 250 mg/kg of *A. hispidum* extract significantly reduced the hyperactivity in the mice (Figure 3), while *C. capitatum* extract gave a dose dependent decrease in the hyper activity of the animals, with 125 mg/kg being more active as compared to other doses (Figure 4). The dose of 125 mg/kg was significantly active in the ambulation study for both plants (Figure 5 and 6).

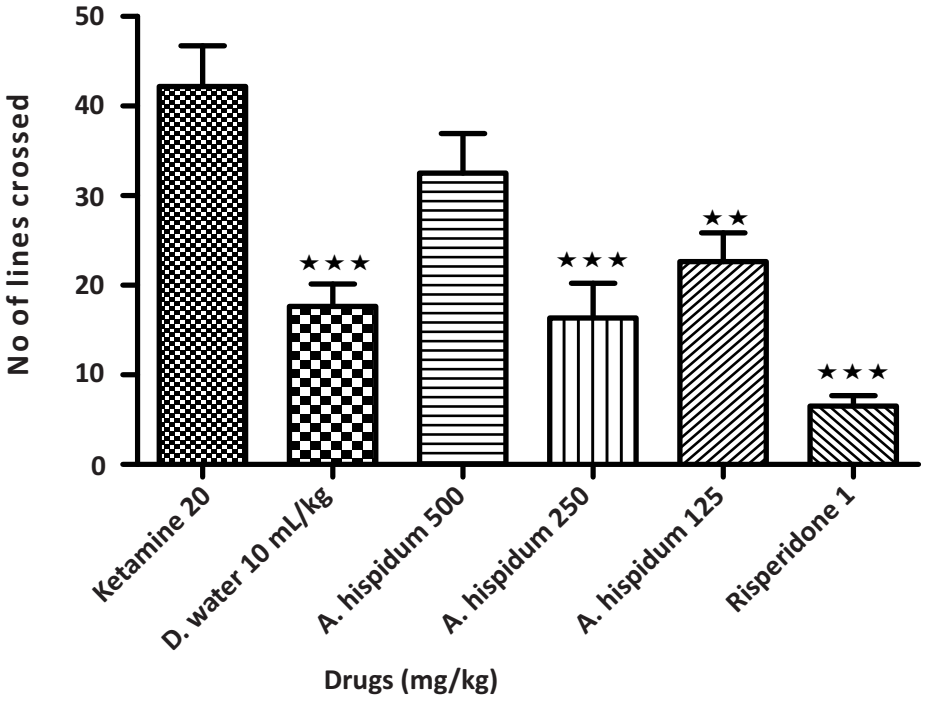

Figure 3: Effect of *Acanthospermum hispidum* crude extract and Risperidone (1 mg/kg b.w) on hyperlocomotion in mice. Each bar represents Mean ± SEM, n = 6; one-way ANOVA followed by Dunnett post hoc test for multiple comparison, **p < 0.01, ***p < 0.001

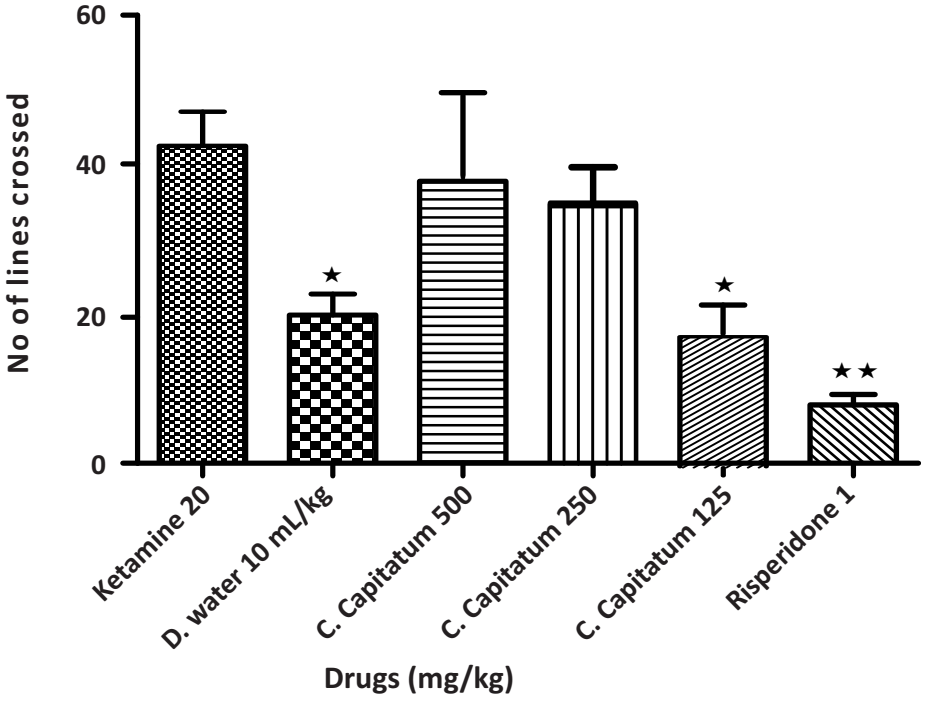

Figure 4: Effect of *Clerodendrum capitatum* crude extract and Risperidone (1 mg/kg b.w) on hyperlocomotion in mice. Each bar represents Mean ± SEM, n = 6; one-way ANOVA followed by Dunnett post hoc test for multiple comparison, *p < 0.05, **p < 0.01

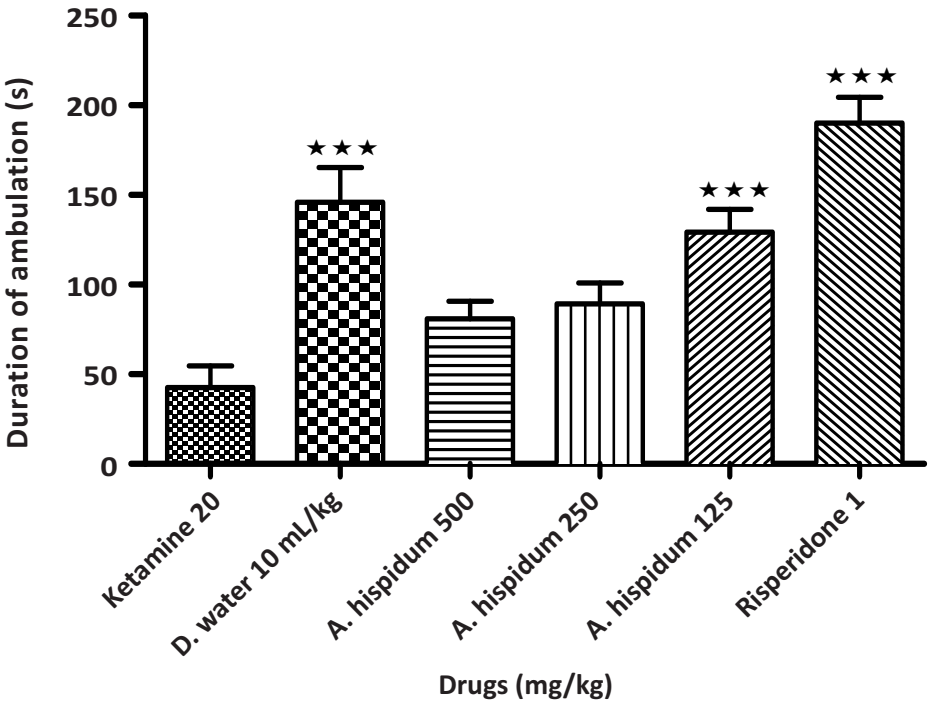

Figure 5: Effect of *Acanthospermum hispidum* crude extract and Risperidone (1 mg/kg b.w) on ambulation in mice. Each bar represents Mean ± SEM, n = 6; one-way ANOVA followed by Dunnett post hoc test for multiple comparison, ***p < 0.001

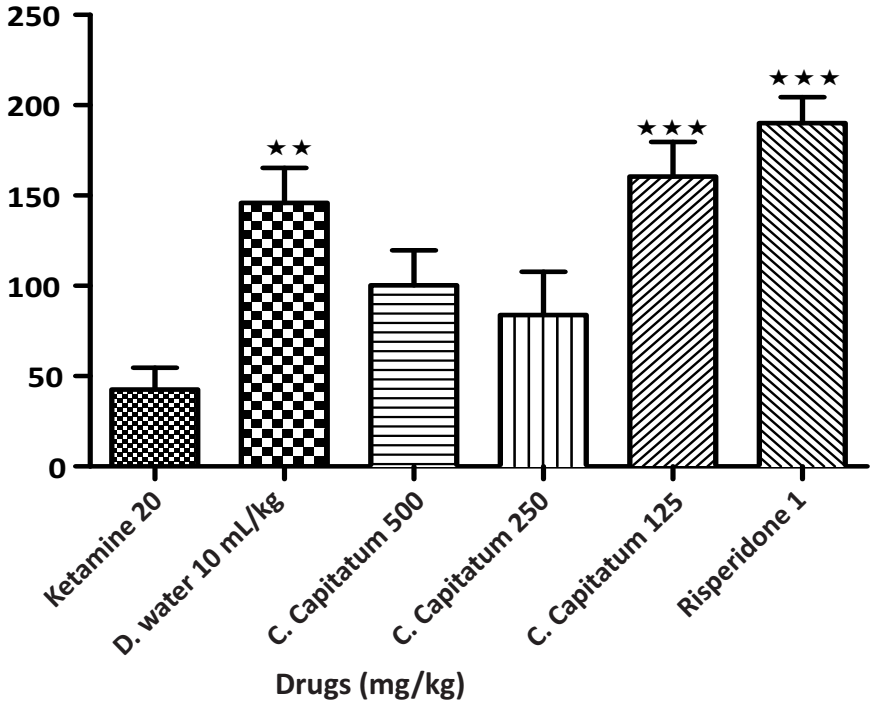

Figure 6: Effects of Clerodendrum capitatum crude extract and Risperidone (1 mg/kg b.w) on ambulation in mice. Each bar represents Mean ± SEM, n = 6; one-way ANOVA followed by Dunnett post hoc test for multiple comparison, **p < 0.01, ***p < 0.001

DISCUSSION

The extraction yields of the selected medicinal plants are recorded in Table 1. The results showed that 0.8 kg and 1.2 kg of *Acanthospermum hispidum* and *Clerodendrum capitatum* yielded 8.4% and 11.7%, respectively.

Qualitative phytochemical screening of both plant materials revealed the presence of alkaloid, saponin, steroids, flavonoids, tannins in appreciable amount, while terpenoid was absent from both plant samples (Table 2).

The results of the effect of the two plant extracts on *A. salina larvae* are expressed as LC_{50} (lethality concentration) and presented in Table 3. *Acanthospermum hispidum* had an LC_{50} value of 402.3±9.1 µg/mL, while *C. capitatum* was 936.3±56.7 µg/mL compared to that of cyclophosphamide, which was 224.7±0.4 µg/mL. The LC_{50} values suggest that CC is relatively non-toxic, while AH is relatively toxic. This agrees with an earlier report by Ogbole *et al*.[34] on the relative toxicity of methanolic extract of *Acanthospermum hispidum* using BSLA with an LC_{50} value 183.7 µg/mL.

The acute toxicity studies of both plants revealed that the LD_{50} of CC was >5000.0 mg/kg, while AH had an LD_{50} value of 2154.1 mg/kg b. w (Table 4). This result further supports the BSLA result suggesting the relative non-toxic nature of CC and relative toxic nature of AH.

Both plant extracts showed the capacity to scavenge the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical (Table 5). *Acanthospermum hispidum* with IC_{50} value of 33.9±1.6 µg/mL exhibited a better DPPH free radical scavenging activity than *Clerodendrum capitatum* (IC_{50}: 219.0±12.1 µg/mL), while ascorbic acid had an IC_{50} value of 6.9±0.2 µg/mL. The antioxidant activity of the crude extracts of *Acanthospermum hispidum* and *Clerodendrum capitatum* leaves may be responsible for its beneficial antipsychotic action as an adjuvant. Also, the total phenolic content of AH (240.3±4.6 µg GAE/g) was higher than that of CC (117.9±1.3 µgGAE/g). Phenolics and polyphenols have been known for their wide spectrum of physiological activities such as antioxidants, antimicrobial and antitumor activities. Phenolic compounds contribute to the overall antioxidant effects of medicinal plant samples mainly due to their redox properties, which enable them to act as reducing agents, hydrogen donors, or metal chelators.

The administration of the crude extracts of AH and CC at different concentrations ranging from 125-500 mg/kg b.w. orally 1 h before the induction of psychosis using intraperitoneal injection of ketamine revealed that the crude extracts were effective in antagonising the stereotype behaviour in mice as reflected by reduced sniffing, head movement, intermittent licking and chewing. Risperidone and ethanolic extracts of AH and CC reduced ketamine-induced stereotype behaviours in mice compared to the control group (Figures 1 and 2). *Acanthospermum hispidum* administered at 250 mg/kg had a better activity (significant at *$p<0.05$) when compared with CC (125 - 500 mg/kg). However, the extent of decrease of the stereotypy activity for *Acanthospermum hispidum* and *Clerodendrum capitatum* was less as compared to the standard drug risperidone (**$p<0.01$). This kind of outcome was indicative of a possibility that the test extracts may be decreasing the Dopamine levels in the brain as is the case for the standard drug risperidone. Thus, the results suggest that these plant extracts possess anti-dopaminergic activity.

For the hyperlocomotion study, Oral administration of the crude extracts of the two plant extracts resulted in significant ($p<0.05$, $p<0.01$ and $p<0.001$) decrease in locomotor activity at different doses (Figures 3 and 4). This shows the CNS depressant activity of different concentrations of AH and CC. Therefore, both the test extracts and the standard drug altered the ketamine induced increase in locomotor activity. The effect of the plant extracts on locomotor activity could suggest that the extracts may be acting on other neurotransmitter systems like glutamatergic or serotonergic systems. This particular model was suggestive of the effectiveness of the test extracts to alleviate the negative symptoms of schizophrenia. It is once again confirmed that risperidone has effect on the negative symptoms of schizophrenia.

In the ambulation study, the ethanol extracts of both AH and CC leaves significantly ($p<0.01$ and $p<0.001$) decreased ambulation in mice as shown in Figures 5 and 6. A central role for D2 receptor occupancy in antipsychotic action is now well established, buttressed by neuroimaging studies using positron emission tomography and single photon emission computed tomography.[35] However, the importance of dopamine receptors in the treatment of psychosis does not by itself constitute proof of the involvement of dopamine in psychosis. Administration of AH and CC may increase the number of dormant receptors, hence resulting in

decrease in dopamine turnover in extracellular spaces in the brain.[36] It derives that alkaloids, tannins and flavonoids are present in the AH and CC, which may possibly be responsible for their psychopharmacological action. However, the dopamine lowering activity for both extracts was less, when compared to risperidone. Nevertheless, the effectiveness of both extracts irrespective of the dose was also a significant observation in this study. It is also well established that it is extremely essential for a molecule to have dopamine antagonistic activity to have any kind of neuroleptic activity. Even the atypical antipsychotic drugs, need to have a certain degree of dopamine reducing activity apart from its interaction with other receptors viz. Serotonergic, Alpha adrenergic or Glutamatergic. So, it can be stated that by virtue of the dopamine lowering effect of the plant crude extracts, they possess the antipsychotic effects.

This study confirms that the leaf extracts of *Acanthospermum hispidum* and *Clerodendrum capitatum* demonstrated cytotoxic, antioxidant and significant antipsychotic activities in mice.

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