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PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTISCHIZOPHRENIC ACTIVITY OF METHANOL LEAF EXTRACT OF *Hymenocardia acida* Tul. (Phyllantaceae)

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ABSTRACT

Schizophrenia is a severe brain disorder marked by distortions in cognition, emotion, language, perception, and thought, affects an estimated 20 million individuals globally. In Nigeria, where a significant population relies on herbal medicines, including those derived from *Hymenocardia acida*, to address mental health needs, there have been traditional claims regarding the plant's efficacy in treating schizophrenia. This study aims to screen phytochemical constituents and establish the anti-schizophrenic potential of the methanol leaf extract of *H. acida*. Phytochemical screening revealed the presence of flavonoids, tannins, terpenoids, and steroids in the methanol leaf extract. The Lorke method determined the intraperitoneal median lethal dose (LD_{50}) of the extract to be above 5000 mg/kg in mice. The anti-schizophrenic activity was assessed through various tests, including apomorphine-induced stereotypic behavior, swim-induced grooming, and haloperidol-induced catalepsy. Results indicated that the methanol leaf extract, at doses of 250, 500, and 1000 mg/kg, did not significantly reduce apomorphine-induced stereotypic behavior. However, it exhibited a significant and dose-dependent reduction in the average number of grooming episodes and the duration of swim-induced grooming behavior across all tested doses. Notably, the highest dose (1000 mg/kg) significantly reduced cataleptic effects at 60 minutes post haloperidol administration compared to the negative control. These findings suggest the potential anti-schizophrenic activity of the methanol leaf extract of *H. acida*, supporting its traditional use in addressing psychiatric disorders.

Keywords: Schizophrenia, Catalepsy, Apomorphine, *Hymenocardia acida*, Phytochemical screening

INTRODUCTION

Schizophrenia is a devastating brain disorder characterized by distortion in cognition, emotion, language, perception and thought (Akinpelu *et al*., 2018). The symptoms of schizophrenia include; delusion, hallucination, anxiety, sleep-disturbance, and depression, (Amoateng *et al*., 2017). An estimated population of 20 million people worldwide is affected with the mental disorder (Oloniniyi *et al*., 2019). Traditional antipsychotic agents example haloperidol are used in the treatment schizophrenia. However, these agents are associated with serious side effect such as extrapyramidal side effects. In addition, atypical antipsychotic agents such as risperidone and olanzapine are reported to possess adverse effects which include postural hypotension, sedation, anticholinergic side effects, and weight gain; these affect patience adherence to medication which in turns result in therapeutic failure. Moreover, relapse occurs in approximately 75 percent of schizophrenic patients within a year if medications are discontinued, and only 25 percent of patients continue their medications (Valenstein *et al*., 2004). Additionally, the overall functional and quality of life outcomes of patients still remain poor after treatment, and there is also the issue of financial burden as these drugs are very expensive. Thus, there is a critical need to search for more effective, affordable and less toxic therapeutic agents from medicinal plants.

In Nigeria, where appreciable number of the native populations rely on medicinal plants to meet their health care needs, herbal medicines formed the cornerstone of therapy for the management of psychiatric disorders including schizophrenia. Different parts of plant species such as leaves, stem, stem bark and sometimes roots are employed in the treatment of psychosis, singly or in combination with different rituals (Ajao *et al*., 2017). *Hymenocardia acida* (Tul), family Phyllantaceae (Tuenter *et al*., 2016) is a very popular plant in African traditional medicine practices and its leaves, roots and barks are used in either infusion or powdered form to treat epilepsy and schizophrenia (Burkill, 1985). This study is therefore designed, to screen phytochemical constituents and provide scientific evidence, for the traditional claim regarding the use *Hymenocardia acida* leaf in the treatment of schizophrenia.

MATERIALS AND METHODS

Chemicals, reagents, solvents and drugs

Methanol 90%v/v, distilled water, Normal saline, haloperidol, apomorphine and ondansetron.

Experimental animals

Adult swiss albino mice of either sex and weight 18-22g were used in this study. The animals were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Bayero University, Kano, Kano state-Nigeria. The mice were maintained on excel feeds and water *ad libitum*, they were housed in propylene cages at room temperature throughout the course of the study. All experimental protocols were in accordance with the Bayero University Research policy; and ethic and regulations governing the care and use of experimental animals as contained in "Principles of laboratory animal care" (National Research Council Guide for the Care and Use of Laboratory Animals 1996). Ethical clearance was obtained from Animal Care and Use Research Committee (ACUREC).

Plant Collection and Identification

The branches of *H.acida* were collected from Lokoidan farm in Diko,Gurara Local Government Area, Niger state in July 2021. Then it was identified and authenticated in the Herbarium unit, Department of Plant Biology, Bayero University Kano. A specimen of the collected plant was given

a Bayero University Kano Herbarium Accession Number (BUKHAN 0058) after comparing with an existing specimen.

Extraction of Plant Materials

The leaves were removed from the branches, washed with distilled water and shade dried. The dried leaves were pulverized using mortar and pestle. The powdered plant material was extracted by cold maceration using 90% methanol for 72 hours. The extract was filtered using Whatmann filter paper (No. 1) and then concentrated using rotatory evaporator at 40 degrees under reduced pressure. The extract was then stored in a desiccator for further use.

Preliminary Phtochemical Screening

Qualitative preliminary screening for the presence of secondary metabolite was carried out according to established methods (Evans, 1996).

Determination of Acute Toxicity (LD_{50}) profile of methanol leaf extract of *H. acida*

The median lethal dose was determined using lorke method 1983. The method comprises of two phases; in the first phase, the mice were grouped into three (3) groups of three (3) mice each.

The extract of *H. acida* was administered at a dose of 10, 100 and 1000 mg/kg body weight intraperitoneally (i,p) to groups 1,2,and 3 respectively. The mice were monitored for toxicity signs, including mortality, within a 24-hour period. In the subsequent phase, guided by phase 1 outcomes, three mice were administered the extract at doses of 1600, 2900, and 5000 mg/kg body weight. Similar observations for toxicity indicators and mortality within a 24-hour timeframe were conducted. The LD_{50} value was determined by calculating the geometric mean between the lowest lethal dose and the highest non-lethal dose.

Determination of antischizophrenic activity

Apomorphine – induced stereotype behaviour in mice

The study was carried out according to the method outlined by Costall et al. (1978) with minor modifications. Each mouse was situated in a cylindrical cage featuring vertical metal bars spaced 1 cm apart, topped with a smooth surface, and allowed to acclimate for at least 10 minutes. The mice received treatments of normal saline (10 ml/kg), plant extract (at specified doses), or haloperidol (1 mg/kg) intraperitoneally (i.p.). After a 30-minute interval post-treatment, each mouse was subcutaneously administered apomorphine (1.5 mg/kg) in 10% sodium metabisulphite. Subsequently, the mice were placed in an observation cage and observed for stereotypic behaviors, primarily sniffing and climbing, at 10, 20, and 30-minute intervals. Climbing behaviors were rated on a scale of four paws on the floor (0), fore feet holding the vertical wall (1), to four paws grasping on the vertical wall (2). The mice were also assessed for repetitive sniffing, with ratings ranging from no sniffing (0), moderate sniffing with minimal snout contact (1), to constant sniffing (2). The average climbing and sniffing scores were determined for each group.

Swim-induced grooming test in mice

After thirty minutes post-administration of normal saline, *H. acida* extract, or haloperidol (1 mg/kg), each mouse was individually introduced into 1,000 ml transparent beaker (15.5 cm in height, 11 cm in diameter) containing warm water (25 °C) up to 6 cm from the bottom. The mouse was allowed to swim for 3 minutes, after which it was dried with a clean towel for 20 seconds and promptly placed in a transparent Plexiglas cage. The mouse's grooming episodes and the overall duration of grooming in seconds were observed and recorded for a 5-minute period (Chesher and Jackson, 1981).]

Haloperidol-induced catalepsy study in mice

The model previously described by Nair *et al*. (2007) was adopted with slight modifications. The mice were grouped into four groups. The first group of mice received normal saline (10 ml/kg). The second, third and fourth groups received the extract at doses of 1000, 500 and 250 mg/kg body weight respectively. After a lapse of thirty minutes, each mouse received intraperitoneal administration of haloperidol at a dose of 1 mg/kg. The extent of catalepsy was measured at 30-minute intervals up to 90 minutes. Catalepsy was determined when the mouse sustained an imposed position with both forelimbs raised and resting on a 4 cm high wooden bar. The endpoint of catalepsy was defined as when the mouse withdrew both front paws from the bar or when it moves its head in an exploratory manner. The animal is considered cataleptic and is scored a point (1 point) if it maintains the imposed position for at least 20 sec. and the animal will be given a score of 2 points if it maintains the posture for 40 sec. A cataleptic score of 2 (equivalent to 40 seconds) served as the cutoff point.

Statistical analysis

The data was expressed as mean ± SEM and it was analyzed using One Way Analysis of Variance (ANOVA) followed by Dunnet's post hoc test where significant difference was observed. The p value ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Preliminary phytochemical studies of methanol leaf extract of *H. acida* showed the presence of flavonoids, tanins, terpenoids/steroids (Table 1).

Table 1: Preliminary phytochemical constituents of methanol leaf extract of *H.acida*

TEST	INFERENCE
TEST FOR FLAVONOIDS	
1. Ferric chloride test	+
2. Sodium hydroxide test	+
TEST FOR TANINS	
1. Lead sub acetate	+
TEST FOR TERPENOIDS/STERIODS	
2. Salkowski's test	+
3. Liebermann-burchards test	+
TEST FOR ALKALOIDS	
2. Dragendoffs test	+
3. Mayers	+

TEST FOR SAPONINS	
4. Frothing	-

Key: present = (+) ; absent = (-)

Acute toxicity

The intraperitoneal (i.p) median lethal dose (LD_{50}) of the extract was deduced to be above 5000 mg/kg body weight in mice. All the mice treated with the extract were dull; clustered together for about three hours following intraperitoneal administration of the extract.

Apomorphine – induced stereotype behaviour in mice

The methanol leaves extract of *H.acida* at all doses (250, 500 and 1000 mg/kg) tested showed no significantly reduction of apomorphine-induced stereotypic behavior (climbing and sniffing). However positive control (haloperidol) significantly reduced the sniffing and climbing episodes (Table 2).

Table 2: Effect of methanol leaves extract of *H.acida* on apomorphine-induced Stereotypic behavior in mice

Drug treatment (mg/kg)	Time (min)	Climbing			Sniffing		
		10	20	30	10	20	30
Normal saline		1.67±0.21	1.50±0.21	1.67±0.33	1.83±0.17	1.00±0.36	1.50±0.34
HA 250		1.00±0.45	1.00±0.45	0.33±21	1.83±0.17	1.50±0.22	0.83±0.17
HA 500		0.83±0.40	1.17±0.40	0.83±0.31	2.00	2.00	1.33±0.21
HA 1000		0.67±0.33	1.00±0.45	0.83±0.40	1.67±0.21	1.67±0.33	0.83±0.37
Haloperidol 1		0.33±0.33*	0.00*	0.17±0.17*	0.17±0.17*	0.00*	0.00*

Data presented as mean ± SEM; Significant decrease *P<0.05 versus normal saline treated control; n=6 (one-way ANOVA followed by Dunnett's multiple comparison post hoc tests).

Swim-induced grooming test in mice

The methanol leaf extract of H. acida demonstrated a significant (P < 0.05) and dose-dependent reduction in both the mean number of grooming episodes and the duration of swim-induced grooming behavior across all tested doses (250, 500, and 1000 mg/kg), (Figure 1 and Figure 2). Haloperidol completely eliminated swim-induced grooming behavior. There is a close relationship between haloperidol and extract at the dose of 1000mg/kg in abolishing grooming behaviors.

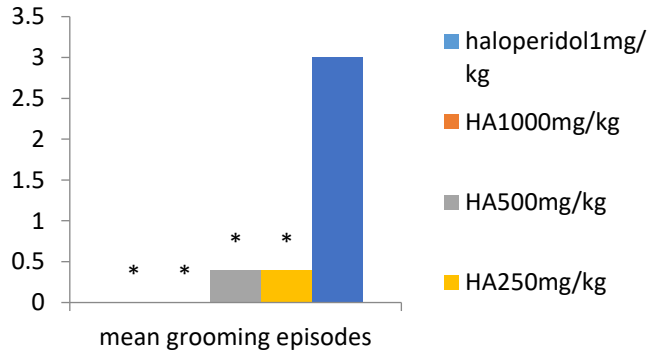

Figure 1: Showing the effect of methanol leaf extract of *H.acida* on swim-induced grooming behaviour in mice. Data presented as mean ± SEM. Significant decrease *P <0.05; versus normal saline-treated control; n=5 group (one-way ANOVA followed by Dunnett's multiple comparison post hoc tests).

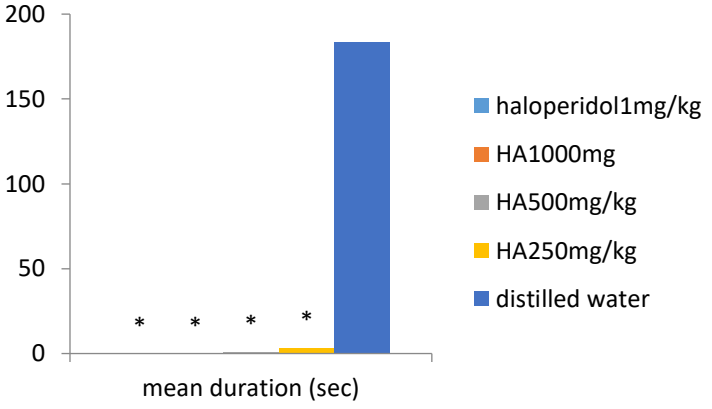

Figure 2: Showing the effect of methanol leaf extract of *H.acida* on swim-induced grooming behaviour in mice. Data presented as mean ± SEM. Significant decrease *P <0.05; versus normal saline-treated control; n=5 group (one-way ANOVA followed by Dunnett's multiple comparison post hoc tests).

Haloperidol-induced catalepsy study in mice

The methanol leaves extract of *H.acida* at highest dose (1000 mg/kg body weight) significantly (P <0.05) reduced cataleptic effect at 60 min post haloperidol administration compared to negative control. However no significant difference was recorded at 30 and 90 min post administration of 1000mg/kg of the extract. The extract at median and lowest doses (500 and 250 mg/kg) did not significantly inhibit or potentiate catalepsy (Figure 3).

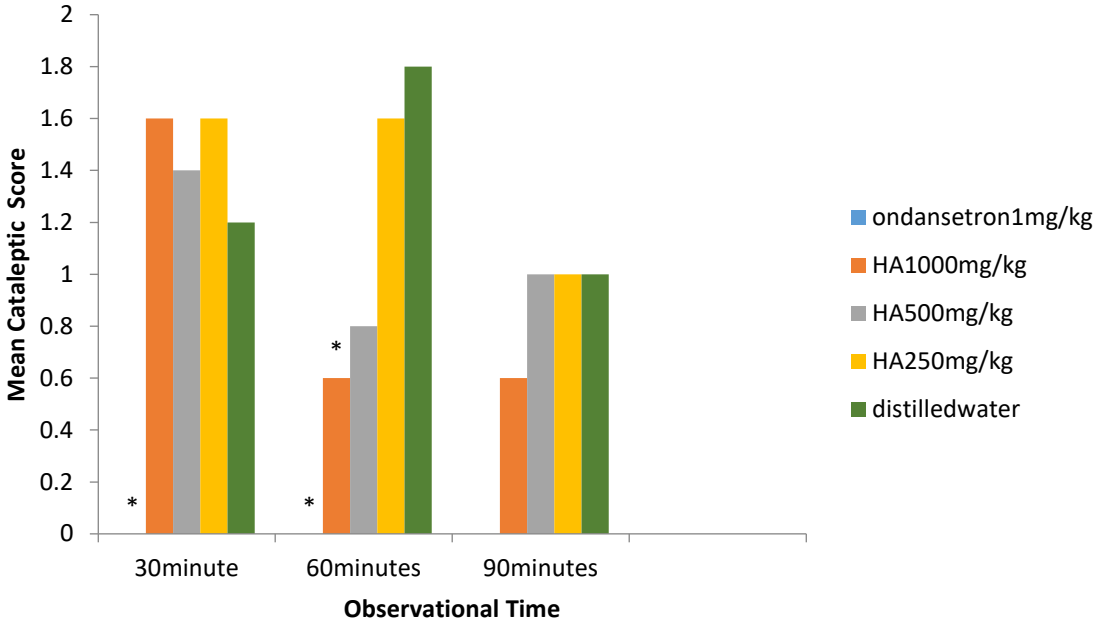

Figure 3: Effect of methanol leaf extract of *H.acida* on Haloperidol-induced Catalepsy in mice; Cataleptic scores were taken 30,60 and 90 minutes after administration of haloperidol; Data are mean ± SEM (n = 5). *P ≤ 0.05 compared with normal saline treated control (one-way ANOVA followed by Dunnett's multiple comparison post hoc test.

Discussion

The intraperitoneal median lethal dose (LD_{50}) of the extract was deduced to be above 5000 mg/kg in mice. The large (LD_{50}) value indicates that the extract is relatively rather non-toxic when administered intraperitoneally (Lorke, 1983). Results obtained from this study is similar to that of Haruna *et.al*., (2017). In the Apomorphine – induced stereotype behaviour in mice test, the methanol leaf extract of *H.acida* did not reduce the apomorphine-induced cage climbing and sniffing behaviour suggesting that it may not have strong affinity for the D2 dopaminergic receptors. Apomorphine, when administered in mice result in increased motility, stereotyped and cage-climbing behaviours (Amoateng *et.al*., 2017). Since apomorphine acts by activating D2 dopamine receptors, it is possible that the methanol leaf extract of *H.acida* has more affinity for dopamine D1 receptor compared to D2. In Swim-induced grooming test, the methanol leaf extract of *H. acida* was found to significantly and dose-dependently reduce the mean number of grooming episodes and duration. The profound and dose-dependent reduction in grooming behavior by the methanol leaf extract of *H. acida* further implies its potential as a D1 dopaminergic antagonist. There is valid evidence that support the role of serotonergic modulation of antipsychotic induced catalepsy and it has been reported that anti-serotonergic activity overcomes the detrimental side effects associated with traditional anti-psychotic therapy (Bethany *et al*., 1992). The inability of the methanol leaf extract of *H. acida* to enhace the haloperidol-induced catalepsy support the earlier finding of this study that its effects might be specifically confined to D1 dopaminergic receptors. Additionally, its ability to attenuate catalepsy at 60 minutes post administration of haloperidol implies that it might have affinity for serotonergic receptors, thus, suggesting similarities with atypical antipsychotics.

Preliminary phytochemical studies of methanol leaf extract of *H. acida* showed the presence of flavonoids, tanins, terpenoids/steroids. The findings of this study is similar to that of Haruna *et.al*., (2017). However in this study saponins were found to be absent. The potential anti-schizophrenic effects exhibited by the methanol leaf extract of *H. acida* are likely attributed to the presence of phytochemicals within the plant. Given the reported presence of bioactive metabolites in *H. acida*, it is plausible that these active compounds contribute to its anti-schizophrenic activity.

CONCLUSION

This study shows that methanol leaf extract of *H. acida* possess anti-psychotic properties. This plant can also be well formulated and sold as herbal medicine preparation for the management schizophrenia.

REFERENCES

Ajao, A. A., Alimi, A. A., Olatunji, O. A, Balogun. O. F., Saheed A. S. (2017). A synopsis of antipsychotic medicinal plants in Nigeria. *Transactions of the Royal Society of South Africa*. DOI:10.1080/0035919X.2017.1386138.

Akinpelu, L. A., Akanmu, M. A., & Obuotor, E. M. (2018). Antipsychotic effects of ethanol leaf extract and fractions of *Milicia excelsa* (Moraceae) in Mice. *Journal of Pharmaceutical Research International*. doi: 10.9734/JPRI/2018/42383.

Amoateng. P., Adjei, S., Osei-safo, D., Kukuia. K. K. E., Oppong Bekoe, E. O., Karikari K. T., Kombian, S. B. (2017).

Extract of *Synedrella nodiflora* (L) Gaertn exhibits antipsychotic properties in murine models of psychosis. *BMC Complementary and Alternative Medicine* 17:389 ;doi 10.1186/s12906-017-1901-2.

Bethany, S., Neal-beliveau, Jeffrey. N., Joyce and Irwan Lucki (1992). Serotonergic involvement in Haloperidol-induced catalepsy. *Journal of Pharmacology and Experimental Therapeutics*. 0022-3565/93/2651-0207S03.00/0

Burkill, H.M. (1985). The useful plants of west tropical Africa, Vol 2 from https://plants.jstor.org/stable/10.5555/al.ap.upwta.2_186

Chesher, G. B., Jackson, D. M. (1981). Swim-induced grooming in mice is mediated by a dopaminergic substrate. *Journal of Neural Transmission* 50(1).

Costall, B., Naylor, R. J., Nohria, V. (1978). Climbing behavior-induced by apomorphine in mice: a potent model for the detection of neuroleptic activity. *European Journal of Pharmacology* 50:39–50.

Davis, K. L., Kahn, R. S. (2019). Dopamine in schizophrenia: a review and reconceptualization. *American Journal of Psychiatry*, 148:1474.

Evans, W. C. (1996). Trease and Evans' Pharmacognosy. WB Saunders, London, 520-521.

Haruna, A., Pateh, U. U., Sule, M. I., Musa, A. M., Sani, Y. M., Mohammed, M., Lawal. M. D., Garba, M. A. (2017). evaluation of anticonvulsant activity of methanol leaf extract of *Hymenocardia acida*, tul (Euphorbiaceae) in laboratory animals. *Journal of Pharmaceutical and Allied Sciences* 14 (5): 2675 – 2683.

Ingale, S. P.,Kasture, S. B. (2012). Psychopharmacological profile of *Passiflora incarnate* Linn in mice. *International Journal of Phytopharmacology*, 3(3), 263-268.

Lorke, D.G. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*, 54:275-287.

Nair, V., Arjuman, A., Dorababu, P., Gopalakrishna, U., Rao, C., Mohan, L. (2007). Effect of NR-ANX-C (a polyherbal formulation) on haloperidol induced catalepsy in albino mice. *Indian Journal of Medicinal Research*, 126:480–484.

Oloninyi, O. I., Akinsulore, A., Aloba, O. O., Mapayi, B. M., Oginni. O. A., Makanjuola, R. (2019). Economic cost of schizophrenia in a Nigerian teaching hospital. *Journal of Neurosciences in Rural Practice*.

Tuenter, E., Exarchou, V., Baldè, A., Cos, P., Maes, L., Apers, S. and Pieters, L.(2016). Cyclopeptide alkaloids from *Hymenocardia acida*. *Journal of natural products*,79(7):1746-1751.

Valenstein, M., Blow, F.C, Copel L. A., McCarthy, J. F., Zeber J. E., QMon L, Bingham, C. R., Stavenger, T. (2004). Poor Antipsychotic adherence among patients with schizophrenia: Medication and Patient Factors. *Schizophrenia Bulletin*, 30(2).