

## Isolation and Structure Elucidation of Cyclohexanepentol, an Anti-Psychotic Agent from *Cissampelos owariensis* (P. Beauv.) Leaves

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### Abstract

**Background:** In Nigeria, an estimated 20 – 30% of our population is believed to suffer from mental disorders. *Cissampelos owariensis* has been used in Southwest Nigerian traditional medicine to treat mental illness.

**Objectives:** This study evaluated the anti-psychotic potential of *Cissampelos owariensis* crude extract, fractions, sub-fractions and isolated compound, using *in vivo* and *in vitro* antipsychotic assays.

**Material and Methods:** Crude extract and fractions of the leaves were investigated against ketamine induced hyper-locomotion and stereotype behaviours in mice. Extrapyramidal effect of bioactive fraction was tested using catalepsy model. D - amino acid oxidase assay was used to test for anti-psychotic property of chromatographic sub-fractions and isolated compound. Percentage inhibition of pooled sub-fractions and compound was measured at an excitation and emission wavelengths of 355 and 460 nm, respectively. Characterization of isolated compound was done using Infrared and Nuclear Magnetic Resonance spectroscopy. One-way ANOVA, followed by Dunnett's posthoc test at  $\alpha_{0.05}$ , was used for data analysis.

**Results:** Crude extract of *Cissampelos owariensis* (125 mg/kg) and fractions showed reduction in hyper-locomotion at different doses. The extract (125 mg/kg) and ethylacetate fraction (500 mg/kg) significantly antagonized stereotypy in mice. The ethyl acetate fraction did not induce catalepsy but rather reversed the cataleptic effect induced by haloperidol. Phytochemical investigation led to the identification of Cyclohexanepentol, which possesses comparable anti-psychotic activity with Risperidone.

**Conclusion:** This paper presents for the first time, anti-psychotic principle from *C. owariensis* and validates its ethno-medicinal use.

**Keywords:** *Cissampelos owariensis*, Cyclohexanepentol, Antipsychotic agent, D-amino acid oxidase, Chromatography

### INTRODUCTION

Psychosis is a chronic neurological disorder characterized by a disconnection from reality (Bangwal *et al.*, 2020). It affects mood, thought and behaviours of people regardless of sex and age group.

Patients experiencing this disorder fail to realize what is real and what is not due to the fact that their reasoning, judgment and insight are lost. They believe that the false experiences are actually happening (Parle

and Sharma, 2013). Psychosis may be caused by side effects or abuse of some drugs, lack of sleep, stress, genetic factors, trauma and spiritual experience (Karamah *et al.*, 2019). Approximately 970 million people worldwide have a mental health disorder, which affects more females (11.9%) than males (9.3%) (Mental health, 2018). Psychotic patients have significant excess mortality. The mortality rate of people with serious mental illnesses, including schizophrenia, is higher than the general population. Hence, they die on average 10 to 15 years earlier than their peers. These disorders were attributed to 14.3% of deaths globally each year (Bitter *et al.*, 2017; Hayes *et al.*, 2017).

In Nigeria, an estimated 20 – 30% of our population is believed to suffer from mental disorders. This is a significant number considering Nigeria with an estimated population of over 200 million. One in four Nigerians (some 50 million people) are suffering from some sort of mental illness, according to the World Health Organization (WHO, 2020). About 70% of people who experience psychosis are not getting adequate and appropriate attention, while almost 90% who are without any form of treatment live in low income or developing countries. Current drug treatments are limited by poor efficacy and tolerability. In addition, financial implication of treatments causes patients or their family to prefer treatment from traditional medical practitioners, because they believe it is safe and less expensive.

In Africa, traditional healers and remedies made from plants play an important role in the health of millions of people. Diseases such as cancer, Parkinson's, diabetes, hypertension among others have been managed using products from natural sources (Al – Dabbagh *et al.*, 2018). Antipsychotic plants are not left out, and have been used since ancient time, as an alternative therapy. Many studies have shown that several chemical agents derived from medicinal plants, have been used in both the prevention and treatment of psychosis. For example, the alkaloidal constituents Reserpiline and  $\alpha$ -Yohimbine were isolated from *Rauwolfia tetraphylla* L. and showed best antipsychotic effect on dopaminergic and serotonergic receptors as well as amphetamine-induced hyperactivity in mice (Gupta *et al.*, 2012). Risperpine, a natural alkaloid was also obtained from the plant *Rauwolfia serpentina* (Curb *et al.*, 1988), the anti-psychotic agent from *Rosmarinus officinallis* L., was identified as Ursolic acid (Machado *et al.*, 2012), and Alstonine from *Picralima nitida* was found to decrease glutamate uptake, which is undoubtedly a good intervention to the glutamatergic deficit

associated with schizophrenia. Furthermore, alstonine expressed its antipsychotic effect by increasing glutathione, a potent radical scavenger reported to be declined in the brain of patients with schizophrenia (Viviane *et al.*, 2011; Herrmann *et al.*, 2012).

The genus *Cissampelos* is from the family Menispermaceae, consisting mainly of about 171 species (The Plant List, 2010). Species from this genus have folkloric use in the treatment of problems related to infertility, such as irregular menstrual cycle and pregnancy related diseases in women (Akande *et al.*, 2013; Erhirhie *et al.*, 2015). *Cissampelos owariensis* is commonly called velvet leaves, and “ewe jenjoko or jokoje” among the Yoruba speaking in Nigeria. The plant is a perennial climbing plant with annual stems that scramble over the ground or twine into the surrounding vegetation for support. It is found mostly in African countries like Nigeria, Sierra Leone, Congo, Tanzania and Angola (Tropical Plants Database, 2021). It is used traditionally for various healing properties such as purgative, fertility, wound healing. In addition, the aerial parts of the plant have been used in the management of mental disorder; juice from the leaf is squeezed and given to patients experiencing psychosis in Nigeria (Sonibare *et al.*, 2008). Yusuf (2003) also reported its use against headache, fever, most especially malaria and typhoid fevers, and powdered leaves are spread in tea, or “pap” to be drunk for the treatment of stomach related problems. Pharmacological studies on this plant revealed that it has anti-proliferative (Omotoso *et al.*, 2021), anti-diabetic (Ekeanyanwu *et al.*, 2012), anti-microbial (Arya, 2011), anti-diarrhea and anti-bacterial (Zumbes *et al.*, 2016) properties. *C. owariensis* leaves are rich in flavonoid, saponins, tannins and moderate amount of alkaloids (Ekeanyanwu *et al.*, 2012), two sesquiterpenes have been isolated from this plant (Efiom, 2010).

Despite the numerous benefits of this plant in traditional medicine to treat mental disorder, there is paucity of information to the best of our knowledge, on the potential anti-psychotic property of the plant. In this study, *in vivo* anti-psychotic property of crude methanol extract and fractions of *Cissampelos owariensis* leaf, as well as *in vitro* analysis of antipsychotic potential of chromatographic pooled sub-fractions and isolated compound were reported. Their antipsychotic properties against ketamine induced mice models predictive of human psychosis provide some justifications for the ethno-medicinal usage of the plant in the management of mental disorder.

## METHODOLOGY

### General experimental procedure

Silica gel 60 F<sub>254</sub> plates (20 × 20 cm, 0.5 mm; Merck, Germany) was used for Thin Layer Chromatography and Preparative Thin Layer Chromatography (TLC and Prep-TLC), Scharlau Silica gel 60 (70 – 230; 200 - 400 mesh sizes; Spain) was used to perform gravity column chromatography. Bruker Ascend 400 MHz Spectrometer (Bruker Instruments Incorporation, Billerica, MA USA) was used for the <sup>1</sup>H and <sup>13</sup>C NMR. The chemical shifts (δ) were expressed in parts per million (ppm), while trimethylsilane (TMS) was used as internal standard.

### Chemicals, reagents and Apparatus

Analytical grade solvents (methanol, *n*-hexane, ethyl acetate, dichloromethane, butanol and acetone), TLC pre-coated plates (Merck, Germany), Silica gel, Enzyme D-amino acid oxidase from Pig Kidney (pKDAO), D - Kynurenine substrate, co-factor FAD (Flavine Adenine Dinucleotide), Dimethyl sulfoxide (DMSO), Tris hydrochloride salt and Bovine Serum Albumin (BSA) were purchased from Sigma. Distilled water was further purified using membrane filter apparatus. Glass column (1000 x 30 mm), UV lamp, micropipette, micropipette tips, and 5 mL glass test tubes were purchased from Thermo Fisher Scientific, (England, UK).

### Collection and preparation of plant material

The leaves of *Cissampelos owariensis* were collected at Ogungbenro village in Akinyele Local Government, Oyo State, Nigeria from January to March, 2016. The plant specimen was identified and authenticated by Dr. O. A. Osiyemi, at Forest Herbarium Ibadan (FHI), Nigeria where specimen was deposited with a voucher number, FHI 110760. Plant specimen was also deposited in the Department of Pharmacognosy Herbarium University of Ibadan (DPHUI), Nigeria. Freshly collected leaves were air-dried under shade for two weeks, oven dried at 40 °C for 3 - 4 h and pulverized with electronic blender.

### Extraction of plant material and fractionation Procedure

Pulverized leaves sample (1.2 kg) of *C. owariensis* was macerated at room temperature with absolute methanol for 72 h and shaken intermittently. The methanol extract was decanted, filtered and concentrated *in vacuo* at 40 °C, using rotary evaporator, regulated steam bath and the obtained crude extract was placed in a desiccator to remove moisture to a constant weight of the extract. Residue

obtained during filtration was re-extracted to obtain optimum yield from the leaves (236 g). The crude methanol extract (230 g) was further separated by solvent – solvent partitioning (see details in Supplementary Chart 1) in increasing polarity of solvents, so as to identify the most active part of the extract. Thin Layer Chromatography (TLC) was used to monitor the termination of each solvent for effective partitioning. All fractions were evaporated to dryness on a regulated steam bath and stored in a cleaned glass bottle with lid at 4 °C for anti-psychotic studies.

### Pre-adsorption and Isolation of compounds

About 40 g of extract was weighed in a beaker and dissolved in methanol, it was transferred into a rotary flask, and silica gel (Sigma-Aldrich, Japan; 70 – 230 mesh size) was added in the evaporating flask, which was then evaporated to dryness. The dried pre-adsorbed extract was gently scraped out of the flask, grinded into fine powder with the help of mortar and pestle.

The pre-adsorbed ethyl acetate fraction of *Cissampelos owariensis* was loaded on a packed gravity column (1000 x 30 mm), and gradiently eluted with a solvent mixture of increasing polarity starting from hexane (non-polar) to methanol (polar). The eluted sub-fractions (222) of 50 mLs each were collected and finally pooled to eleven sub-fractions (I - XI) using TLC. Crystals were observed from pooled sub-fraction V and yielded compound 1 after purification.

### Preparation of extract/fractions/compound for antipsychotic study

Extract and fractions from the leaves of *C. owariensis* were dissolved in distilled water to give the doses of 125 – 500 mg/kg (extract/fractions) and 0.2 mg/kg for standards (Haloperidol and Risperidone) in the *in vivo* study. For *in vitro* study, 0.1 mg/mL of Haloperidol and Risperidone standards and 0.2 mg/mL of the pooled sub-fractions and isolated pure compound were the concentrations used. Each sample was mixed thoroughly using a vortex mixer for about 2 min to ensure content uniformity before use.

### *In vitro* D-amino acid oxidase enzymatic assay

About 5 µL of 0.1 mg/mL D-amino acid oxidase was pipetted in a 96 well plate using a micro pipette and four replicate, thereafter, 6 µL of 200 µM Flavin Adenine Dinucleotide (FAD) was added, 5 µL of 2.0 mg/mL. Bovine Serum Albumin (BSA) was also

added, 27  $\mu$ L of 0.4 M Tris buffer (P<sup>H</sup> 8.3) and 5  $\mu$ L of Haloperidol, pooled sub-fractions I – XI, isolated compound or buffer was added. The mixture was incubated at 37 °C for 20 min, after 20 min, the sample was pre read in a fluorescence spectrophotometer and recorded. Furthermore, 5  $\mu$ L of 7.0 mM D-Kynurenine (D-KYN) substrate was added and incubated for 60 min at 37 °C. It was mixed and measured finally in the fluorescence spectrophotometer at an excitation and emission wavelengths of 355 and 460 nm, respectively.

### Experimental Animals

Balb C albino male mice (25 – 30 g) used for the study were purchased from National Institute of Health, Islamabad, Pakistan. They were housed twelve per cage at controlled room temperature. Wood shavings were used as beddings to absorb excretory products from animals. The animals were allowed to acclimatize for a week minimum before the start of experiment. The animals had regular access to food pellets and water *ad libitum* with a 12 – 12 h light/dark cycle. They were randomly distributed into treatment groups (number of mice was six per group), and acclimatized to each experimental procedure. The number of animals used were reduced by using the same animal for both hyper locomotion and stereotype behavioural models. The experiments were performed in accordance to the National Institutes of Health Guide for care and use of laboratory animals. Also, efforts were made to minimize suffering of the animals during experiments and experiments were performed between working hours of 8 a.m. – 4 p.m., each day in the neuropharmacology laboratory unit, COMSATS Institute of Information Technology (CIIT), Abbottabad, Pakistan.

### Drugs and Treatments

Ketamine was purchased from Indus Pharma, Karachi, Pakistan, Haloperidol (injection) from The Searle Company Limited, Karachi, Pakistan and Risperidone (Risperidal, tablet, powdered and dissolved in distilled water) from West-Coast Pharmaceutical, India. Haloperidol and Risperidone (0.2 mg/kg) were used as standards. Mice were divided and selected randomly into various groups (six animals per group), and pre-treated with either distilled water (group 1), crude extract (group 2,3,4), *n*-hexane (group 5,6,7), DCM (group 8,9,10), EtOAc (group 11,12,13), BuOH (group 14,15,16), Aqueous (group 17,18,19) fractions, Haloperidol (group 20) or Risperidone (group 21). Plant extracts were dissolved with distilled water except *n*-hexane, which was dissolved with 2 drops of 20 % tween 80. The drugs were administered at a dose of 125, 250 and 500 mg/kg, based on results from

preliminary studies. Results were recorded based on observed behaviors without any bias.

### In vivo Behavioural Assays

Hyper-locomotion and stereotype behavioural paradigms were performed according to previously described methods (Bourin *et al.*, 1986; Arowona *et al.*, 2014).

#### Hyper-locomotion in mice

Hyper locomotion was observed in the animals using open field test. A total of 126 animals (n=6/group in an experiment of 21 groups), were pre-treated orally with distilled water, crude extract of CO, fractions (Hex, DCM, EtOAc, BuOH or AQ) at doses of 125, 250 and 500 mg/kg *b.w.* and standards; Haloperidol and Risperidone (0.2 mg/kg) each, 60 min before ketamine (30 mg/kg, *i.p.*) injection. Thereafter, they were singly placed immediately at the centre of an open field chamber. The total number of lines crossed and duration of ambulation (time at which animal did not move) were recorded for 5 min using a mobile video.

#### Stereotype behaviour in mice

The antagonistic effect of the crude extract of the screened plant (*Cissampelos owariensis*) and fractions were tested on ketamine induced stereotype behaviour in mice. Five (5) minutes after observation of animals in open field test, animals were placed in transparent observation chamber (L X B X H: 16 cm x 10 cm x 6 cm), to observe stereotype behaviours, and repetitive behaviours were recorded for a period of 2 min at 5, 10, 15, 20, 30, 45, and 60 min. Stereotype behaviours were scored as 0 = absence of stereotype behaviour, 1 = presence of stereotype movements of the head, 2 = intermittent sniffing, 3 = chewing, and 4 = intense licking.

#### Catalepsy

The cataleptic effect of CO ethyl acetate fraction was investigated. Mice in group 1 received distilled water (10 mL/kg) and served as positive control, group 2 received haloperidol (1 mg/kg; *i.p.*) only and served as standard drug, groups 3 and 4 received EtOAc fraction of CO (125 mg/kg and 500 mg/kg; *p.o.*), groups 5 and 6 received haloperidol (1 mg/kg; *i.p.*) with EtOAc fraction at lower and higher doses respectively. Mouse was placed with both forepaws resting on a 4 cm high glass horizontal bar (1.0 cm diameter) and the time it maintained an imposed position was recorded in seconds. Time recording was terminated when both front paws were removed from the bar or if the mouse moves its head in an exploratory way, and a cut-off time of 5 min was applied. All observations were taken at 0, 30, 60, 90, and 120 min after drug

administration (Pemminati *et al.*, 2007). A total of 36 animals were used for this model.

### Statistical analysis

Stereotype behaviour scores were calculated manually, followed; by one-way analysis of variance (ANOVA), after which Dunnett post hoc test was employed to

compare all groups against the negative control group. Values were presented as mean  $\pm$  standard error of mean, and a p – value of 0.05, 0.01 or 0.001 was considered statistically significant

## RESULTS AND DISCUSSION

### Bioactivity of crude extract/fractions/compound

The results of the Ketamine-induced hyper-locomotion and stereotype behaviours, as well as catalepsy are presented in Table 1 (for extract/fractions), Figure 1 and 2, respectively. Figure 3 showed result of % inhibition of D-amino acid oxidase by isolated compound and standards. Administration of ketamine was shown to enhance the release of dopamine and glutamate in the brain striatal region (Hons *et al.*, 2010). Ketamine, an NMDAR antagonist induces hyper locomotion by increasing the number of lines crossed and reducing the ambulation time in mice (Sartim *et al.*, 2021; Zhang *et al.*, 2021). The crude extract of *Cissampelos owariensis* alongside its fractions were screened for their effect on

behavioural studies. The *in vivo* animal models used in this study include ketamine – induced hyper locomotion, ketamine-induced stereotype behaviour as described by Chatterjee *et al.* (2015) and catalepsy (Ior *et al.*, 2021; Arowona *et al.*, 2014). The crude extract of CO significantly lowered the number of lines crossed at 125 mg/kg (\*p <0.05). Reduction in number of lines crossed was also observed in the *n*-hexane (500 mg/kg), dichloromethane (250 mg/kg), as well as aqueous (500 mg/kg) fractions of CO, although, not comparable to the standards (Haloperidol and Risperidone) (Table 1). This indicates that the extract and fractions gave calming effect by reducing hyperactivity in the animals.

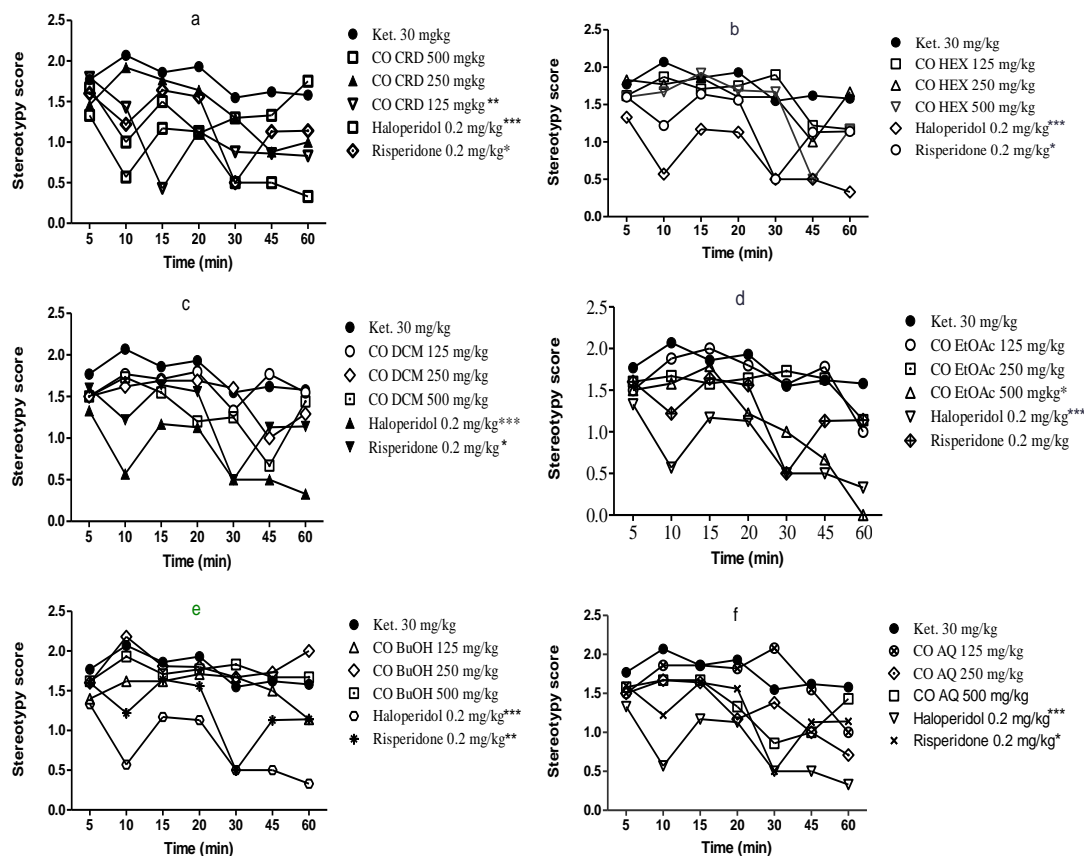
**Table 1: Effect of CO crude extract and fractions, Haloperidol, Risperidone and negative control on hyper-locomotion in mice**

Treatment	Dose (mg/kg)	No. of lines crossed	Ambulation time (s)
Ketamine	30	64.00 $\pm$ 9.63	56.33 $\pm$ 6.96
CO Crude extract	125	32.83 $\pm$ 6.99*	164.00 $\pm$ 16.95*
	250	49.83 $\pm$ 7.64	104.20 $\pm$ 12.50
	500	36.33 $\pm$ 12.26	136.50 $\pm$ 37.97
CO Hexane	125	51.00 $\pm$ 14.95	124.30 $\pm$ 38.75
	250	59.33 $\pm$ 11.49	84.50 $\pm$ 11.84
	500	39.17 $\pm$ 9.17	115.30 $\pm$ 25.94
CO Dichloromethane	125	57.83 $\pm$ 18.71	94.50 $\pm$ 23.52
	250	40.50 $\pm$ 9.18	114.80 $\pm$ 28.63
	500	46.50 $\pm$ 5.86	95.83 $\pm$ 25.81
CO Ethyl acetate	125	70.33 $\pm$ 14.39	121.00 $\pm$ 21.91
	250	56.33 $\pm$ 13.93	85.50 $\pm$ 5.25
	500	60.83 $\pm$ 10.18	88.00 $\pm$ 17.78
CO Butanol	125	62.67 $\pm$ 16.32	99.33 $\pm$ 21.40
	250	69.17 $\pm$ 4.58	96.67 $\pm$ 17.21
	500	54.33 $\pm$ 17.06	134.70 $\pm$ 34.05
CO Aqueous	125	66.33 $\pm$ 10.09	94.67 $\pm$ 10.06
	250	48.17 $\pm$ 12.17	84.83 $\pm$ 11.95
	500	22.00 $\pm$ 5.51**	173.30 $\pm$ 27.20***
Haloperidol	0.2	0.17 $\pm$ 0.17***	297.80 $\pm$ 1.17***
Risperidone	0.2	9.00 $\pm$ 5.87***	222.50 $\pm$ 35.09***

\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001

Stereotypy has mostly been utilized in the screening of potential neuroleptic agents, it is a prominent model for positive symptoms of psychosis. Ketamine also induces stereotype behaviours in animals (Radford *et al.*, 2020; Chatterjee *et al.*, 2015), showing repetitive head movement, intense licking, chewing and intermittent sniffing. The animals maintained this stereotype behavior up till 60 min of experiment as seen in the stereotypy score. However, *Cissampelos owariensis* crude extract (125 mg/kg, \* $p < 0.05$ ) decreased the stereotypy behavior induced by Ketamine significantly, likewise Haloperidol and Risperidone (0.2 mg/kg) the standard typical and

atypical antipsychotics, respectively (Figure 1). In addition, the hexane and DCM fractions of CO at 500 mg/kg, suppressed the stereotypy behaviour induced by ketamine (30 mg/kg) (Figure 1b and 1c). The EtOAc fraction significantly (\* $p < 0.05$ ) gave time dependent reduction at 500 mg/kg, and no stereotypy behaviours were observed in the animals at 60 min of experiment (Figure 1d). Inhibition of stereotypy behaviour was not observed in the butanol fraction, but the aqueous fraction at 500 mg/kg antagonised the stereotypy behaviour induced by Ketamine (30 mg/kg), (Figure 1f). In essence, EtOAc fraction was effective in antagonizing the repetitive behaviour induced by ketamine.



**Figure 1:** Effect of CO crude extract (a), *n*-hexane (b), Dichloromethane (c), Ethyl acetate (d), Butanol (e), and Aqueous (f) fractions on Ketamine induced stereotypy behavior. Stereotypy score were calculated manually ( $n = 6$ ) and one-way ANOVA followed by Dunnnett's multiple comparison post hoc test was used for analysis (Data were mean  $\pm$  SEM \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , compared with negative control group).

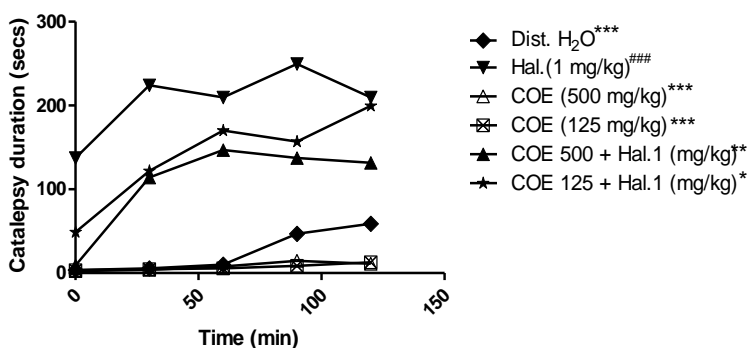
The activity of *n*-hexane, DCM, EtOAc, and aqueous fractions of CO in the behavioural models, suggests synergism in the antipsychotic effect. The antagonism of ketamine-induced hyper-locomotion and stereotypy behaviours by CO crude extract and fractions, suggest antipsychotic property of the plant. This observation was supported by earlier reports in which *Terminalia*

*macroptera* (Ior *et al.*, 2021), *Terminalia ivorensis* (Ben-Azu *et al.*, 2016), *Bacopa monniera* (Chatterjee *et al.*, 2015) and *Viscum album* (Gupta *et al.*, 2012) inhibited ketamine-induced behavioral paradigms in mice.

Efficiency of most antipsychotic drugs are limited to the extra pyramidal effect associated with it, this effect is said to result from lower dopamine activity in the brain striatal. A preferred blockade of D<sub>1</sub> and D<sub>2</sub> striatal dopamine receptors by neuroleptics relieves patients from psychosis and also causes little or no side effects (Porsolt *et al.*, 2010).

Increase in catalepsy was observed to occur in normal non-treated rats, after repeated experiment in testing activities (Sanberg *et al.*, 1988), and was also noticed at 90 min and 120 min of the experiment in normal

untreated animals during this study. The EtOAc fraction of CO (Figure 2) did not induce catalepsy in both higher and lower doses of 500 mg/kg and 125 mg/kg. This fraction also reduced the cataleptic effect produced by Haloperidol at both doses. The implication of this result is that, the ethyl acetate fraction of CO may give a preferential blockade of D<sub>2</sub> receptor in the striatal region, suggesting the antipsychotic property of the plant with little or no extrapyramidal effect.



**Figure 2:** Effect of *Cissampelos owariensis* ethyl acetate fraction (COE), and Haloperidol on catalepsy in mice. Connecting lines showed effect of COE, and Haloperidol at 0, 30, 60, 90 and 120 min of the experiment. Values were calculated as mean  $\pm$  SEM (n=6) in each group. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, compared with Haloperidol group, ###P < 0.001 versus the control group (Distilled H<sub>2</sub>O).

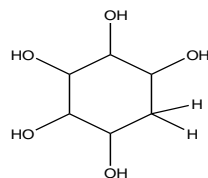
Ior *et al.*, (2021) revealed that administration of ethyl acetate fraction of *Terminalia macroptera* leaf orally in mice at different doses did not increase catalepsy in mice after 60 and 90 min post treatments. Furthermore, different doses of *Terminalia ivorensis* ethanol extract was reported to show no prolongation

in the time of akinesia in mice (Ben-Azu *et al.*, 2016) and (Parwez *et al.*, 2011) showed that ethanol extract of black tea significantly reduced catalepsy score in mice, and may be useful in preventing drug-induced catalepsy. These findings support our results in the present study.

### Characterization of isolated compound

Gradient column chromatographic elution and purification of the ethyl acetate fraction of the leaves of *C. owariensis* led to the isolation of cyclohexanepentol (Figure 3). The compound was obtained as a white needle shape crystal from pooled sub-fraction V. Six prominent peaks were observed in the carbon NMR spectrum at  $\delta$  68.1 (C1),  $\delta$  73.5 (C2),  $\delta$  72.5 (C3),  $\delta$  77.2 (C4),  $\delta$  68.2 (C5) and  $\delta$  34.8 (C6). Angyal and Odier, (1982) reported similar signals in the <sup>13</sup>C NMR signals of cyclohexanepentol at  $\delta$  67.2 (C1),  $\delta$  73.6 (C2),  $\delta$  72.5 (C3),  $\delta$  75.0 (C4),  $\delta$  70.0 (C5) and  $\delta$  34.6 (C6), respectively. The corresponding proton NMR spectrum signal was observed at  $\delta$  3.99 (q, J = 3.2, 3.16; <sup>1</sup>H),  $\delta$  3.41 (dd, J = 3.1, 6.8; <sup>1</sup>H),  $\delta$

3.49 (t, J = 8.96, 10.00; <sup>1</sup>H),  $\delta$  3.17 (t, 9.2; <sup>1</sup>H),  $\delta$  3.69 (m, <sup>1</sup>H),  $\delta$  2.01 (m, <sup>1</sup>H) and  $\delta$  1.48 (m, <sup>1</sup>H) for C1, C2, C3, C4, C5 and C6 respectively (Table 2). The <sup>1</sup>H signals at  $\delta$  2.01 and  $\delta$  1.48 revealed a -CH<sub>2</sub> carbon at C6. The <sup>1</sup>H-<sup>1</sup>H correlation spectroscopy (COSY) spectrum indicated a cyclic spin pattern suggesting a cyclohexanepentol. The HSQC <sup>1</sup>H - <sup>13</sup>C correlation revealed direct bond between proton at  $\delta$  1.48,  $\delta$  2.01,  $\delta$  3.17,  $\delta$  3.41,  $\delta$  3.49,  $\delta$  3.69,  $\delta$  3.99 and carbon at  $\delta$  35.0,  $\delta$  35.0, 77.2, 73.5, 72.5, 68.2, 68.1 respectively. The OH- stretch at 3394.0 in the IR spectroscopy confirms the presence of alcohol in the compound. Compared with literature, isolated compound was identified as 1, 2, 3, 4, 5-cyclohexanepentol (Table 2).



**Figure 3:** Isolated compound from ethyl acetate fraction of *Cissampelos owariensis* leaves

**Table 2:**  $^1\text{H}$  and  $^{13}\text{C}$  NMR of isolated compound and with respect to literature

Position	$^1\text{H}$ NMR (ppm), $J$ (Hz)	$^{13}\text{C}$ NMR (ppm)	Literature (Angyal and Odier, 1982)
1	3.99 q, (3.2, 3.16) $^1\text{H}$	68.1	67.2
2	3.41 dd (3.1, 6.8) $^1\text{H}$	73.5	73.6
3	3.49 t, (8.96, 10.0) $^1\text{H}$	72.5	72.5
4	3.17 t, (9.2) $^1\text{H}$	77.2	75.0
5	3.69 m, $^1\text{H}$	68.2	70.0
6	2.01 m, $^1\text{H}$ ; 1.48 m, $^1\text{H}$	34.8	34.6

dd – double doublet, t – triplet, q – quadruplet, m - multiplet

The pooled sub-fractions from EtOAc fractions of CO, at 0.2 mg/mL as well as the standards, (Haloperidol and Risperidone) at 0.1 mg/mL, inhibited Pig kidney D-amino acid oxidase enzyme *in vitro*. From the result, pooled sub-fraction IX (33.7%) exhibited the

highest percentage inhibition, which was close to the standard risperidone (43.2%). Percentage inhibition obtained from pooled sub-fractions VII (19.0%), VIII (22.1%) and X (21.3%) are comparable to that of haloperidol (18.3%) as presented in Table 3.

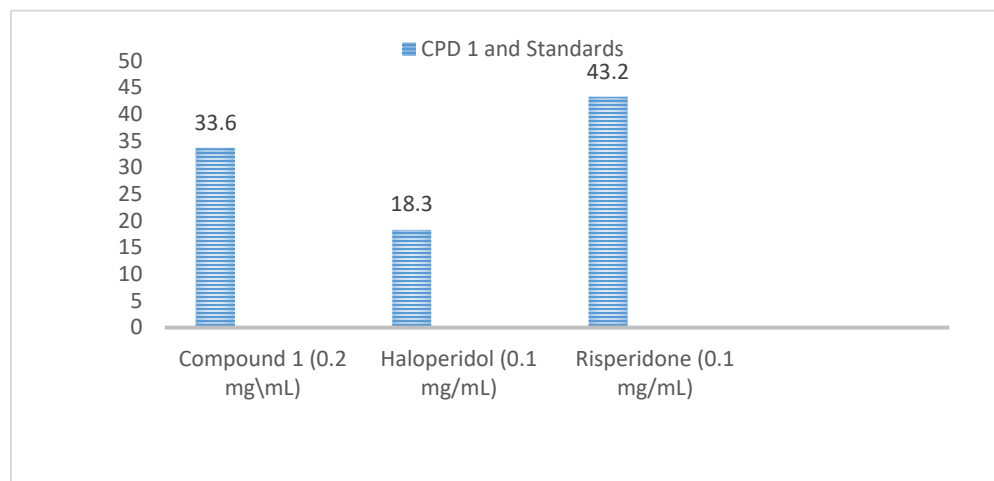
**Table 3:** Pooled sub-fractions from EtOAc fraction of *Cissampelos owariensis*

Pooled fractions	Range of Pooled sub-fractions	Solvent system (ratio)	Characteristics colour of sub-fractions	Yield (mg)	% inhibition @ 0.2 mg/mL
I	W – 68	DCM : EtOAc (1.5:3.5)	Light yellow	4.7	2.4
II	69 – 82	DCM : EtOAc (3.5:1.5)	Light cream	3.9	ND
III	83 – 88	DCM : EtOAc (3.5:1.5)	Light cream	3.9	ND
IV	89 – 108	DCM : EtOAc (3.5:1.5)	Orange	85.1	15.6
V	109 – 111	DCM : EtOAc (0.5:4.5)	Greenish Yellow	70.5	13.8
VI	112 – 117	DCM : EtOAc (0.5:4.5)	Yellow	107.8	-75.1
VII	118 – 124	DCM : EtOAc (0.5:4.5)	Yellow	162.7	19.0
VIII	125 – 147	EtOAc: MeOH : H <sub>2</sub> O (0.5:3.0:1.5)	Orange red	627.9	22.1
IX	148 – 172	EtOAc: MeOH : H <sub>2</sub> O (0.5:3.0:1.5)	Dark red	17703.3	33.7
X	173 – 189	EtOAc: MeOH : H <sub>2</sub> O (0.5:3.0:1.5)	Dark brown	5570.7	21.3
XI	190 - 222	EtOAc: MeOH : H <sub>2</sub> O (0.5:3.0:1.5)	Dark brown	6380.6	45.4
Haloperidol (0.1 mg/mL)	-	-	-	-	18.3
Risperidone (0.1 mg/mL)	-	-	-	-	43.2



The antipsychotic potential of the pooled sub-fractions and isolated compound (cyclohexanepentol), was determined using the DAO inhibitory assay. This is important because there is need for safe and effective alternative therapy in the treatment of psychotic disease. The result of percentage inhibition of the

enzyme D – amino acid oxidase by isolated compound as well as standards (Risperidone and Haloperidol) are shown in Figure 4. The compound at 0.2 mg/mL showed 33.6% inhibition and the standards, Risperidone and Haloperidol at 0.1 mg/mL gave percentage inhibition of 43.2% and 18.3% respectively.



**Figure 4:** % inhibition of D-amino acid oxidase enzyme by cyclohexanepentol (isolated compound), haloperidol and risperidone (antipsychotic standards)

Recently, cyclic small molecule compounds have been reported to inhibit DAO *in vitro* (Ferraris and Tsukamoto, 2011), benzoic acid binds parallel to the co-factor (flavine ring) and interacts with the amino acid Tyr224 through hydrogen bond. The isolated compound is also a small molecule classified in the carbohydrate (alcohol) group. It may exerts its inhibitory action through intermolecular hydrogen

bond between the hydroxyl group of the compound and the cofactor of the enzyme. The result herein shows that cyclohexanepentol possesses pkDAO-inhibitory effect *in vitro*. Therefore, the antipsychotic properties of *Cissampelos owariensis* crude extracts and fractions *in vivo*, the DAO inhibitory activity of cyclohexanepentol may contribute to the antipsychotic properties of this plant.

## CONCLUSION

*Cissampelos owariensis* crude extract and fractions possess anti-psychotic property in the behavioural studies. The compound, cyclohexanepentol, a cyclic alcohol identified from *Cissampelos owariensis* EtOAc fraction, was shown to inhibit D-amino acid

oxidase, an enzyme responsible for the hypo functioning of the NMDA receptor in the brain. This suggests that this compound may contribute to the antipsychotic activity of the plant in traditional medicine, and is reported firstly in this study.

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## ETHICAL APPROVAL

The experiments were performed after protocol approval by the Ethics Committee of the COMSATS Institute of Information Technology (CIIT), Abbottabad, Pakistan, with reference number Phm.Eth/SP17-CS-M10/17-010-69.

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