



Anti Psychotic Evaluation and GC-MS Analysis of *Cassia occidentalis* Leaves

D. Suchitra^{1*}, D. Kilimozhi², B. A. Vishwanath¹ and Venkatesan Natarajan¹

¹Aditya Bangalore Institute of Pharmacy Education and Research,
Bangalore – 560 064, Karnataka, India.

²Department of Pharmacy, Annamalai University, Annamalai Nagar- 608 002,
Tamil Nadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author DS carried out the experiments analyzed the data and wrote the manuscript. Author VN supervised the experimental design and laboratory analysis. Author DK corrected the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the effect of an active fraction from *Cassia occidentalis* leaves on Wistar rats against psychosis were investigated.

Place and Duration of Study: This study was carried out in the Aditya Bangalore Institute of Pharmacy Education and Research, Bangalore, India between June -2019 to July – 2020.

Methods: For the assessment of neuroleptic activity of the *Cassia occidentalis* leaves with different antipsychotic animal models, Chloroform and ethanol extract (200 mg.kg⁻¹) were used for the study with different animal models. The extract showing higher anti psychotic activity was subjected to column chromatography and led to the isolation of an active fraction and examined in GC-MS analysis.

Results: A significant decrease of amphetamine-caused stereotype and conditioned avoidance reaction turned into found with extract treated animals as in comparison to control. Phencyclidine induced weird sample of locomotor activity and social withdrawal test in test extracts does no longer proven any significant activity as in comparison to control. Minor symptoms of catalepsy have been

*Corresponding author: E-mail: k.suchi07@gmail.com;

seen extract-treated group and decreased dopamine level in the frontal cortex and corpus striatum in comparison to the control group. GC-MS evaluation was identified two active principles present in the eluted fraction.

Conclusion: The study revealed that the ethanol extract of *Cassia occidentalis* may possess the property to alleviate the positive symptoms of psychosis.

Keywords: *Cassia occidentalis*; neuroleptic; column chromatography; GC-MS analysis.

1. INTRODUCTION

Psychopharmacology is the systematic study of the effects of drugs on mood, sensation, thinking and behavior [1]. Psychiatry denotes to a field of medicine focused specifically on the mind, aiming to study, prevent, and treat mental disorders in humans [2]. The condition often co-exists with other chronic ailments that amount to even greater morbidity and mortality rates. According to the WHO, disability due to mental illnesses is greater than cancer and heart disease in developed countries [3]. Public concern on mental health has noticeably increased given the high prevalence of neuropsychiatric disorders. WHO reports approximately 450 million of people suffer by mental or behavioral disorder [4]. Two-thirds of the anxious, depressed or psychotic patients react to the currently available treatments; but their clinical uses are limited by their side effects such as psychomotor injury, potentiating of other central depressant drugs and dependence liability. In the hunt for novel therapeutics for the management of neurological disorders, medicinal plant research has also contributed by demonstrating pharmacological effectiveness of different herbs in various animals models [5,6]. When one looks at the history of psychiatric treatments, prior to availability of electroconvulsive therapy (ECT), measures like magic, restraints, bloodletting, emetics, purgatives, surgical operations on various organs, removal of foci of infections, vaccines and endocrines were tried as treatment options for schizophrenia. [7]. However, the era of pharmacotherapy for treatment of schizophrenia started with use of chlorpromazine by Delay and Deniker for the treatment of patients suffering from schizophrenia in early 1950's. Over the next half century, a large number of drugs have been evaluated and marketed as antipsychotics. This class of drugs also helped in understanding the neurobiology of schizophrenia to some extent. This class of drug has also changed the attitude of the clinicians towards the expected outcome of the disorder.[8] Herbal treatments are gaining emergent attention because of their cost-effective, eco- friendly features and true relief

from illness. Since antique tense the herbal remedies are effective in the control of some complaints. Various plants have a folklore claim in the dealing of some dreadful syndromes, but they are not scientifically exploited and/or incorrectly used. Thus, this plant dose demerit particularized contemplation in the luster of neoteric cure [9].*Cassia occidentalis* has been found to possess significant anti- bacterial, antifungal [10,11], antimalarial [12], anti-inflammatory[13], antimutagenic/anticarcinogenic [14], immunostimulant, laxative, analgesic, chloretic, and diuretic properties [15]. The main phytochemicals in *C.occidentalis* include achrosin, aloe-emodin, emodin, islandicine, kaempferol, obtusifolin, obtusin, physcion [16], anthraquinones, apigenin, aurantiobutinin, campesterol, cassiollin, chrysoobutinin, chrysophanic acid, chrysarobin, chrysophanol [17], chrysoeriol, funiculosin [18], quercetin, rhamnosides, rhein, rubrofusarin, sitosterols, tannins, and xanthorine [19]. A study of phytochemicals of *C. occidentalis* reveals that the nature and amount of phytochemical vary according to the climate and soil conditions of the growing location. For example, stems, leaves, and root bark of the plant from Ivory Coast, Africa, contain no alkaloids, while a large amount of alkaloids was found in the samples collected from India [20]. Even though *C. occidentalis* finds a large number of uses in traditional medicine as well as in ethnic food, its physico- chemical properties and nutritional value remains unexplored. Hence, in the present paper, attempts were made to evaluate the anti psychotic activity and GC-MS analysis of ethanolic extract of *cassia occidentalis* leaves.

2. MATERIALS AND METHODS

2.1 Preparation of Different Plant Extracts

Cassia occidentalis leaves were collected from the forest of kalakatu, Tirunelveli District, India. Taxonomic identification was made from botanical survey of medicinal plants, Siddha Unit, Government of India, Palayamkottai, authenticated by ChelladuraiBotonist. A voucher specimen No (CCRAS-1015/2018). Fresh plant leaves were shade dried at room temperature,

ground into fine powder and stored in airtight containers. Then extracted (amount 500 g) with solvents of increasing polarity such as petroleum ether, chloroform and ethanol, for 72 hours with each solvent, by continuous hot extraction using the soxhlet apparatus at a temperature of 60°C. The extracts were concentrated under reduced pressure using a rotary evaporator to constant weight. The extracts were collected and preserved in a desiccator until used for further studies.

2.2 Drugs and Chemicals

Phencyclidine and amphetamine were purchased from India mart, Bangalore. Risperidone sample was gifted by mylan Lab, Bangalore, India.

2.3 Preliminary Phytochemical Screening

Cassia occidentalis leaves extract was subjected to various chemical tests for determination of its phytochemical constituents according to standard method [21].

2.4 Experimental Animals

Wistar rats were weighing 150-200 gm, were used for all sets of experiments in four groups of six animals. They were maintained at controlled room temperature (25±2°C) on 12 hour light/dark cycle and allowed free access to food and water.

2.4.1 Acute toxicity study

Acute toxicity study was performed for chloroform and ethanol extracts of *Cassia occidentalis* according to the acute toxic classic method as per guidelines prescribed by OECD-423. 2000 mg/kg of extract was administered as per OECD guidelines per orally to 6 mice. Effects were observed on behavior for 72 hours. Mice were examined for behavioral effects 45 minutes post administration of the extracts. No change in behavior or any abnormality in behavior was observed and no mortality was seen. Thus it was concluded that chloroform and ethanol extract of *Cassia occidentalis* was nontoxic up to 2000 mg/kg doses. Then 1/10th of the administered dose was selected for future studies as per OECD-423 guidelines.

2.5 Treatment

For assessment of antipsychotic activity of *Cassia occidentalis* extracts, the animals were divided in to four (I-IV) groups. Group I served as control and administered with the vehicle (normal saline 1 ml/100 g/b.w.) Group II rats were

received standard drug (Risperidone) Group III rats were received chloroform extract of *Cassia occidentalis* at dose of 200 mg.kg⁻¹ and Group IV rats were received ethanol extract of *Cassia occidentalis* at dose of 200 mg.kg⁻¹p.o respectively

2.6 Assessment of Antipsychotic Activity

2.6.1 Amphetamine induced stereotype in rats

Amphetamine is an oblique sympathomimetic agent. It induces licking, gnawing, grooming, sniffing (stereotype) in rats which may be efficiently avoided through classical neuroleptic agents. This take a look at is predictive of antipsychotic drug, for D2 receptor antagonism. Two groups (n=6) of adult wistar rats had been taken weighing among 150 to 200gm and had been handled with both test extracts or the standard drug (Risperidone) after which kept in individual cages. They had been injected with amphetamine (5 mg.kg-1, intra peritoneal) after 30 minutes. The onset of stereotypic behavior was evaluated at 30 minutes interval for three hours. The discount in mean stereotype rating is indicative of antipsychotic effect [22].

2.6.2 Phencyclidine induced bizarre pattern of locomotor activity

Phencyclidine is a glutamate receptor antagonist. Administration of phencyclidine has been observed to set off locomotor hyperactivity in rodents and is antagonized by antipsychotic drugs. Wistar rats weighing 150-200gm have been housed in cages. Animals have been divided into 3 groups (n=6), for test extracts and standard drug. 30 minutes earlier than the begin of the experiment, the animals have been administered with the test extracts and standard drug. Phencyclidine (2mg.kg-1) became administered to the animals of all of the groups just begin of the experiment. Then the locomotor activity of the animals may be measured in photoactometer for a consultation lasting for 90 minutes. Drugs antagonizing the phencyclidine induced activity are anticipated to behave by different receptor viz. Glutamatergic and Serotonergic in place of dopaminergic receptors [23].

2.6.3 Phencyclidine induced social withdrawal test

This test allows exposing the effectiveness of capability antipsychotic drugs towards terrible signs of schizophrenia. Phencyclidine decreases the time of social interaction in the rats. Wistar

rats had been housed in pairs for 10 days previous to the begin of the test. During the test one, cage mate is eliminated and new one is kept with inside the cage for 20 minutes. The quantity of social interaction is measured as the over all quantity of time spent on diverse factors of inter action i.e. social exploration, and anogenital investigation. Phencyclidine can be administered 5 minutes earlier than the begin of the experiment while the take a test extracts and the usual standard drug can be given 30 minutes earlier than the experiment [23].

2.6.4 Conditioned avoidance response in rats

Perhaps the oldest animal model to be expecting ability antipsychotic drug efficacy is the conditioned avoidance reaction. In the conditioned reinforcement model, experimental animals are trained to carry out a certain response i.e. to keep away from a slight shock. Trained avoidance responses can be active (pressing a lever, mountaineering a pole, or leaping out of a box). Classical antipsychotic drugs decrease avoidance responding at doses that don't impair natural (untrained) escape. Three groups of rats (every having twenty rats) weighing 150-250 gm have been examined on this model for test extracts and standard drug. 10 days of training duration have been finished earlier than the experiment, and a complete of 20 sessions of training have been imparted to every rat earlier than the experiment. Test extracts and standard drugs have been administered 30 minutes earlier than the begin of the experiment [24].

2.6.5 Induction of catalepsy in rats

Wistar rats weighing a 150 to 200 gm each are randomly divided in 3 groups (test extracts and standrad drug). After the adequate pretreatment time of the drug, every rat is examined for with respect to the right and left the front paws which can be first placed on columns, first 3 cm after which 9 cm height. The cataleptic phase became taken into considerationif the rat continues the bizarre posture for 10 sec or more. The scoring became completed in line withthe subsequent 0- The rat movements typically whilst located on a desk. 1-Rats flow most effective whilst touched or pushed. 1+1=2 – Rats located on a desk with the front paws set alternately on a 3 cm excessive block fails to accurate the posture in 10 secs, scored as 1 factor for every paw, with a complete of 2 for each paws. 1+1=2 – Rats located on a desk with the front paws set alternately on a 9 cm excessive block fails to

accurate the posture in 10 secs, scored as 1 factor for every paw, with a complete of 2 for each paws. This version predicts the extra pyramidal aspect outcome of the test extracts [24].

2.6.6 Estimation of dopamine in different regions of the brain

The following day of drug administration, the rats were decapitated and the brains were removed immediately according to the method described by Glowinski and Iversen [25]. The striatum and the frontal cortex areas had been removed and had been without delay frozen on dry ice and saved at -80°C. Striatal and frontal cortical tissues had been sonicated in 0.1 M of perchloric acid (approximately a hundred µl/mg tissues). The supernatant fluids had been taken for measurements of stages of dopamine through HPLC. Briefly, 20 µl of supernatant fluid become isocratically eluted via a 4.6-mm C18 column containing paracetamol (100 mg/ml) as the internal standard with a mobile phase containing 50 mM ammonium phosphate pH 4.6, 25 mM hexane sulfonic acid pH 4.04, and 5% acetonitrile and detected through a UV spectrophotometer detector. The flow rate become 1 ml/ min. Concentration of dopamine become expressed as nanograms consistent with gram of tissue [26].

2.7 Isolation of the Active Principle from the Ethanol Extract of *Cassia occidentalis*

After the pharmacological screening, it was found that among all the extracts, the ethanol extract of *Cassia occidentalis* showed a maximum anti psychotic effect. Hence, it was subjected to column chromatography and active principle was isolated. The ethanol extract of *Cassia occidentalis* (10g) was subjected to chromatography over a column of silica gel (60 – 120) mesh. The column was eluted successively with petroleum ether, petroleum ether – ethyl acetate mixtures and chloroform – ethanol mixtures in different proportions in the order of increasing polarity. Fractions with same Rf values on TLC were combined and evaporated under reduced pressure. The major active fraction (2.5 g) was obtained after elution with chloroform:ethanol (60 : 40) and was further purified by chromatography over a column of silica gel (100 – 200 mesh) resulting in a yellow amorphous solid (2.0 g) after elution with chloroform:ethanol (50 : 50). The isolated fraction was examined by GC-MS analysis [27].

2.8 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis and Identification of the Active Principle

After the pharmacological screening, it was found that among both the extracts, the ethanol extract of *Cassia occidentalis* showed a maximum antipsychotic effect. Hence ethanol extract was subjected in GC-MS analysis and active principles were identified. GC-MS analysis of ethanol extract of *Cassia occidentalis* was followed by method Milne, 1971[28]. The spectrum of unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

2.9 Statistical Analysis

Data are expressed as $\bar{x} \pm \text{SEM}$. Statistical analysis was performed by one-way analysis of variance (ANOVA). The least significant difference test was used for mean comparisons and $p < 0.05$ was considered to be statistically significant.

3. RESULTS

3.1 Phytochemical Screening

Phytochemical Screening of *Cassia occidentalis* leaves has indicated the presence of Saponins, flavonoids, proteins, alkaloids, tannins and terpenoids.

3.2 Acute Toxicity Study

2000mg/kg of extract was administered as per OECD guidelines per orally to 6 mice. Effects were observed on behavior for 72 hours. Mice were examined for behavioral effects 45 minutes post administration of the extracts. No change in behavior or any abnormality in behavior was observed and no mortality was seen. Thus it was concluded that chloroform and extract of *Cassia occidentalis* was non toxic up to 2000 mg/kg doses. Then $1/10^{\text{th}}$ of the administered dose was selected for future studies as per OECD-423 guidelines.

3.3 Assessment of Antipsychotic Activity

3.3.1 Amphetamine induced stereotype in rats

Results from this examine suggests that each one stereotypic activities like sniffing, rearing and licking had been decreased significantly ($p < 0.05$) in the std drug and ethanol extract (200 mg/kg^{-1}) whilst in comparison with control group, however

no widespread reduction with the chloroform (200 mg.kg^{-1}) extract whilst in comparison with control and the data are summarized in Table 1.

3.3.2 Phencyclidine induced bizarre pattern of locomotor activity

Results from this study observe that locomotor activity has now no longer been confirmed any tremendous activity ($p < 0.05$) in test extracts and STD drug while in comparison with control group and the data are reported in Table 2.

3.3.3 Phencyclidine induced social withdrawal test

Results from this examine study that social exploration and the anogenital inspection activity has now no longer been confirmed any tremendous activity ($p < 0.05$) in the STD drug and test extracts whilst as compared with control group and the data are presented in Table 3.

3.3.4 Conditioned avoidance response in rats

All the treated groups significantly showed the avoidance response activity compared to the control group ($p < 0.05$) and the data are summarized in Table 4.

3.3.5 Induction of catalepsy in rats

All the treatment groups increased the mean cataleptic scores significantly ($p < 0.05$) compared with the control group and the data are presented in Table 5.

3.3.6 Estimation of dopamine in different brain regions

Estimation of dopamine in the two areas of the mind counseled that the dopamine stages reduced in the frontal cortex for all of the treatment groups including the standard. But the lower in dopamine awareness became extra for the standard drug than the chloroform and the ethanol extract while in comparison with the control ($p < 0.05$). In any other hand there have been no tremendous changes in the striatum dopamine levels of the animals treated with the chloroform and ethanol extract while in comparison with the control group, however there has been a significant ($p < 0.05$) changes in striatum dopamine level in the standard drug while in comparison with the control group and the data are summarized in Table 6.

3.4 Identification of the Active Principle

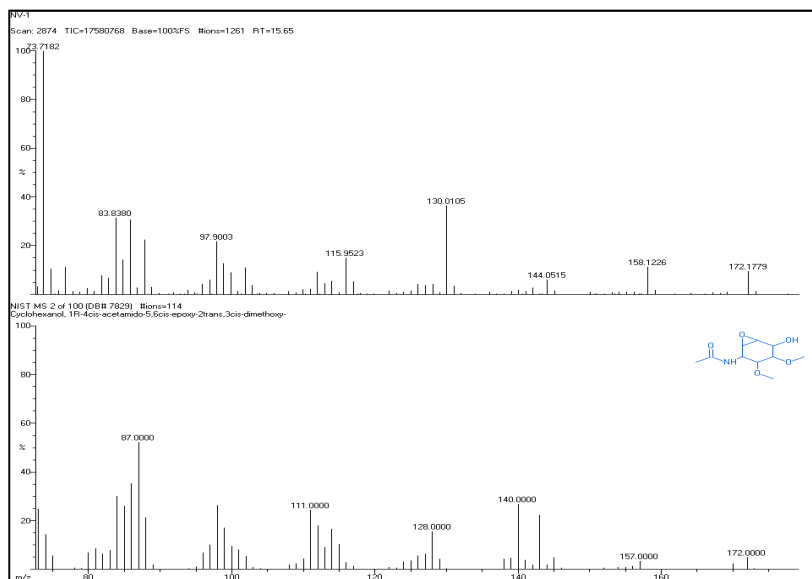
Ethanol extract of *Cassia occidentalis* was found to be the active principles confirmed by

GC-MS analysis. Complete structure of compounds which was identified by a mixture of cyclohexanol and nonadecene, as shown in Fig 1.

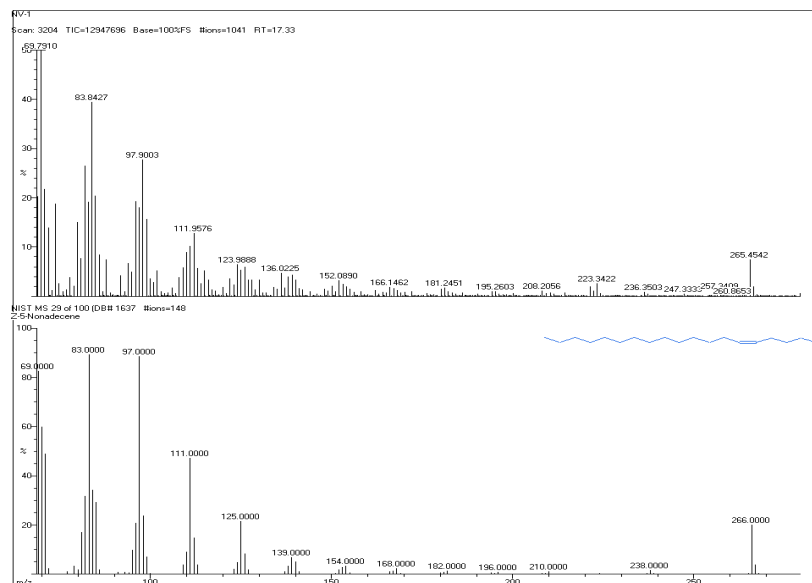
4. DISCUSSION

Improving the effectiveness of antipsychotics seems to require right and precise modulation of the numerous Dopamine pathways. For Instance, lessened extrapyramidal signs located with newer agents are idea to be consequent to differential

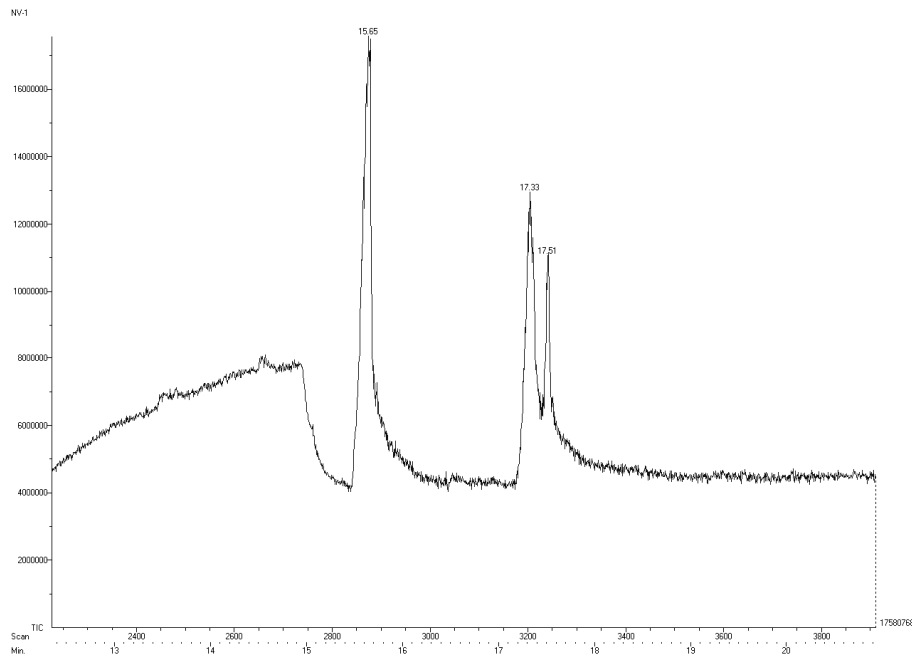
results at the striatum and frontal cortex, respectively [29]. Risperidone and each extract of *Cassia occidentalis* confirmed lower in amphetamine precipitated stereotype as in comparison to the control group. However the extend of lower of the stereotypic activity for each the extract of *Cassia occidentalis* changed into much less in comparison to the standard drug risperidone. This type of final results changed into indicative of an opportunity that the test extracts can



(a) Mass spectrum of isolated fraction Cyclohexanol



(b) Mass spectrum of isolated fraction Nonadecene



(c) Gas chromatogram of isolated fraction of ethanol extract

Fig. 1. (a) Mass spectrum of isolated fraction Cyclohexanol; (b) Mass spectrum of isolated fraction Nonadecene; (c) Gas chromatogram of isolated fraction of ethanol extract**Table 1. Inhibition of amphetamine induced stereotype ($\bar{x} \pm \text{SEM}$, $n = 6$)**

Treatment	Sniffing	Rearing	Licking
Group I (Control)	21.5 \pm 1.26	8.96 \pm 0.72	5.69 \pm 0.08
Group II (Std Drug Risperidone 10 mg.kg ⁻¹)	12.6 \pm 1.54*	5.64 \pm 0.34*	4.13 \pm 0.16*
Group III (Chloroform Extract 200 mg.kg ⁻¹)	19.4 \pm 2.61	9.23 \pm 0.13	5.78 \pm 0.12
Group IV (Ethanol Extract 200 mg.kg ⁻¹)	14.6 \pm 1.71*	6.42 \pm 0.28*	4.09 \pm 0.98*

N=6, *P<0.05 when compared with control

Table 2. Phencyclidine induced bizarre pattern of locomotor activity ($\bar{x} \pm \text{SEM}$, $n = 6$)

Treatment	Locomotor activity scores
Group I (Control)	415.56 \pm 2.08
Group II (Std Drug Risperidone 10 mg.kg ⁻¹)	425.66 \pm 3.11
Group III (Chloroform Extract 200 mg.kg ⁻¹)	410.82 \pm 2.56
Group IV (Ethanol Extract 200 mg.kg ⁻¹)	418.73 \pm 1.09

N=6, *P<0.05 when compared with control

Table 3. Phencyclidine induced social withdrawal test ($\bar{x} \pm \text{SEM}$, $n = 6$)

Treatment	Social exploration	Anogenital inspection
Group I (Control)	18.4 \pm 2.12	7.43 \pm 1.12
Group II (Std Drug Risperidone 10 mg.kg ⁻¹)	16.1 \pm 1.14	6.91 \pm 0.34
Group III (Chloroform Extract 200 mg.kg ⁻¹)	18.3 \pm 3.04	7.78 \pm 1.81
Group IV (Ethanol Extract 200 mg.kg ⁻¹)	17.8 \pm 2.76	7.23 \pm 0.27

N=6, *P<0.05 when compared with control

Table 4. Conditioned avoidance response in rats ($\bar{x} \pm$ SEM, $n = 6$)

Treatment	No. of times escaped
Group I (Control)	24.2 \pm 0.14
Group II (Std Drug Risperidone 10 mg.kg ⁻¹)	18.3 \pm 1.23*
Group III (Chloroform Extract 200 mg.kg ⁻¹)	20.8 \pm 0.57*
Group IV (Ethanol Extract 200 mg.kg ⁻¹)	19.9 \pm 1.51*

*N=6, *P<0.05 when compared with control*

Table 5. Induction of catalepsy in rats ($\bar{x} \pm$ SEM, $n = 6$)

Treatment	Mean cataleptic scores
Group I (Control)	1.23 \pm 0.12
Group II (Std Drug Risperidone 10 mg.kg ⁻¹)	4.21 \pm 0.21*
Group III (Chloroform Extract 200 mg.kg ⁻¹)	2.02 \pm 0.42
Group IV (Ethanol Extract 200 mg.kg ⁻¹)	3.58 \pm 0.87*

*N=6, *P<0.05 when compared with control*

Table 6. Estimation of dopamine in different regions of the brain ($\bar{x} \pm$ SEM, $n = 6$)

Treatment	Frontal Cortex (ng/gm)	Corpus Striatum (ng/gm)
Group I (Control)	0.53 \pm 0.1	13.9 \pm 0.6
Group II (Std Drug Risperidone 10 mg.kg ⁻¹)	0.37 \pm 0.2*	9.06 \pm 0.7*
Group III (Chloroform Extract 200 mg.kg ⁻¹)	0.42 \pm 0.4	13.7 \pm 0.6
Group IV (Ethanol Extract 200 mg.kg ⁻¹)	0.40 \pm 0.2	12.0 \pm 0.2

*N=6, *P<0.05 when compared with control*

Be lowering the dopamine level in the brain. The test extracts and standard drug altered the phencyclidine induced increase in locomotor activity. In effectiveness of the extracts to expose any effect in this model recommended that the extracts might not be performing on different neurotransmitter structures like glutamatergic or serotonergic structures [30]. The test extracts along with the standard drug did not longer have any effect at the phencyclidine induced social interaction test. This unique model changed into so suggestive of the ineffectiveness of the test extracts to relieve the poor signs of schizophrenia [31]. It is another time showed that risperidone has no impact at the poor signs of schizophrenia. The test extracts in addition to the standard drug decreased the conditioned avoidance response; but the significance of discount changed in to much less for the test extracts than standard drug after they have been as in comparison with the control group. This type of outcomes for the standard drug and the test extracts once more indicated the alleviating results of positive signs of schizophrenia. The induction of catalepsy another time mentioned the truth that each the extracts like standard drug might be performing at the dopaminergic neurons of the brain. Risperidone is understood to lower the dopamine level at the numerous dopaminergic pathways of the brain that's the

purpose for added pyramidal motor disorders. The reduction of the dopamine in the frontal cortical areas of the brain changed into a type of confirmatory end result to set up the mode of antipsychotic movement of the *Cassia occidentalis* leaves extracts. This changed in to the important locating of the observe which changed into indicative and assertive of the mode of movement of the *Cassia occidentalis* leaves extracts. However the dopamine reducing interest for each the extracts changed in to much less, while as in comparison to Risperidone. Nevertheless the unchanged dopamine levels in the corpus striatum for each the extracts no matter the dose changed into additionally a significant statement for this observation. This observation pin pointing the truth that the tests extract might not have an effect on any type of motor in coordination like that of the standard neuroleptic drugs [32]. Discuss all of the above information in to consideration; it is able to be secure to mention that the ethanolic extract of the *Cassia occidentalis* leaves lower the dopamine level in the frontal cortical region of the brain. Among both extracts ethanol extract showed more anti psychotic activity compared with chloroform extract, so the ethanol extract was fractionated by column chromatography which led to the isolation of several specific compounds from the isolated fraction and

identification by GC-MS. After performing a GC-MS study of the ethanol extract of *Cassia occidentalis* the GC chromatogram revealed two products at retention times of 15.65 and 17.33 min, respectively. Further, the mass spectrum of the compound having RT 15.65 min confirmed the structure of cyclohexanol and at RT 17.33 min was nonadecene from the mass spectrum. At present, the exact mechanism of action of the active principle of cyclohexanol and nonadecene is not yet known and will be the subject of further studies.

5. CONCLUSION

This observation found that the ethanol extract of *Cassia occidentalis* can also additionally own the assets to relieve the high quality signs and symptoms of psychosis. The extract might be in addition isolated and purified the active constituents liable for this type of activity which also can be foremost area of future research.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experiments were performed after the experimental protocols approved by the Institutional Animal Ethics Committee of Aditya Bangalore Institute of Pharmacy Education and Research (Approval No. 43/1611/CPCSEA).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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