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Isolation and Characterization of Anti Psychotic Compound From *Cassia Occidentalis* Leaf

Suchitra D[*1], Kilimozhi D[2], Vishwanath B A[1], Venkatesan Natarajan[1]

[1]Department of Pharmaceutical Chemistry, Aditya Bangalore Institute of Pharmacy Education and Research, Bangalore – 560064, Karnataka, India
[2]Department of Pharmacy, Annamalai University, Annamalai Nagar- 608002, Tamil Nadu, India

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ABSTRACT

The aim of the current work was mainly to find the neuroleptic effects of responsible compounds isolated from *Cassia occidentalis* leaves, on condiontioning avoidance and catalepsy induced psychotic wistar rats. Psychosis was induced by condiontioning avoidance and catalepsy induced in albino wistar rats. An isolated fraction of the ethanolic extract of *Cassia occidentalis* was ingested orally at 100 mg/kg, p.o dose. Haloperidol was considered as standard neuroleptic drug (10 mg/kg, p.o.). The isolated fractions from the column chromatography showing for the higher neuroleptic activity that facilitated for isolating the pure constituent, that was named trivially as name CO- 1.Precise analysis of the previous investigations with Ethanolic extract of *Cassia occidentalis* (ETCO) have encouraged us to isolate anti-psychotic responsible consituents from the leaves of *Cassia occidentalis* for managing the anti-psychotic activity. Collected pooled fractions were subjected to anti-psychotic activity in conditioning avoidance and catalepsy induced wistar rats. The fraction F from ETCO showed strong neuroleptic activity when compared with the commercial standard drug Haloperidol. To propose the constituents that possess anti psychotic activity related to F respectively. Besides, column chromatography analysis was performed with 'F' using different solvents and the isolated compound which is named as CO-1. This amorphous powder was extracted from the column with decomposition point. CO-1 is a phenolic compound that was confirmed by IR, Mass Spectrum and NMR analysis.

*Corresponding Author

Name: Suchitra D
Phone: +91-9611564505
Email: k.suchi07@gmail.com

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INTRODUCTION

Natural herbal products, for example, plants isolates, and unadulterated compounds or as normalized separates, give more opportunities to new medication discovery due to the unequaled accessibility of chemically diverse constituents (Cos *et al.*, 2006). Based on the World Health Organization (WHO) report, over 80% of the total population depends on traditional medication for their essential medical care needs. The utilization of natural medicine in Asia speaks to a long history of human communications with the earth. Plants utilized for traditional medication contain a wide scope of substances that can be utilized to regard interminable just as irresistible diseases (Duraipandiyan *et al.*, 2006). Because of the advancement of antagonistic impacts and microbial resistance from the synthetic medications, human turned to ethno-pharmacognosy. They discovered actually a large number of phyto-

chemicals from plants as protected and extensively powerful options with less adverse impact. Various helpful natural movement for example, antimicrobial, anticancer, antidiarrheal, cancer prevention agent, analgesic and wound-healing action were reported. By and large the individuals guarantee the great advantage of certain natural medicines. Nonetheless, clinical studies are important to show the adequacy of a bioactive compound to confirm this conventional case. Clinical studies are carefully planned to defend the wellbeing of the members just as answer explicit exploration investigations by assession for both quick and long term symptoms and their respective results are proposed before the drug is generally applied to patients. As per the World Health Organization (WHO), about 20,000 restorative herbs present in more than 90 nations including the 12 super biodiversity countries (Sasidharan *et al.*, 2010). Psychosis is a mental illness issue described by different side effects influencing thought, feeling, perception,volition and general prosperity of the victims. Serious mental illness patients in India are generally overseen by conventional clinical experts, who utilize assortments of therapeutic used of medicinal plants. *Cassia occidentalis* (Caesalpiniaceae) plant has been broadly utilized in indigenous and folklore medication framework in India. In Indian arrangement of medication the plant has been archived as thermogenic, purgative, expectorant, diuretic, and utilized in the treatment of leprosy, erysipelas, ulcers, bronchitis, obstruction, dyspepsia, menstrual issues and tuberculosis (Al-Snafi, 2015). So target of our objective was to isolate the neuroleptic active compound from ethanolic extract *of Cassia occidentalis* leaves.

MATERIALS AND METHODS

Preparation of various plant extracts

Cassia occidentalis leaves had been gathered from the wooded area of, Tirunalveli District, India. The Taxonomic identification was performed from the botanical survey of medicinal plants, Unit of Siddha Medicine, aunthenticated by Chelladurai, Botanist (Voucher specimen No. CCRAS-1015/2018). Collected plant leaves had been dried under sun shade, grind into powder and stored in sealed shut holders. Then extracted in (500 g powdered drug) with solvents of varying polarity of Pet ether, ethyl acetate & ethanol for about 72 hours with every solvent by hot continuous extraction by the utilization of the soxhlet apparatus at 60^{o}C temperature. The extracts were concentrated underneath diminished pressure utilizing a rotating evaporator to consistent weight. The extracts had been amassed and safeguarded in a desiccator until utilized for additional studies (Natarajan and Dhas, 2013).

Post extraction processing of the chemical compounds from the ethanol extract

Chromatographic procedures have been utilized for isolation of the pure compound from the available fractions. The column chromatography procedure mostly used for separation of compounds into various fractions in step with the partiality or solvating limit of the compounds to the solvents used. The structure of the compound had been endeavored to set up through spectroscopic techniques (Huie, 2002).

Study design

Perform a column chromatography; a solvent was mounted with the guide of developing TLC procedure. The stationary phase was made using silica gel powder. This was passed through 100-200 mesh size of filter and slurry was prepared with the solvent system that was introduced ahead of time. The slurry became emptied along with the time into the segment cautiously and the silica gel became permitted to settle right down to from an even pressing. At that point the stop-cock of the section changed into opened and the abundance of solvents over the segment head became permitted to run. The dry crude ethanol extract (10g) changed into blended with limited quantity of silica gel powder in a mortar to get a free streaming powder. The powdered sample changed into then executed carefully at the zenith of the readied section and productively eluted with solvet/solvent system the usage of different solvent structures comprising of Pet. ether, Pet. ether: chloroform, chloroform:ethyl-acetate, ethyl-acetate alone, ethylacetate: methanol and methanol alone to isolate the eluate. The eluate with equivalent R_f value are pooled collected and vanished to dryness. At the point when the blend of solvent system utilized, the proportion of ratio are set up by lowering and equalizing the concentrations as follows. 10: 90, 90:10,80:20,70:30,60:40,50:50,40:60,30:70,20:80. Elutes had been assembled in some of beaker set apart from parts 1-100. Elutes had been seen effectively on TLC Plate and the beakers having comparative spots were combined with others (Venkatesan and Smith, 2014).

Experimental Animals

Wistar rats (150-200 gm), were employed for the experimental section with six rats in four groups. They were acclimatized in a controlled room environment where temp is maintained at (25±2°C) on 12-hour night and light cycle and allowed for a

free access to feed and water. These investigations were performed after getting approved by the Institutional Animal Ethics Committee of Aditya Bangalore Institute of Pharmacy Education and Research (Approval No. 43/1611/CPCSEA) for all the experimental protocols.

Acute Oral Toxicity study

Acute oral toxicity study was studied for different fraction of ethanol extract of *Cassia occidentalis* (ETCO) as per the acute toxic classic method according to rules recommended by OECD-423. 1000 mg/kg of extract was directed according to OECD guidelines in oral route to 6 mice. Impacts were seen on conduct for 72 hours. Mice were analyzed for social impacts 45 mins after the administration of extracts. No changes in behaviour or any abnormality in conduct was noticed and mortality was not seen. In this manner it was reasoned that chloroform and ethanol extract of *Cassia occidentalis* was nontoxic up to 1000 mg/kg dosed. At that point 1/tenth of the ingested dose was selected for future examinations according to OECD-423 guidelines.

Treatment

For assessment of antipsychotic activity of various fractions of ethanol extracts of *Cassia occidentalis*, the animals were divided into four (I - VIII) groups. Group I had served as control group and administered with the vehicle solution (normal saline 1 ml/100 g/b.w.) Group II rats were received standard drug (Haloperidol) Group III - VIII rats were received various isolated fractions of ethanol extracts of *cassia occidentalis* at dose of 100 $mg.kg^{-1}$ p.o respectively.

Assessment of antipsychotic activity

Evaluation of Conditioned avoidance in rats

In this model, experimental rats are prepared to perform a specific response, for example to stay away from a mild shock. Prepared avoidance responses might be dynamic (squeezing a lever, climbing a shaft, or jump out a box). Traditional antipsychotic drugs diminish avoidance response at doses that do not debilitate a normal escape. Three groups of rats (each having 6 rats) weighing 150-250 gm were investigated for test extract and standard drug. 10 days of preparing period were completed before the test, and a sum of 20 sessions of preparing were given to each rat before the start of test. Test concentrates and the standard drug were administered 30-mins before the beginning of the test (Matthysse, 1986).

Catalepsy Induction in Rats

Wistar rats were weighing 150 to 200 gm each are arbitrarily divided into three groups (test extracts and standard drug). After a suitable pre-treatment time of the constituent, every rat is screened for concerning the privilege and left side front paws are first kept on columns, the initial 3cm and then 9cm tallness. The state of catalepsy was considered if the rat remains in the normal posture for 10 sec or more. The scores were finished by the accompanying 0- The rodent moves regularly when set on a experimental table. 1-Rats adjust only when contacted or pushed. 1+1=2 – Rats set on a table with their front paws that were set then again on a 3 cm high square neglect to address the posture in 10secs, scores1-point for each paw, with 2 for the two paws. 1+1=2 – Rats set on the table with front paws kept then again on a 9cm high square neglect to correct the posture in 10 secs, scores as 1point for each paw, 2 for the two paws. This model infers the extrapyramidal symptoms of the test extract (Matthysse, 1986).

Isolated Fraction Purifying

About 1 gm of isolated fraction is measured and blended in with silica-gel and filled the column. The column was eluted with various solvents on the basis of polarity. Aliphatic fractions are isolated via the solvent Pet ether. In the solvent, few distinctive bands are created. That fraction is gathered and desiccated till dry. The aromatic parts of fractions are isolated by the mixture of n-hexane:ethyl-acetate in a ratio of 1:1. The polar portion situate in the extract is eluted by use of chloroform. The remaining polar part of fraction of the extract is eluted by utilization of methanol (Cannell, 1998).

Fraction analysis

The fraction became characterized via spectrum techniques like FT-IR, NMR spectrum and Mass spectrometer (Harbone, 1984).

Statistical analysis

Data is represented as mean±SEM. The analysis was carried out using one-way ANOVA employing by Dunnett's multiple comparison tests by using Instat-3 software package (Graph pad), Prism Ltd, USA.

RESULT

Column Chromatography Study with Ethanolic extract of *Cassia occidentalis* (ETCO)

The column chromatography has been performed with ETCO to split the eluates specifically F1 - F 40 utilizing pet. Ether alone as a solvent system. F 41 – F75 are the eluates remoted the use of pet. Ether: chloroform solvent, F 76 - F 105 are the eluates remoted the use of chloroform solvent alone, F 106 – F 140 are the eluates remoted the utilization of chloroform: ethyl acetate solvent system, F 141 – F

160 are the eluates remoted the use of ethyl acetate solvent alone, F 161 – F183 are the eluates remoted the use of ethyl acetate: methanol, Finally methanol alone is utilized the elauate F 184 – F200.The volume of the eluate is 50ml. The drying of eluates was done which ran with similar R_f value. They were mixed and then evaporated till dry. The mixture of fractions of ETCO such as F1 – F40 named as A, F41 - F75 named as B, F76 – F105 named as C, F106 – F140 named as D, F141 – F160 named as E, F161-F183 named as F and F184 – F200. The combined eluates of all the fractions mentioned above had been screened in condition avoidance response and induction of catalepsy in rats.

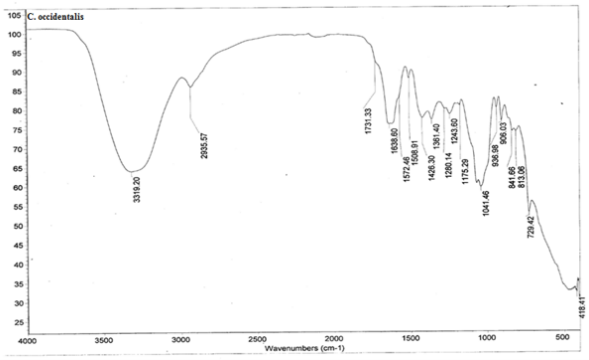

Figure 1: IR Spectrum corresponding to isolated compound CO-1

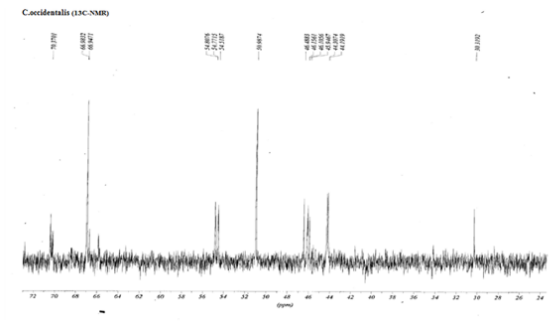

Figure 2: 13C-NMR Spectrum corresponding to isolated compound CO-1

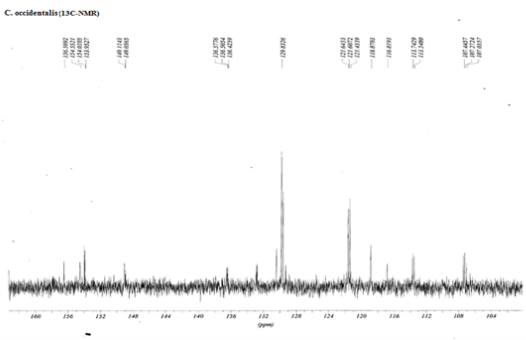

Figure 3: 13C-NMR Spectrum corresponding to isolated compound CO-1

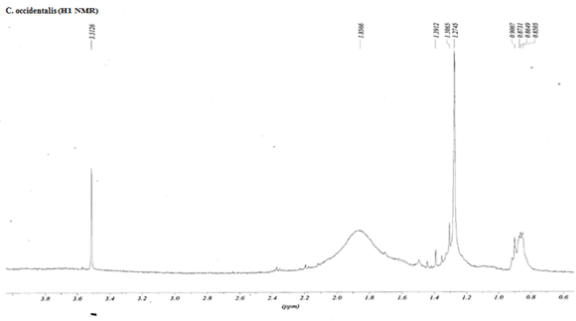

Figure 4: H1-NMR Spectrum corresponding to isolated compound CO-1

Acute toxicity study

1000mg/kg of the extract was ingested as per OECD regulations orally to 6 mice. Effects were noticed for change in behavior for 3days. Mice were observed for the behavioral effects 45mins after the administration of the extracts. There was no change in behavior or no abnormality in behavior was seen. The rats exhibited no mortality. It can be asserted that isolated compound from *cassia occidentalis* was non-toxic up to 1000 mg/kg doses. Then $1/10^{th}$ of the administered amount of drug was fixed for further studies as per OECD-423 guidelines.

Response of Conditioned Avoidance in rats

The investigated groups showed the avoidance response with significant activity in comparison to control batch ($p< 0.05$) and the data are summarized in Table 1.

Induction of catalepsy in rats

The catalepsy score of all the groups were elevated significantly ($p< 0.05$) compared with the control group and the data are presented in Table 2.

Purification of fractions of ETCO by using column chromatography

Based on the observation of the anti-diabetic activity, one of the fractions"F" from ETCO demonstrated more effect. So, the fraction "F" is subjected for further purification of the usage of CC-Column Chromatography, followed by a TLC method. The natures of the fractions are summarized in Table 3.

Characterization of various compounds using analytical methods

IR Spectrum corresponding to isolated compound

The IR spectra displayed characteristic absorption bands at 3319 cm-1 for a – OH streching, Characteristic absorption bands at 1731 cm-1 which showed that the compound has C=O group. The IR spectra exhibited characteristic absorption bands at 1638

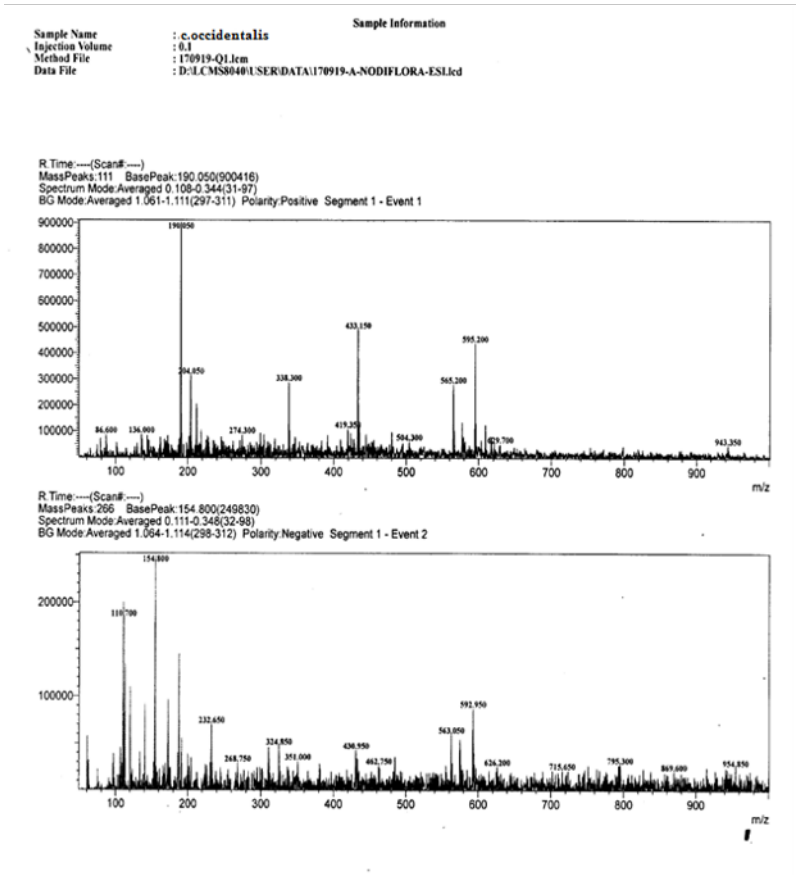

Figure 5: Mass Spectrum corresponding to isolated compound CO-1

Table 1: Conditioned avoidance response in rats ($\bar{x}\pm$ SEM, n = 6)

Treated Group	No. of times escaped
Group I (Control)	32.6 ± 1.45
Group II (Std drug Haloperidol 10 mg/kg)	29.5 ± 2.13*
Group III (Fraction A)	38.5 ± 3.71
Group IV (Fraction B)	39.4 ± 1.83
Group V (Fraction C)	41.2 ± 0.98
Group VI (Fraction D)	40.4 ± 0.88
Group VII (Fraction E)	37.5 ± 1.93
Group VIII (Fraction F)	30.8 ± 0.43*

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

Table 2: Induction of catalepsy in rats ($\bar{x}\pm$ SEM, n= 6)

Treated Groups	Mean cataleptic scores
Group I (Control)	4.45 ± 1.23
Group II (Std drug Haloperidol 10 mg/kg)	5.46 ± 2.08*
Group III (Fraction A)	1.47 ± 1.07
Group IV (Fraction B)	0.45 ± 0.11
Group V (Fraction C)	1.98 ± 1.12
Group VI (Fraction D)	1.43 ± 0.87
Group VII (Fraction E)	0.98 ± 0.12
Group VIII (Fraction F)	4.95 ± 1.59*

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

Table 3: The Column Chromatography Fractions of 'F' from ETCO and their TLC Analysis

S. No	Column solvent system elution	Eluated fractions	Volumes of pooled eluate (ml)	TLC Solvent system	Nature of the compound
1.	Ethyl acetate: Hexane(25:75)	F 1 – F 5	300	Methanol:Ethyl-acetate(20:20)	
2.	Ethyl acetate: Hexane(50:50)	F 6 – F 10	300	Methanol:Ethyl-acetate(20:20)	
3.	Ethyl acetate: Hexane(75:25)	F 11 – F 19	300	Methanol:Ethyl-acetate(20:20)	
4.	Ethyl acetate alone (100)	F 20 – F 25	300	Methanol:Ethyl-acetate(20:20)	
5.	Methanol: Ethyl acetate(5:95)	F 26 – F 31	300	Methanol:Ethyl-acetate(20:20)	
6.	Methanol: Ethyl acetate(10:90)	F 32 – F 41	300	Methanol:Ethyl-acetate(20:20)	
7.	Methanol: Ethyl acetate(20:80)	F 42 – F 45	300	Methanol:Ethyl-acetate(20:20)	
8.	Methanol: Ethyl acetate(50:50)	F 46 – F 47	300	Methanol:Ethyl-acetate(20:20)	Amorphous powder with decomposition point *
9.	Methanol alone(100)	F 48 – F 50	100	Methanol:Ethyl-acetate(20:20)	

* is the compound eluted from the fraction of (F46 - 47) named as CO -1

cm-1 for a C=C extending. IR spectrum corresponding to isolated compound was shown in Figure 1.

^{13} C-NMR Spectrum corresponding to isolated compound

From the ^{13}C-NMR spectrum was seen that, spectrum showed various signals. It revealed that the chemical shifts were at δ 107.27, δ 113.54, δ 118.87, δ 121.43, δ 121.60, δ 121.60, δ 121.64, δ 129.00, δ 153.95, δ 154.01 and δ 156.50 shows the presence of at least five C=C groups.

The signals at δ 66.94, δ 66.98 and δ 70.37 recommend the presence of three carbons under oxygen functional groups. (- CH-O or – CH2 OH). The signals at δ44.19, δ44.30, δ46.38, δ50.98, δ54.51, δ54.27 and δ54.80 ppm show the presence of carbon and oxygen functional group. 13C-NMR Spectrum corresponding to isolated compound was shown in Figures 2 and 3.

^{1} H – NMR Spectrum corresponding to isolated compound

From the spectra it was shown that the compound showed signal at δ 0.85ppm for a methyl group, a wide singlet at d 1.27 ppm with a signal at δ 1.85ppm for a long chain of methylene group and signal at δ 3.50 ppm for protons under oxygen function group. 1H-NMR Spectrum of isolated compound was displayed in Figure 4.

Mass Spectrum of isolated compound

From the mass spectrum of isolated compound, Spectrum was seen that a molecular ion peak at signal m/z 595.20 (ESI MS positive mode) and a peak at m/z 563.05 (ESI MS negative mode) recommending a molecular weight of 594.0. The fragmentation pattern observed indicates that there is a similarity of a compound that has a similar aromatic region. Mass spectrum of separated compound was shown in Figure 5.

DISCUSSION

Presently the interest of natural medicines is developing quickly, mainly a plant part of drug discovery process (Atanasov *et al.*, 2015). In continuation of the research, interest was shown to separate pure compounds that are exhibiting for the anti psychotic activity. The preliminary studies of GC-MS examination showed the result of ETCO have fifteen compounds. An endeavor was made to do isolation of the pure compound that exhibits anti psychotic activity utilization of *Cassia occidentalis*. One of the fractions "F" eluted from ETCO exhibited more neuroleptic activity compared with marketed commercial standard drug haloperidol. To confirm the compound responsible for neuroleptic activity associated with fraction "F" respectively. Further fraction "F" exposed to column chromatography by utilizing

various solvent systems. Isolate the pure compound trivial named as CO-1 from column that became amorphous powder with the point of decomposition. CO-1 is a natural phenolic compound affirmed by IR, NMR, and Mass spectrum analysis. This sort of result was characteristic of a chance a isolated compound might be diminishing the dopamine levels in the brain.

CONCLUSION

The isolated compound from fraction F from ETCO showed strong neuroleptic activity on a par with the standard drug haloperidol. To ensure the compounds responsible for antipsychotic activities associated with F respectively. In addition, a column chromatographic analysis was carried out with F using various solvent systems and isolated compound named as CO-1 from the column which was amorphous powders with decomposition point. CO-1 is phenolic compound nature confirmed by spectral analysis. Reduction in the dopamine by CO-1 indicates that CO-1 has neuroleptic effect and provides a scientific rationale for the use as an antipsychotic agent.

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Conflict of Interest

The authors declare that there is no conflict of interest for this study.

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