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Research Article

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Assessment of psychoactive potential of *Flemingia macrophylla* involving neurotransmitter receptor assay

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ABSTRACT

The aim of the present work is to study the in vitro screening of psychoactive potential of F. macrophylla using neurotransmitter receptor binding assay and in vivo screening of anti- dementia activity in mouse model scopolamine induced memory deficits .Different extracts were prepared on the basis of polarity and these extracts were assessed for psychoactive potential. It was found thatin vitro receptor binding assay exhibited more than 60% inhibition on anti-dementia and anti –anxiety activity while in vivo it was not significantly active on anti- dementia activity in the model of scopolamine(i.p) induced memory deficits in passive avoidance test in mice.

Key words: Neurotransmitter, receptor binding assay, psychoactive potential

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INTRODUCTION

Neurotransmitters, their receptors and cellular signaling play an important role in the functioning of the CNS.Alterations in their level at any step may lead to neurological and psychiatric disorders like Parkinson's disease, Alzheimer's disease, schizophrenia mental retardation, depression and others. Study of neurotransmitters and their receptors has assumed a great significance in understanding the mechanism of disease and also revolutionized the drug discovery in developing suitable therapeutic agents which could mimic such effects with none or little side effects.[1]At present there has been great thrust to do research and discovery in the fields of psychotropic medication.

The role of herbal medicine in the treatment of various psychological disorders has become well established over past few decades. Investigation of psychoactive plants and their mechanism of action have provided insight in to the neurochemistry of many CNS diseases as well as the chemistry of consciousness. Assay of neurotransmitter receptors has been preferred worldwide as ascreening tool for the psychoactive potential of synthetic compounds,crude extracts and new chemical entities as a rapid and reliable *in vitro* tool.[2]

MATERIALS AND METHODS

The whole plant of *Flemingia macrophylla* was collected from Regional Research Institute Almora. Uttarakand. The root of the plant was air dried under shade to get completely dried, the dried root parts were coarsely powdered and sieved (60#) to get fine and uniform size powder.

Extraction procedure
Requirements
Plant Material: Dried root powder of *Flemingia macrophylla*

Apparatus: Percolator, Rotavapour and lyophilizer

Solvents Used for extraction;- Hexane, chloroform, Butanol, Ethanol, Distilled water

Method of Extraction
The extraction from the root (250 mg) of *Flemingia macrophylla* was carried out from less polar to more polar solvents by using a Percolator apparatus for continuous cool percolation procedure with the help of alcohol. The extract was dried with the help of Rotavapour and Lyophilizer and fractionated through different solvents.

Hexane
The dried, Powdered root of *Flemingia macrophylla*(250 gm) was extracted with hexane in a percolator for 72 hrs. After that extract (free from solvent) were processed under reduced pressure at 40°C by using a rotary evaporator. The dried hexane powdered extract was stored in an air tight container.

Chloroform
The marc left after hexane Extraction was dried and then extracted with chloroform (55-60°C) until the extract is complete, the filtered extract was dried (free from solvent) by using a rotary evaporator under reduced pressure at 40°C and stored in air tight container.

Butanol Extract
The marc was dried and extracted for 72 hrs butanol is more polar solvent, active constituents of the root were extracted in this extraction. The filtered extract was dried under reduced pressure 40°C using a rotatory evaporator. The dried butanol extract was transferred into air tight container.

Ethanol extract
The marc left after butanol extract was again dried and ethanol was poured and extracted by percolation for 72 hrs, after that it was filtered and the filtrate was dried under reduced pressure using a rotary evaporator and then freezedried and preserved in an airtight container.

Water extract
The marc left after ethanol extract was again dried and distilled water was poured and extracted by percolation for 72 hrs. After that it was filtered and the filtrate was dried under reduced pressure at 50°C using a rotary evaporator and then freeze dried and preserved in an air tight container.

Test animals
Adult rats of Wistar strain (200±20 gm) or albino mice of either sex (25±2 gm body weight) were obtained from animal- breeding colony of Indian Institute of Toxicology Research (IITR), Lucknow. The animals were housed in plastic propylene cages under standard animal house conditions with a 12 hrs light/dark cycle at 25± 2°C. The animals had access to pellet diet *ad libitum* (Ashirwad industries Pvt. Ltd, India) and drinking water. All experimental protocols were approved by the animal Care and Ethics Committee of IITR, Lucknow. The Ethical Committee number is ITRC/IAEC/06/07

Instruments used for the studies were Robotic Liquid Handling System (Packard Biosciences USA), Top count NXTSystem (Packard Biosciences USA), Milli-Q water purification assembly (Millipore, USA),RC-5B Sorvall high speed centrifuge, Computerized shuttle box(Coloumbus Instruments, Ohio, USA), UV-Visible Spectrophotometer (Thermospectronic ,USA).

Specific Radio Ligands of high specific activity-3H-Spiperone (Specific activity-15Ci/mmol),3H-Quinuclidinyl benzilate (Specific activity-52 Ci/mmol), 3H-Ketanserin(Specific activity-72.50 Ci/mmol) and 3H-Flunitrazepam (Specific Activity-85.20 Ci/mmol) were used for the assay of Dopamine –D2, Cholinergic- muscarinic, serotonic (5-HT)-2A and benzodiazepine receptors respectively. These radiochemicals were procured from M/s Perkin Elmer,USA. Specific competitors like haloperidol, atropine sulphate, cinanserin and diazepam used were procured from M/s Sigma Aldrich, St. Louis, USA. All other chemicals and reagents used in the study were of pure analytical reagent (AR) grade, which were available locally and obtained from various commercial establishments. Multiwell filter plates used were obtained from Millipore, USA. Bovine serum albumin (BSA) was purchased from SRL, India. Radio ligands receptor binding assay using Multi Probe II Exrobotics liquid handling system. The details of the radioligands and competitors that will be used in the study are given below in the Table-1

Table 1: Details of radioligand, competitors and brain regions involved in the assay of neurotransmitter receptors.[3]

S. No:	Receptor	Activity	Brain Region	Radio ligand	Competitor
1.	Dopamine D2	Anti-Parkinson	Corpus Striatum	^{3}H-Spiperone ($1X10^{-9}$ M)	Haloperidol ($1X10^{-6}$ M)
2.	Cholinergic- muscarinic	Anti- Dementia	Frontal Cortex	^{3}H-QNB ($1x10^{-9}$ M)	Atropine sulphate ($1X10^{-6}$M)
3.	Benzodiazepine	Anti- Anxiety	Frontal Cortex	^{3} H- Flunitrazapam ($0.5x\ 10^{-9}$ M)	Diazapam ($1X10^{-6}$M)
4.	Serotonin-2A	Anti- Depression	Frontal Cortex	^{3} H- Ketanserin ($0.5x\ 10^{-9}$ M)	Cinanserin ($1X10^{-6}$M)

Assessment of psychoactive potential of different extracts of *F. macrophylla* using *in vitro* neurotransmitter Receptor Assay.

Preparation of different plant extracts of *F. macrophylla*:

Plant extracts of different solvent extract were dissolved in dimethyl sulphoxide as per information provided for solubility of samples. The final concentrations of samples corresponded 10 mg in 1 ml of solvent. After vortex samples were transferred into 96 deep well plates (Bekman).

Preparation of crude synaptic membrane:

Brain from anaesthetized rats were isolated and dissected into discrete brain regions following the standard protocol. Crude synaptic membrane from corpus striatum and frontal cortex regions were prepared separately following the procedure of Khanna et al (1994)

i) Brain region was weighed and homogenized in 19 volumes of 5mM Tris-HCl buffer (pH 7.4)

ii)The homogenate was centrifuged at 50,000 X g for 20 minutes at 4°C and the supernatant was removed

iii) The pellet was dispersed in the buffer, centrifuged at 50,000 x g for 20 minutes at 4°C again for washing. The supernatant was discarded and the pellet was finally suspended in 40 mMTris – HCl buffer (p H 7.4), stored at -20 °C and used as a source of receptor for in vitro screening of the samples.

Radioligand receptor binding assay procedure

Briefly, the lab wares were arranged and programming was done using the WINPREP software. Due care was taken that the amount of aspiration and dispense position of a particular reagent (buffer, membrane, competitor, radio ligand and sample) was fixed and programmedaccording to the experiment. The test was executed after confirming that the programming is set right.

The reaction was run in triplicate for each concentration o these extracts in multi screen plates. For the assay of total binding , the incubation mixture in a final volume of 250 µl contained buffer (40 mMTris- HCl, PH 7.4), appropriate radioligand and membrane specific in each case (described in Table- 2)was incubated at 37˚C for 15 minutes at the plate incubator (Heidolph Tetramax 100, France). To assess the level of competition of different plant extracts and its action on the specific receptors, 20 µl of plant extract were also added in parallel in triplicate individually. The contents were filtered over the vacuum manifold soon after the incubation was over. The plates were washed twice with Tris buffer (40 Mm Tris-HCl pH 7.4), dried and left overnight. The final volume of radioligands, crude synaptic membrane and competitors used for *in vitro* neurotransmitter receptor assay has been indicated in Table 2.

Table 2: Details of buffer, competitors and plant extracts added in the multiwell plates

Receptor binding	Tris buffer(40 Mm) PH 7.4	Radioligand	membrane	competitor	Plant extract	Total volume
Total Binding	160 µl	40 µl	50 µl	-------	-------	250 µl
Non specific Binding	140 µl	40 µl	50 µl	20 µl	-------	250 µl
Binding with plant Extracts	140 µl	40 µl	50 µl	------	--------	250 µl

Incubation was carried out in afinal volume of 250 µl

Plates were dried overnight and finally 70 µl scintillation cocktail (Microscint) was added to all the wells. Plates were stabilized by keeping these for 12 hours and counted on Top count-NXT-Microplate scintillation and luminescence counter for the determination of radioactivity. The Count per minutes (CPM) was detected for each well and % inhibition in binding was calculated. The plant extracts that showed 60% or more inhibition on the binding of receptors were considered active.

Specific Binding = Total Binding –Non- Specific Binding

$$\% \text{ Inhibition in binding} = \frac{\text{Specific binding X 100}}{\text{Total Binding}}$$

***In vivo* Screening of anti- dementia activity in mouse model of scopolamine induced memory deficits in Passive avoidance test**

Passive avoidance Test:

The mice were subjected to a single trial passive avoidance test as described by Das et al.(2000). Briefly an animal was placed in the lighted compartment of a computerized shuttle box (Columbus Instruments, Ohio, USA) provided with a software programme PACS 30. An automated guillotine door isolates the compartment lighted at intensity of 8 (scale of 0-off and 10-brightest provided in the PACS 30 software) from the dark department. After an acclimatization period of 30 s the guillotine door automatically opened and after entry into the dark compartment automatically shut the door and the subject received a low intensity foot shock (0.5m A;10 S). Infrared sensors monitored the transfer of animal from one compartment to another, which was recorded as transfer latency time (TLT) in seconds.[4]

The extract of *F. macrophylla* that is chloroform, Butanol, Ethanol and Water respectively at a dose of 100 mg/kg orally dissolved in normal saline pretreated with 1st, 2nd and 3rd day. In 3rd pretreatment of plant extracts after 55 minutes mice given scopolamine then after 5 minutes count acquisition time on shuttle box passive avoidance system. The control mice treated with normal saline as a vehicle. The another group treated with scopolamine 5 minutes before the experiment and after 5 minutes count acquisition time in shuttle box passive avoidance system. The 1st trial was for acquisition and retention was tested by a 2nd trial given 24 hour after the 1st trial. More trials after 2nd trial at 24 hour interval were given to test retention in treated mice. The shock was not delivered in the retention trials to avoid reacquisition. The criterion for learning was taken as an increase in the TLT on retention (2nd or subsequent) trial as compared to acquisition (1st) trial.[5]

Statistical analysis

Mean Significant difference in the treatment groups was determined using t-Test using nonparametric unpaired test. Values of $p<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

I. Assessment of psychoactive potential of *Flemingia macrophylla* using in vitro neurotransmitter receptor binding assay

Table 3:- Details of different solvent extracts activity

Extraction/Fraction ID	% inhibition of receptor binding assay			
	DA-D2 receptor	Cholinergic receptor	Serotonin receptor	Benzodiazepine receptor
Hexane	44.79	41.08	53.03	73.81
Chloroform	44.16	94.21	46.27	90.83
Butanol	54.92	83.87	44.23	82.84
Ethanol	56.77	83.46	50.95	83.85
Water	52.13	84.68	58.51	80.69

II. Assessment of psychoactive potential of *F. macrophylla* using scopolamine induced memory deficits in passive avoidance test.

Table 4: Significant difference of extract of F. macrophylla form t-test values are shown in Mean± S. Error

S. No:	Group	Acquisition(Mean±SE)	Retention(Mean±SE)
1	Control	71±16.59	224. ±39.8
2	Scopolamine	69.8±9.92	98.8±19.5
3	Chloroform extract	75.40±10.73	166.0±46.58
4	Butanol extract	79.2±5.65	94.8±35.0
5	Ethanol extract	83.75±3.25	180.5±49.17
6	Water extract	75.6±7.66	141±53.7

GRAPHICAL REPRESENTATION:-

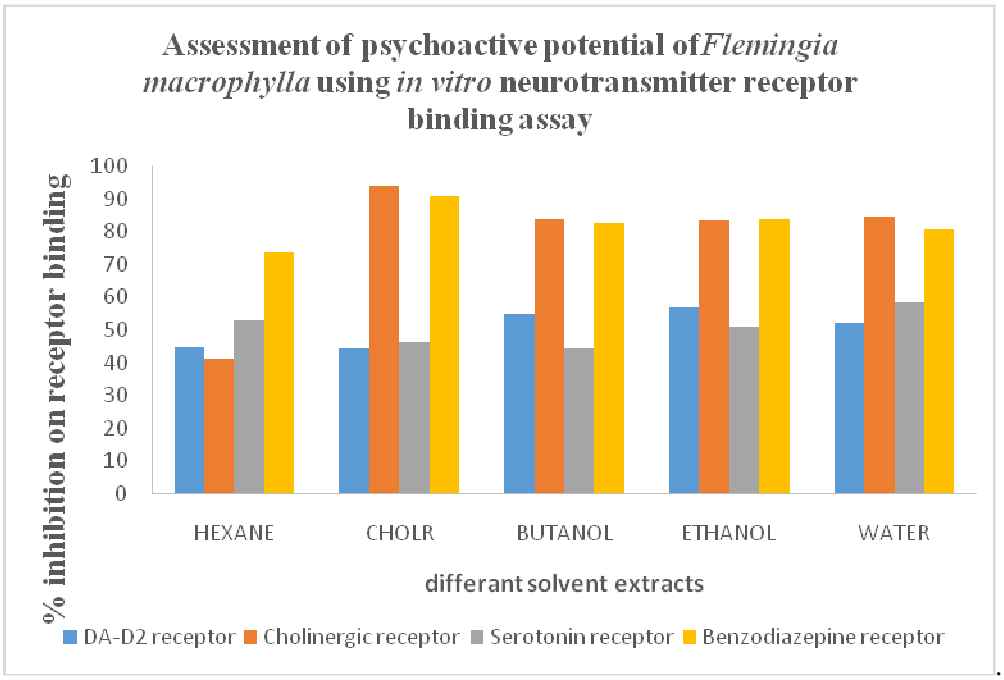

Fig:-1(Table 3): Effect of different extracts of *F. macrophylla* on the binding of inhibitors on various receptors

The plant extracts that showed more than 60% inhibition on the binding receptors were considered to be active and selected for in vivo screening on animal models of neurological and psychiatric disorders.

B. Assessment of *invivo* screening of *Flemingia macrophylla*

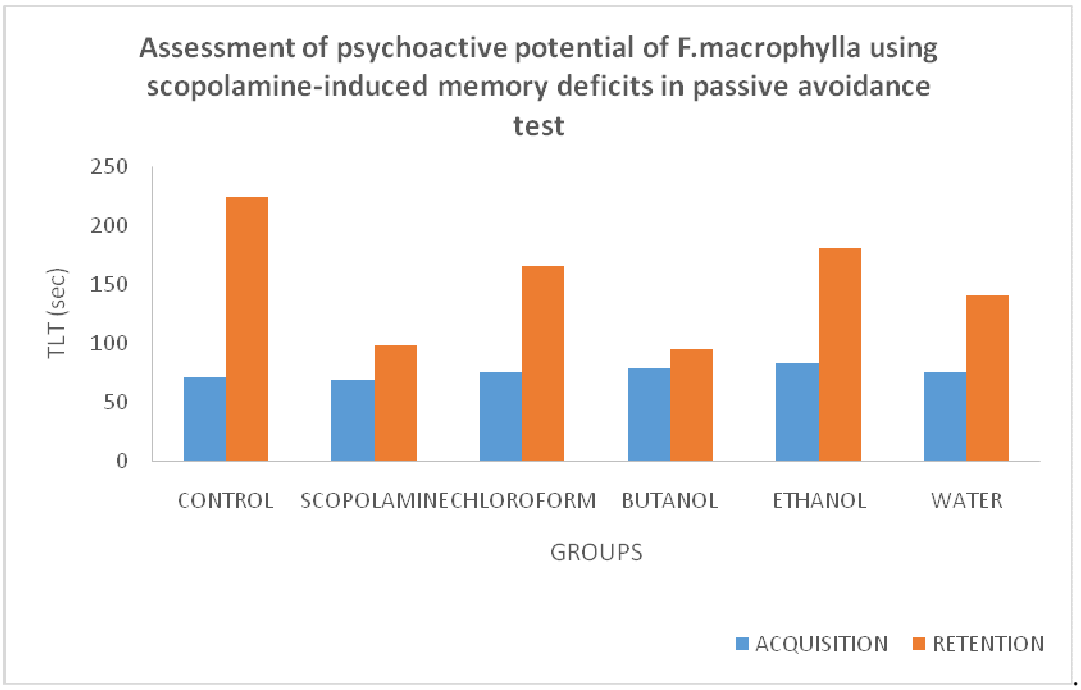

Fig 2(Table 4): Effect of different extract of F.macrophylla (100 mg/kg body weight p.o) on scopolamine (3 mg/kg body weight i.p) induced deficit in memory in passive avoidance test. Significant difference (p<0.001)s from the Acquisition (Students t-test)

Psychoactive potential of different extracts of *Flemingia macrophylla* were assessed in vitro involving assays of dopamine D2, Cholinergic – muscarinic, Serotonine-2A, Benzodiazepine receptor.

The choloroform, butanol, ethanol and water extracts exhibited significant inhibition on the binding of Cholinergic – muscarinic receptors in rat frontocortical membranes indicating Anti-Dementia activity. No significant effect of different extract of *F. macrophylla* on the binding of dopamine –D2 receptors in rat corpus striatum membranes indicating Anti-parkinson's activity. The chloroform, butanol, ethanol and water extracts exhibited significant inhibition on the binding of benzodiazepine receptors in rat frontocortical membranes indicating Anti-anxiety activity.No significant effect of different extract of F. macrophylla on the binding of serotonin receptors in rat corpus frontocortical membranes indicating Anti-depressant activity.

The standard drugs/ competitors were haloperidol, atropine sulphate and cinanserine antagonists on dopamine receptor, cholinergic receptor and serotonin receptor respectively and diazepam acting on agonist on benzodiazepine receptors. After screening of different extracts of *F. macrophylla* using in vitro neurotransmitter receptor binding assay involving different receptor activity we have found that four extract of *F. macrophylla* (chloroform, butanol, ethanol and water) were active on anti-dementia activity. Further these extract were screened for scopolamine – induced memory deficits in passive avoidance test in mice. The transfer latency time (TLT) was significantly increased on the 1st retention as compared to the acquisition time in control group ($p<0.007$, $t=3.55$). In scopolamine treated group there was no significant increase in TLT on the retention as compared to the acquisition ($p<0.05$, $t=1.32$). However the effect of different plant extracts were not significantly found 100 mg/kg body weight p.o., the result of chloroform, butanol, ethanol and water extracts were ($p>0.05$, $t=1.89$), ($p>0.05$, $t=0.43$), ($p>0.05$, $t=1.96$) and ($p>0.05$, $t=1.20$) respectively. There was no significant difference in TLT on the 1st retention as compared to the acquisition time in different plant extracts group.

CONCLUSION

The present study clearly demonstrates that *F. macrophylla* in *invitro* neurotransmitter receptor binding assay significantly exhibited on anti-dementia and anti- anxiety activity while *in vivo* screening not significantly exhibited and restored the cognitive impairment that can be attributed to the enhancement of cholinergic influence.

REFERENCES

[1] C.M. Tanner et al, Epidemiology of Parkinson's Diseases, *Adv.Neurol.***1999**;80:153-159
[2] Lake,James L. Psychotic medications from Natural products: A review of promising research and recommendations. *Alternative Therapies* **2000**, 6(3):p. 36-60.
[3] Fibigerer HC. Central Cholinergic System and memory In:The Biology of memeory. Symposia medica Hoechst. New York: Springer-Verlag.**1990**.p.35-47.
[4] Kuroki.T. et al. Effects of Anti psychotic drugs on extracellular dopamine levels in rat medical prefrontal cortex and nucleus accumbemns. *J.Pharmacol.Exp.Ther*.288, 774-781.
[5] Glowenski, J and Iversen, L.L. Regional studies of catecholamines in the rat brin. The disposition of 3H-norepinephrin, 3H-Dopamine and 3H- Dopa in Various regions of the Brain. *J. Neurochemistry* 13,655-659.