ORIGINAL ARTICLE

Antidepressant and Anticataleptic Effects of *Eucalyptus tereticornis* in Rats and Mice

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Abstract:

Background: Terpenoids possess antidepressant, memory enhancer, neuroprotective and other Central Nervous System (CNS) actions. *Eucalyptus tereticornis* is a rich source of terpenoids. *Aim and Objectives:* The aim of the present study is to evaluate anti-depressant and anti-cataleptic effect of *Eucalyptus tereticornis*. Molecular docking studies were also employed to study the mechanism of action. *Material and Methods:* Locomotor activity and muscle coordination was studied by using actophotometer and rotarod apparatus in rats. The evaluation of anti-depressant activity was studied by forced swimming and tail suspension test in mice. Haloperidol induced catalepsy model was used to evaluate anti-cataleptic effect. Different phyto-constituents present in *Eucalyptus tereticornis* like citronellal, geranyl acetate and terpine-4-ol were screened through computational assessment with the Lipinski's rule of 5, ADMET properties and molecular docking studies. Computational docking analysis was performed by AutoDock Vina 4.2.6 version. *Results: Eucalyptus tereticornis* significantly increases the locomotor activity and improves motor coordination. At 100 mg/kg hydroalcoholic extract of *Eucalyptus tereticornis* significantly shows anti-depressant and anti-cataleptic activity. Phytoconstituents like citronellal, geranyl acetate and terpinen-4-ol exhibit non-selective Monoamine Oxidase (MAO) inhibition activity. *Conclusion:* Hydroalcoholic extract of *Eucalyptus tereticornis* may possess anti-depressant and anti-cataleptic activity with efficient CNS stimulant action which may be attributed to non-selective MAO inhibition activity of its phytoconstituents like geranyl acetate, citronellal and terpinen-4-ol.

Keywords: Catalepsy, Depression, Dopamine, Haloperidol, Monoamine Oxidase

Introduction:

According to World Health Organization, neurological disorders affect more than billions of people worldwide and become an economic burden in the present scenario [1]. Depression is a heterogeneous mental disorder that affects a person's mood, physical health, and behaviour and has become the risk factor of suicide among peoples of different countries [2]. Depression is associated with deficiency in monoamine neurotransmitters like norepinephrine and serotonin [3]. Catalepsy is defined as a condition of diminished responsiveness which can be characterized by muscular rigidity, constant immobility, fixity of posture and loss of voluntary movement and consciousness. Many neuroleptics on prolonged use cause catatonic activity characterized by extra-pyramidal side effects due to blockage of dopaminergic transmission [4-5]. Haloperidol induces catalepsy by blocking dopaminergic D_2 receptor [6-7].

Terpenoids possess a wide range of biological activities such as anti-inflammatory, anti-anxiety, antidepressant, memory enhancer, antinociceptive, neuroprotective and other Central Nervous System (CNS) actions [8]. *Eucalyptus* is one of the most

commonly and widely used important genera of Myrtaceae family [9]. The aerial parts of the fresh leaves of *Eucalyptus tereticornis* contains essential oils which are rich source of various phyto-chemicals like monoterpenes, sesquiterpenes, carvone, citronellal, α, β-pinene, geranyl acetate, linalool oxide, triterpene esters [9-13]. It is reported that the essential oils and triterpenoids present in the *Eucalyptus tereticornis* increases the amount of dopamine in the substantial nigra par compact in the brain and also possess the anti-oxidant property [14].

Thorough literature survey revealed no such neuropharmacological activity in *Eucalyptus tereticornis*. So, the present study was undertaken to evaluate the neuropharmacological effect of *Eucalyptus tereticornis*. The anti-depressant and anti-cataleptic activity in the leaves of *Eucalyptus tereticornis* was studied. Furthermore, using the advanced computational tools several relevant chemical characters and drug-likeliness properties of different phytoconstituents present in *E. tereticornis* was carried out through Lipinski's rule of 5. Molecular docking studies were also employed to study their mechanism of action.

Material and Methods:

Plant Material Collection and Authentication:

Healthy and disease free, mature fresh leaves of *Eucalyptus tereticornis* (Smith) were collected locally during day time from Ratnapur, Chhattisgarh, India. The plants were taxonomically identified by Dr. P. C. Panda, Principal Scientist of Regional Plant Resource Centre (RPRC), Bhubaneswar, Odisha, India (accession field no. PT-01 E. Tereticornis). The authenticated plant was kept in the herbarium file for the record purpose.

Preparation of Plant Extract:

The fresh leaves of *Eucalyptus tereticornis* (Smith) were shade dried at room temp (27°C) for a week without any contamination. Then they are weighed for several times until constant weight achieved. The dried aerial parts were then grinded into fine powder and the powder was preserved in an air tight container for extraction procedure. The dried powder (250 g) of aerial part was extracted with methanol in Soxhlet apparatus at 60-70°C for 10-12 hours. Extraction was continued till clear solvent were observed in Siphon tube. Extracts were concentrated in water bath at 40°C and was dried in hot air oven. Dried extract was stored in sterile amber coloured air tight container in a refrigerator till its use in the experiment.

Experimental Animals:

Twenty-four Wistar albino rats of 120-250 g and albino balb/c mice of 25-30 g respectively were taken from the animal house of School of Pharmaceutical Sciences, Siksha 'O' Anusandhan Deemed to be University, Bhubaneswar for the experimental purpose after due approval from Institutional Animal Ethics Committee of School of Pharmaceutical Sciences. All animals were kept under standard environmental conditions 25 ± 30°C, 45-55% relative humidity and light and dark cycle of 12 hours. They were given free access of food and water and kept under strict hygienic conditions. The animals in each experiment were divided into four groups. Group - I was control, which received vehicle (10 ml/kg), Groups - II, III and IV received hydroalcoholic extract of *Eucalyptus tereticornis* (ET) 100, 200 and 400 mg/kg (p.o.) respectively. Haloperidol, 1mg/kg (i.p.) was used to induce catatonia. Haloperidol was administered intraperitonially

half an hour after administration of the extract orally. For the evaluation of other parameters like locomotor activity (actophotometer), motor coordination (rotarod) and time of immobility [Forced Swimming Test (FST), Tail Suspension Test (TST)] only the extract at different doses were used.

Neuropharmacological Activities:

Locomotor Activity:

The locomotor activity (horizontal activity) was measured by using an actophotometer. The animals were allowed to explore the inside environment and when the animal moves it cuts off the beam of light falling on the photocell and the count was recorded and displayed digitally. The animals were divided into four groups and each group consisted of six animals. Each rat was individually placed inside the actophotometer for 10 min and basal activity score was observed and noted. The vehicle (10 ml/kg, p.o.), and hydroalcoholic extract of *Eucalyptus tereticornis* in a dose of 100, 200 and 400 mg/kg, p.o., were administered daily for 21 days. On day 7, 14 and 21 after 60 min of drug administration the rats were placed in the actophotometer for recording of the activity score [15].

Effect on Motor Coordination:

Neuromuscular coordination activity was measured by using a Rota-rod (Inco) apparatus with the rotation speed of 20 rpm. The rats were divided into four groups, each group consisting of six animals. Then each rat was placed on the rotating rod and the time of falls in seconds was noted, which was considered as the basal reading. The administration of vehicle (10 ml/kg, p.o.), hydroalcoholic extract (100, 200 and 400 mg/kg, p.o.) was done daily for 21 days. On day 7, 14 and 21 after 60 min of drug administration the rats were again placed on the rota rod and the time of fall was recorded [15].

FST:

For this experiment, a 2000 ml beaker was used. The beaker was filled two third with fresh water at 25 ± 1°C. The animals were divided into four groups. The different groups were treated with saline (control) and the extract of test drug at different doses i.e., 100, 200 and 400mg/kg p.o. were taken. During the exposure, mice were placed in the beaker for 5 mins and the immobility time was observed. A mouse was considered to be immobile when it was remained floating in the water, without struggling, making only the movement necessary to keep its head above water [16]. The duration of immobility was observed on days 0, 7, 14 and 21.

TST:

Mice were first prepared for the test by taking them to the testing area in their own cages and allowed to adapt to the new environment for 1 hour before testing. Animals were divided into four groups and treated with the test compounds or the vehicle by oral administration 1 hour prior to testing. For the test, the mice were suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded for a period of 6 mins. Mice were considered immobile when they hang passively and completely motionless [17]. The duration of immobility was observed on days 0, 7, 14 and 21.

Haloperidol Induced Catalepsy (HIC) on Wooden Block:

Catalepsy, defined as a reduced ability to initiate movement and a failure to correct abnormal

posture. The catatonia was induced by administration of haloperidol (1mg/kg i.p.), 1 hour after administration of the drug and the catatonic score was observed at 15, 30, 45, 60, 90 and 120 minutes after administration of the haloperidol. All the test groups were compared with the control. Maximum catatonic score was 3.5. The catatonic response was observed as follows:

Score 0 = Rat moves normally; Score 0.5 = Rat moves only when touched or pushed; Score 1 = Rat placed on the table with front paws set alternatively on a 3 cm high block fails to correct the posture in 10 seconds, score 0.5 for each paw with a total of 1 for this stage; Score 2 = Rat placed on the table with front paws set alternatively on a 9 cm highblock fails to correct the posture in 10 seconds, score 1 for each paw with a total of 2 for this stage [18].

Computational Study:

Ligand and Target Protein Preparation:

The structure of the novel bioactive compounds from the plant *E. tereticorinis* (Family: Myrtaceae) namely, citronellal, geranyl acetate, α-pinene, β-pinene, 1,8-cineole, limonene, terpinen-4-ol, α-phellandrene, β-phellandrene, tereticornate A, α-terpinen and p-cymene were drawn by Chem draw ultra-tool (chem bio office. 12.0 suite), which has associated with 2 Dimensional (2D) orientations and the structure were drawn with proper geometry features and the evaluation was carried out for any error. The 2D orientations of the drawn figures were checked using ACD Labs freeware 2015, and minimization of energy was done in Open Babel program 2.4.1. The required protein for the docking process was accessed from the PDB (Protein Data Bank) (http://www.rcsb.org/pdb/home/home.do). The protein crystals which are taken into account with PDB Id and resolution power were dopamine (6CM4-2.87Å, 5AER-2.19 Å), MAO-A (2Z5X-2.2 Å) and MAO-B (3PO7-1.8 Å). After that the heteroatoms, water molecules and other ligands that bind to the protein crystals were removed and missing side chains were added by using What-If server before the starting of docking process [19].

***In-silico* ADMET Prediction:**

In-silico study offers prediction about the pharmacokinetic profile, drug-likeliness property and optimization of the different phytoconstituents present in the *Eucalyptus tereticornis*. In the present study molecular weight, number of H-bond donor, H-bond acceptor, LogP and the topological polar surface area (TPSA) of various constituents were calculated according to the Lipinski's rule of 5 (RO5) by web-server based Molinspiration property tool. Concomitantly, the percentage of Absorption (%ABS) was calculated by the reported standard formula %ABS=109-(0.345×TPSA) [19-20].

Molecular Docking:

In this study, molecular docking was carried out to predict the binding interactions and free energy molecules of the phytoconstituents of *E. tereticornis* (Citronellal, Geranyl acetate and Terpinen-4-ol), MAO A inhibitor (Moclobemide), MAO B inhibitor (Selegiline), Dopamine (D_2) agonist (Ropinirole) and Dopamine (D_2) antagonist (Haloperidol) against the target receptor through AutoDock suite of programs. The most popular molecular docking tool, AutoDock version 4.2.6 was selected for docking and scoring functions due to its high reproductivity results with accuracy. The grid of the receptor (55 × 55 × 55) was determined as ligand binding site search region and an

enclosed box which was similar to that of co-crystallized ligand were used to capture the phytoconstituents and different target molecules that to be docked. The optimized binding interactions was determined with empirical free energy scoring function in Lamarckian genetic algorithm of 1,000,000 energy evaluation for each run with a maximum of 27,000 generations. The number of individuals in a population was established as 100 and the rate of crossover was 0.8. All docked molecular confirmations were grouped with root mean square of 2.0Å. A total of 100 docking runs was supported and from that the top-ranked binding confirmations was selected according to the computed binding free energy values. Receptor-ligand binding interactions were evaluated by using BIOVIA Discovery Studio Visualizer version 4.5 and PyMOL (The PyMOL Molecular Graphics System, Version 2.0 Schrodinger, LLC) [21-22].

Statistical Analysis:

The above experimental data were expressed in mean ± SEM and analysed by one-way variance ANOVA followed by Tukey's t-test. The value of $p<0.05$ was considered to be significant.

Results:

Assessment of Locomotor Activity:

Hydroalcoholic extract of *E. tereticornis* (100, 200 and 400 mg/kg) significantly ($p<0.05$) increased the locomotor activity as compared to control (Table 1). The activity score is found to be increased significantly ($p<0.05$) by all the three doses of plant extract *E. tereticornis* at day 7, 14 and 21. On day 14 and day 21, a dose of 200 mg/kg and 400 mg/kg showed significant increase in locomotor activity as compared to 100 mg/kg. The results, however, were not dose-dependent. There was no significant difference between 200 mg/kg and 400 mg/kg.

Assessment of Time of Fall from Rotarod Apparatus:

Hydroalcoholic extracts of *E. tereticornis* (100, 200 and 400 mg/kg) showed significant increase ($p<0.05$) in motor coordination as compared to control (Table 2). The time of fall is found to be increased significantly ($p<0.05$) by all the three doses of plant extract *E. tereticornis* at day 7, 14 and 21. On day 14 and day 21, a dose of 200 mg/kg and 400 mg/kg showed significant increase in time of fall as compared to 100 mg/kg. The results,

Table 1: Effect of *E. tereticornis* on Locomotor Activity Using Actophotometer

No. of days	Day 0	Day 7	Day 14	Day 21
Group	**No. of count (activity score)**			
Control	147.16 ± 2.63	146.66 ± 2.40	147.66 ± 2.05	150.33 ± 1.88
ET 100 mg/kg	149.16 ± 2.92	171.83 ± 1.78*	180 ± 2.58*	191.66 ± 2.01*
ET 200 mg/kg	144.83 ± 2.58	173 ± 1.73*	209.33 ± 2.17$^{*\#}$	214.16 ± 2.77$^{*\#}$
ET 400 mg/kg	154.16 ± 2.08	178.33 ± 2.34*	212.83 ± 2.67$^{*\#}$	230.5 ± 1.80$^{*\#}$

Values are expressed as mean ± SEM; n = 6 animal, one-way ANOVA followed by Tukey's multiple comparison t test.
**Indicates $p<0.05$ when compared to control. $^{\#}$indicates $p<0.05$ when compared to 100mg/kg group of animals.*

Table 2: Effect of *E. tereticornis* on Motor Coordination Using Rotarod Apparatus

No. of days	Day 0	Day 7	Day 14	Day 21
Group	**Time of fall in seconds**			
Control	55.83 ± 1.52	53.50 ± 1.46	57.33 ± 1.56	59.33 ± 1.66
ET 100 mg/kg	54.5 ± 1.41	75.66 ± 2.03*	90.33 ± 1.59*	109 ± 1.73*
ET 200 mg/kg	57 ± 1.16	80.66 ± 2.23*	111.66 ± 2.52*#	135.16 ± 2.90*#
ET 400 mg/kg	52 ± 1.13	78.16 ± 1.82*	110.5 ± 2.01*#	130.66 ± 3.02*

Values are expressed as mean ± SEM; n = 6 animal, one-way ANOVA followed by Tukey's multiple comparison t test.
**Indicates p<0.05 when compared to control. #indicates p<0.05 when compared to 100mg/kg group of animals.*

however, were not dose-dependent. There was no significant difference between 200 mg/kg and 400 mg/kg.

Assessment of FST:

In the FST, the *E. tereticornis* extract (200 and 400 mg/kg) produced significant (p<0.05) decrease in the duration of immobility as compared to the control group on days 7, 14 and 21 (Table 3). The duration of immobility is found to be decreased significantly (p<0.05) by a dose of 100 mg/kg at day 14. On day 14, a dose of 400 mg/kg showed significant decrease in the duration of immobility as compared to 100 mg/kg. On day 21, dose of 200 mg/kg and 400 mg/kg showed significant decrease in the duration of immobility as compared to 100 mg/kg. The results, however, were not dose-dependent. There was no significant difference between 200 mg/kg and 400 mg/kg.

Assessment of TST:

In case of TST, the *E. tereticornis* extract (200 and 400 mg/kg) produced significant (p<0.05) decrease in the duration of immobility as compared to the control group on days 7, 14 and 21 (Table 4). The duration of immobility is found to be decreased significantly (p<0.05) by a dose of 100 mg/kg at day 14 only. The results, however, were not dose-dependent.

Table 3: Effect of *E. tereticornis* on Duration of Immobility Using FST

No. of days	Day 0	Day 7	Day 14	Day 21
Group	**Duration of Immobility (in seconds)**			
Control	120.66 ± 1.92	122.66 ± 1.98	125.5 ± 2.51	123.5 ± 2.34
ET 100 mg/kg	117.16 ± 3.11	107.5 ± 2.52	100.16 ± 2.16*	85.16 ± 2.26*
ET 200 mg/kg	119.33 ± 1.63	98.16 ± 2.08*	91.66 ± 1.78*	78.66 ± 2.48*#
ET 400 mg/kg	123 ± 3.76	95.83 ± 2.75*	78.16 ± 2.53*#	67 ± 2.18*#

Values are expressed as mean ± SEM; n = 6 animal, one-way ANOVA followed by Tukey's multiple comparison t test.
**Indicates p<0.05 when compared to control. #indicates p<0.05 when compared to 100mg/kg group of animals.*

Table 4: Effect of *E. tereticornis* on Duration of Immobility Using TST

No. of days	Day 0	Day 7	Day 14	Day 21
Group	**Duration of Immobility (in seconds)**			
Control	153.34 ± 2.54	155.5 ± 1.67	156.5 ± 1.79	153.33 ± 1.92
ET 100 mg/kg	152.33 ± 3.61	137.33 ± 2.36	132 ± 2.40*	117.34 ± 2.06*
ET 200 mg/kg	154.5 ± 2.75	128.16 ± 2.18*	118.33 ± 1.90*#	100.66 ± 3.17*
ET 400 mg/kg	156.83 ± 3.06	130.5 ± 2.54*	125.83 ± 2.41*	107 ± 2.19*

Values are expressed as mean ± SEM; n = 6 animal, one-way ANOVA followed by Tukey's multiple comparison t test.
**Indicates p<0.05 when compared to control. #indicates p<0.05 when compared to 100mg/kg group of animals.*

Assessment of HIC Test on Wooden Block:

The control group showed maximum cataleptic score of 3.5 after 90 min of haloperidol (1 mg/kg) administration. *E. tereticornis* at 400 mg/kg significantly reduced the cataleptic score after 45 min of drug administration. *E. tereticornis* (100 and 200 mg/kg) significantly reduced the cataleptic score after 90 and 60 min of drug administration respectively (Table 5).

Computational Study:

***In-silico* ADMET Prediction**

The molecular weight, number of H-bond donor, H-bond acceptor, LogP and the Topological Polar Surface Area (TPSA) of various phytoconstituents present in the *Eucalyptus tereticornis* were calculated according to the Lipinski's rule of 5 (RO5). Concomitantly, the percentage of absorption (%ABS) was calculated by the reported standard formula %ABS=109-(0.345×TPSA) and given in Table 6.

Table 5: Effect of *E. tereticornis* (100, 200, 400mg/kg; p.o) against HIC (1mg/kg; i.p) after 15, 30, 45, 60, 90 and120 Min of Drug Administration

No. of mins	15 min	30 min	45 min	60 min	90 min	120 min
Group	**No. of cataleptic score**					
Control	2.16 ± 0.06	2.41 ± 0.15	2.83 ± 0.10	2.86 ± 0.10	3.16 ± 0.06	2.75 ± 0.13
ET 100 mg/kg	2.25 ± 0.12	2.58 ± 0.09	2.41 ± 0.12	2.08 ± 0.12	2.16 ± 0.11*	1.33 ± 0.86*
ET 200 mg/kg	2.00 ± 0.11	2.83 ± 0.10	2.33 ± 0.14	1.75 ± 0.08*	1.36 ± 0.06*#	1.58 ± 0.13*
ET 400 mg/kg	1.75 ± 0.04*#	2.25 ± 0.08	1.66 ± 0.08*	1.33 ± 0.85*	1.25 ± 0.88*#	0.83 ± 0.09*$

Values are expressed as mean ± SEM; n = 6 animal, one-way ANOVA followed by Tukey's multiple comparison t test.
**Indicates p<0.05 when compared to control. #indicates p<0.05 when compared to 100mg/kg group of animals and $Indicates p<0.05 when compared to 200mg/kg group of animals.*

Table 6: Molecular Weight, Number of H-bond Donor, H-bond Acceptor, LogP, the Topological Polar Surface Area (TPSA) and the Percentage of Absorption (%ABS) of Various Phytoconstituents Present in the *Eucalyptus tereticornis*

Compound Name	Lipinski rule of five (RO5)					%ABS
	MW (⩽500g/mol)	No. of H-ba(⩽10)	No. of H-bd(⩽5)	cLogP value (⩽5)	tPSA (Å)	
Citronellal	**154**	**1**	**0**	**370**	**14.61**	103.95
Geranyl acetate	**196**	**2**	**0**	**4.07**	**21.66**	101.52
β-pinene	136	0	0	4.14	0.00	109
α-pinene	150	0	0	4.22	0.00	109
1,8-cineole	154	1	0	2.61	6.37	106.80
Limonene	136	0	0	4.53	0.00	109
Terpinen-4-ol	**154**	**1**	**1**	**3.39**	**15.54**	103.63
α phellandrene	136	0	0	4.01	0.00	109
β phellandrene	136	0	0	3.76	0.00	109
Tereticornate A	630	6	1	8.70	64.78	86.65
α terpinene	136	0	0	4.23	0.00	109
p-cymene	134	0	0	4.05	0.00	109

Molecular Docking:

Based on the results of ADMET study, only 3 phytoconstituents were selected for further study. Molecular docking score of these phyto-constituents was noted in Table 7. The binding interactions of citronellal, geranyl acetate and terpinen-4-ol (Table 8) were compared with commercially standard drugs i.e. Ropinirole (Dopamine 2 receptor agonist), Moclobemide (MAO-A inhibitor), Selegiline (MAO-B inhibitor) and a chemical to induce catalepsy i.e. Haloperidol (Dopamine 2 receptor antagonist). Moreover, the binding interactions of ligand and protein with H-bonding interactions were described in Fig. 1. Geranyl acetate, terpinen-4-ol and citronellal possess both MAO-A and MAO-B inhibition activity as evident from the docking score. It was observed that geranyl acetate possesses better docking activity score for MAO-A and MAO-B i.e. 7.2 and 6.8 respectively than citronellal and terpinen-4-ol. The phytoconstituent geranyl acetate also has H-bond interactions with several amino acids like Thr300, Gly41, Glu62, Ile301, Leu169, Phe341 etc and possesses similar activity when compared with the available marketed

MAO-A inhibitor (Moclobemide) and MAO-B inhibitor (Selegiline). However, the molecular docking score and binding interactions of phytoconstituents of *E. tereticornis* on dopamine receptor have not shown good docking scores when compared to dopamine agonist (ropinirole) and dopamine antagonist (haloperidol).

Table 7: Phytoconstituents of *E. tereticornis* and Standard Drugs with Docking Score

Name of the compounds	Receptors/ Enzymes	Docking score (Kcal/mol)
Citronellal	D_2 MAO-A MAO-B	-5.4 -7.0 -5.4
Geranyl acetate	D_2 MAO-A MAO-B	-5.7 -7.2 -6.8
Terpinen-4-ol	D_2 MAO-A MAO-B	-5.8 -6.6 -6.9
Haloperidol	D_2	-9.0
Ropinirole	D_2	-7.3
Moclobemide	MAO-A	-7.1
Selegiline	MAO-B	-8.4

Table 8: Binding Interactions with Amino Acids for Phytoconstituents of *E. tereticornis* and Standard Drugs

Name of the compounds	Receptors	Binding interactions
Citronellal	Dopamine	TRP 374: 6.11, ASP 79: 6.35, PHE 378, 377: 6.31, 5.09, VAL 80: 4.02, HIS 381: 4.75, CYS 83: 4.94
	MAO-A	GLY 40, 41: 3.64, 3.63, PRO 271, 302: 4.92, 5.24, ALA 63: 4.40, VAL 272: 5.02, PHE 418: 5.20, ILE 38, 301: 4.68, 5.58
	MAO-B	TYR 58, 324, 433: 5.25, 5.62&5.34, 4.96, PHE 341: 5.94, LEU 169, 326: 4.51, 5.47,

Continued...

Name of the compounds	Receptors	Binding interactions
Geranyl acetate	Dopamine	PHE 75: 6.20, VAL 55: 5.01, LEU 58: 4.10, ILE 152: 3.84
	MAO-A	THR 300: 4.25, GLY 41: 3.43, GLU 62: 5.52, PRO 271, 302: 4.83, 5.24, ALA 63: 4.90, PHE 418: 4.38, ILE 301: 4.16, 4.64 & 5.63
	MAO-B	TYR 58, 324, 433, 396: 5.36, 5.21 & 5.66, 4.52, 4.01, LEU 169, 326: 4.44, 5.69, PHE 341: 5.98
Terpinen-4-ol	Dopamine	ASP 79: 3.99, PHE 75, 377: 6.56, 6.06, ILE 152: 4.61, VAL 55: 4.01 & 4.21, LEU 58: 4.43 & 6.61
	MAO-A	ILE 460: 4.41, PHE 459: 5.15, GLY 85: 3.94, TRP 223, 423: 6.96, 4.84 & 4.88
	MAO-B	SER 57: 4.20, PHE 341: 6.69, TYR 58, 396, 433: 4.54, 4.09, 4.26 & 4.47, 3.58 & 4.41
Haloperidol	Dopamine	LEU 58: 5.17, 4.76, ASP 79: 4.55, VAL 55, 158: 5.60, 3.80, PHE 377: 6.23, HIS 381: 5.96, SER 162: 4.05, ILE 152: 5.02
Ropinirole	Dopamine	SER 161: 3.52, ILE 152: 4.88, VAL 76, 158: 3.74, 5.47, HIS 381: 5.99, PHE 75, 377: 5.13, 6.20, TYR 405: 5.79, ASP 79: 6.11
Moclobemide	MAO-A	ALA 63, 463: 5.61, 7.51, ARG 64, 70: 4.18, 3.75, ASN 449: 4.05, THR 300: 3.96, GLY 39: 3.86, PHE 418: 4.94, VAL 305: 5.49, ILE 309, 501: 5.02, 4.82
Selegiline	MAO-B	ARG 40: 3.57, TYR 433: 4.36, MET 434: 4.27, GLY 432: 5.01, TYR 396: 4.03

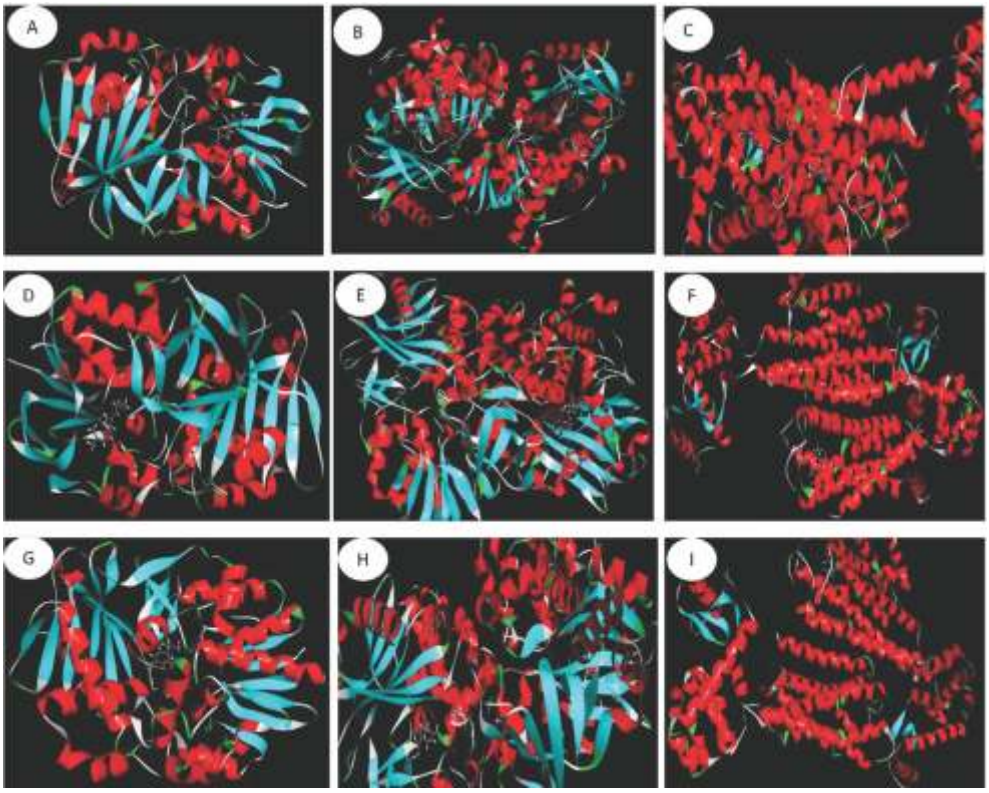

Fig. 1: Visualization of Ligand-protein Interactions by PyMol Tool
Where A represents: Citronellal-dopamine complex, B: Citronellal-MAO-A complex, C: Citronellal-MAO-B complex, D: Geranyl acetate- dopamine complex, E: Geranyl acetate- MAO-A complex, F: Geranyl acetate- MAO-B complex, G: Terpinen-4-ol-dopamine complex, H: Terpinen-4-ol- MAO-A complex, I: Terpinen-4-ol-MAO-B complex.

Discussion:

Actophotometer is a model which studies the locomotor activity of animals in response to drugs. It identifies the CNS stimulant/depressant action of a drug [23]. Decrease in locomotor activity is considered to cause sedation which is closely related to the depression of CNS [24]. The activity score is found to be increased significantly ($p<0.05$) by plant extract *E. tereticornis* at a dose of 100, 200 and 400 mg/kg at day 7. So, the plant showed CNS stimulant action. There are earlier reports of the CNS stimulant action of plant *E. tereticornis*. Our study is also in agreement with the earlier studies [25].

Neuromuscular coordination property of an animal can be evaluated by rotarod apparatus, which also identifies motor coordination effect of a drug. The time of fall from rotating rod at 20 rpm is found to be increased significantly ($p<0.05$) by plant extract *E. tereticornis* at a dose of 100, 200 and 400 mg/kg. The results, however, were not dose-dependent. Improvement in motor co-ordination is an essential characteristic of a drug. Lack of motor co-ordination makes a drug unsuitable for use. So, in our present study the plant *E. tereticornis* improves motor co-ordination which may be correlated with its CNS stimulant action.

FST and TST were carried out to evaluate anti-depressant action of a drug. An antidepressant drug decreases the duration of immobility [26]. *E. tereticornis* significantly decreased ($p<0.05$) the immobility duration thereby showing anti-depressant action. HIC animal model is used to evaluate Parkinson's disease as it causes blockade in the dopamine receptor and increases the cataleptic function [27]. The plant extract *E. tereticornis* at 100, 200 and 400 mg/kg significantly ($p<0.05$) reduced cataleptic score. The control group produced maximum cataleptic score i.e. 3.5. So, from the above observation we can say that *E. tereticornis* effectively protects against HIC.

E. tereticornis consists of several phytochemical constituents like triterpenoids, tannins, saponins, flavonoids, fatty acid, tannins, lignins, inositol derivatives and phenolic compounds which are responsible for various therapeutic and pharmacological properties [13, 28]. Patients with major depression have symptoms which cause changes in brain monoamine transmitters specifically norepinephrine, serotonin and dopamine neurotransmitters [3]. According to monoamine theory of depression, depression is mainly associated with monoaminergic neurotransmitter i.e. noradrenaline and serotonin deficiency [29]. Haloperidol is an antipsychotic which acts by blocking D_2 receptor [30]. The haloperidol-induced catalepsy is associated with blockage of transmission of dopaminergic neurons [6-7].

Monoamines like noradrenaline and serotonin (5-HT) are MAO-A selective whereas dopamine is degraded by both MAO-A and MAO-B [31]. In our molecular docking study, phytoconstituents like geranyl acetate, terpinen-4-ol and citronellal possess potent non-selective MAO-A and MAO-B inhibition activity. Geranyl acetate possesses better docking activity score for both MAO-A and MAO-B. The H-bond interactions of geranyl acetate, terpinen-4-ol and citronellal with several amino acids like Thr300, Ala63, Phe418 etc were similar with the MAO-A inhibitor (Moclobemide). So, the phytoconstituents present in *Eucalyptus tereticornis* by inhibiting MAO-A can increase the level of noradrenaline and 5-HT [32] and show antidepressant activity whereas by inhibiting MAO-B they can increase the level of dopamine [33] and show anticataleptic activity. The triterpenoids present in the *E. tereticornis* increases the amount of dopamine in the substantial nigra par compact in the brain [14]. Terpenoids possess a wide range of biological activities such as anti-inflammatory, anti-anxiety, antidepressant, memory enhancer, antinociceptive, neuro-protective and other CNS actions [8]. It was also observed that *E. tereticornis* hydroalcoholic extract at 100 mg/kg extract may possess CNS stimulant, antidepressant anti-cataleptic action. So, the CNS activity of *E. tereticornis* may be attributed to the terpenoids present in it.

Conclusion:

Hydro-alcoholic extract of *E. tereticornis* stimulates the CNS and improves motor co-ordination. It shows significant antidepressant and anti-cataleptic actions which may be attributed to non selective MAO inhibition activity of its phytoconstituents like geranyl acetate, terpinen-4-ol and citronellal.

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