Indian Journal of Natural Products and Resources
Vol. 13(1), March 2022, pp. 23-31

Antidepressant and anxiolytic activities of *Cochlospermum religiosum* leaf extract, synergism with antidepressants, and molecular docking studies

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Received 03 July 2020; Revised 09 January 2022

The leaves of *Cochlospermum religiosum* were investigated for the antidepressant and anxiolytic activities in mice using behavioural models like spontaneous locomotor activity, forced swim test, tail suspension test, elevated plus maze and marble burying behaviour. The mechanism was studied using Reserpine-induced hypothermia (RIH) model and *in silico* molecular docking. The leaf extract exhibited significant antidepressant and anxiolytic effects (P <0.05 for 50 mg/kgb.w.,p.o./P <0.01 for 100 mg/kgb.w., p.o.) in mice without an impact on baseline locomotor activity. The result from Reserpine-induced hypothermia rat model revealed that the leaf extract (50 mg/kgb.w., p.o.) significantly antagonized the effect (P <0.05) of Reserpine. Furthermore, synergistic effect was evaluated by coadministration of the leaf extracts with fluoxetine (10 mg/kg, i.p.) and imipramine (10 mg/kg, i.p.) at sub-therapeutic dose levels. Synergistic effect of the leaf extract was significant (P <0.05) for both antidepressant and anxiolytic activities as compared to therapeutic doses of extract, imipramine, and fluoxetine. The molecular docking studies for the chemical constituents of the leaves on 5HT1B, 5HT2A, β1 and β2 crystal structures revealed that pentagalloyl glucose showed typical binding with higher affinity on 5HT1B (-10.79) and 5HT2A(-10.33) than fluoxetine and imipramine. Cynarine docked on β-2 receptor with score of -13.582 at binding site of timolol, and similarly, it binds with 5HT1B and 5HT2A at serotonin binding site.

Keywords: Antidepressant, Anxiolytic, *Cochlospermum religiosum*, Molecular docking, Reserpine, Synergism.

IPC code; Int. cl. (2021.01)-A61K 36/00, A61K 127/00, A61P 25/00, A61P 25/22, A61P 25/24

Introduction

Depression is one of the prevalent chronic disorders of mental health and it is rising globally every year and expected to be a leading mental disorder by the year 2030. It is characterized by symptoms like sadness, loss of interest, feelings of guilt, low self-worth, disturbed sleep or appetite, feelings of tiredness, poor concentration, and suicidal tendency[1,2]. Every year, about 8,00,000 people die due to depression induced suicide. Thus, depression induced suicide is the second leading cause of death in the age group between 15-29 years. The World Health Organisation (WHO) predicted that depression will rank second in global disease burdens and it is estimated that more than 350 million people are affected worldwide[3]. Natural product discovery is being the most attractive area for discovering new drugs for all mental disorders including depression, anxiety, alzheimer, schizophrenia etc., due to wide biological accessibility, and low toxicity of natural molecules. There are reports on phytochemical constituents belonging to the class of saponins[4], flavanoids[5], and alkaloids[6] as potential antidepressants.

The plant *Cochlospermum religiosum* (Family: Cochlospermaceae; Synonyms: *Cochlospermum gossypium* DC) was chosen in this study, based on evidence in folk medicine in India. The gum of the tree was used in coughs and gonorrhoea[7]. The *Cochlospermum religiosum* (CSR) leaves have sedative, antibacterial, antifungal, antioxidant, memory enhancers, anxiolytic and antidepressant activity[8]. The existing therapy for depression are not curative and have low effectiveness with high toxicity. In addition to above, the existence of co-morbidity diseases among depression patients is becoming a major challenge in diagnosis and treatment[9] and is leading to the condition of drug resistant depression[10]. On the other hand, focus on herbal

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therapy, the interactions with synthetic antidepressants is also an important concern for safety and efficacious therapy[11,12]. There are no preclinical and clinical investigation reports on antidepressant and anxiolytic efficacy. Indeed, CSR has been enlisted in the endangered medicinal plant list CSR is commonly known as 'Golden silk cotton', a flowering plant widely found in the tropical region of Southeast Asia and the Indian subcontinent[13,14]. The leaves contain phytochemical constituents such as myricetin, quercetin, isorhamnetin 3-glucoside, cynarine ellagic acid, methysergide, lysergic acid, syringaldehyde 2,3-dihydrobenzoic acid, gallic acid, thymol, ascorbic acid, and pentagalloylglucose[15]. The present study was designed to investigate the leaves of CSR for antidepressant and anxiolytic property in mice models. This extrapolated the mechanism study for CSR leaves in rats by Reserpine induced hypothermia model. the extract of CSR leaves has been investigated for synergistic effect with fluoxetine and imipramine using mice models at sub-therapeutic dose level. At the end, phytochemical constituents of the CSR leaves were subjected to in silico molecular docking to predict the possible binding targets and its binding mechanism.

Materials and Methods

Plant material collection and identification

The CSR leaves were collected from Sri Venkateshwara University Tirupathi, Andhra Pradesh, India (13.6293° N, 79.4056° E) during September 2018. The plant specimen was identified by Dr Madhava Chetty and the specimen sample (Voucher no 742) was deposited in the Herbarium-cum-Museum of Department of Botany, Sri Venkateshwara University.

Chemicals, reagents, and standards

Solvent used in the extraction 95% v/v ethanol. All other reagents used in this study were analytical grade (AR grade) obtained from Sigma Aldrich, India. Fluoxetine (99.99%, Sanify Healthcare, India) and imipramine (99.99%, Cipla, India) were used as standard drugs in the *in vivo* animal experimental screening. The standard drug Reserpine (99.99%, Sigma Aldrich) was used as the mechanistic model to induce hypothermia in rats. All the standard drugs and leaf extract solution used were freshly prepared in saline containing 5% of Tween 80, on the day of the experiments.

Preparation of extracts

About 500 g of coarsely powdered leaves was cold macerated using 95% v/v ethanol. Utmost care was taken that the temperature of the extraction process did not exceeded 50 °C. After the extraction, the excess solvent was removed by rotary vacuum evaporator and the dried extract was stored in a refrigerator until it was used for animal experiments and phytochemical screening tests. The percentage yield was 9.21% w/w. The extract appeared as dark brown and sticky semisolid mass.

Animals

Albino mice (25±2 g, male and female), and Wistar rats (250±20 g, male and female) were obtained from Veterinary College, Bangalore, India. All animal experimental procedures were carried out in adherence to the guidelines of CPCSEA, Govt. of India and the protocol was approved by the Institutional Animal Ethics Committee of M.S Ramaiah University of Applied Sciences, Bangalore, India (IAEC (XVI/MSRFPH/M-002/20.01.2016). The procured animals were acclimatized to animal room conditions at an optimum temperature of 23±2 °C and relative humidity of 50–60% under 12:12 h light/dark cycle for at least 10 days prior to the experiments. The animals were allowed free access to food and water ad libitum. Behavioural studies were carried out during the light phase (9.00 a.m. to 2.00 p.m.). The albino mice were used for antidepressant and antianxiety screening. The Wistar rats were used in the Reserpine induced hypothermia animal model for mechanism study.

Preparation of dose

A normal saline containing 5% of Tween 80 was used as a vehicle for preparing the extract and standard drugs. The required amount of test extract and standard drugs were suspended in appropriate volume of vehicle for administration.

Acute toxicity study of extracts

The oral acute toxicity study of ethanol leaf extract was conducted using Albino rats as per OECD guidelines (423)[13]. After 12 h of fasting, the animal groups were treated with an oral dose of 500, 1000, and 2000 mg/kg b.w, p.o, and then immediately after dosing, all animals were observed for any sign of toxicity during the first 0, 1, 2, 3, 4, 8 and 12 h at every 24 h for a period of 14 days. During the period, behavioural parameters, tremors, lethargy and death were considered to assess the acute oral toxicity.

Antidepressant activity

A total of 24 Albino mice were used in the evaluation of antidepressant activity. The mice were

divided into 4 groups namely, control (vehicle), standard (fluoxetine 20 mg/kg, i.p), CSR-50 (leaf extract at 50 mg/kg b.w., p.o.) and CSR-100 (leaf extract, 100 mg/kg b.w., p.o.). Three experiments were conducted for antidepressant activities namely Spontaneous locomotor activity (SLA), Forced swim test (FST), and Tail suspension test (TST).

Spontaneous locomotor activity (SLA)

The SLA was evaluated using Actophotometer which consisted a dark chamber (30 cm x 30 cm). The photocells were checked before and after each experiment. They were cleaned with 70% v/v alcohol and dried for each trial. The CSR extract doses of 50 and 100 mg/kg, i.p. and standard (fluoxetine 20 mg/kg, i.p.) were administered at the 0 minute and 30 minutes after the experiments started. The familiarization period of initial 2 minutes was allowed and then digital locomotors score (LMS) was recorded for the next 8 minutes of the total 10 minutes test.

Forced swim test (FST)

The experiments were conducted as per earlier methods[15,16]. The animals were dipped individually into a glass jar (20 cm height and 12 cm diameter) filled with water to a depth of 15 cm, at a temperature of 23–25 °C. Initially, for about 2 minutes, animals showed vigorous activity to escape an immobile posture, making only those movements necessary to keep the head above the water. The test extracts (CSR-50, CSR-100) and standard (fluoxetine 20 mg/kg, i.p.) were administered 30 minutes before the start of experiments. Duration of immobility was recorded in the last 4 minutes of the 6 minutes test. All the test animals were trained for 15 minutes before the experiments. The immobility time or the duration of immobility (DIM) was recorded as difference in the time period between the immersion time of mice and the time where the mice adopt necessary movements to keep its head above the water.

Tail suspension test (TST)

Test extract doses (CSR 50 and 100 mg/kg b.w., p.o.) and standard (fluoxetine 20 mg/kg, i.p.) were administered 30 minutes before the start of experiments[17,18]. In this TST model, each animal was suspended 60 cm above the floor using a Scotch tape. Initially, the mice exhibited several escape oriented behaviours. The mouse was considered as immobile, only when it hung passively without motion. The duration of immobility (DIM) was assessed in the last 4 minutes of the total 6 minutes test.

Anxiolytic activity

Anxiolytic potential of the leaf extract was evaluated using two models *viz*. Elevated plus maze (EPM)[19] and Marble burying behaviour (MBB)[20]. Animals groups, test extracts (doses), and treatment protocols adopted in screening of anxiolytic activity were same as that of antidepressant activity.

Elevated plus maze (EPM)

The test CSR extracts (50 and 100 mg/kg b.w., p.o.) and standard (fluoxetine 20 mg/kg, i.p) were administered 30 minutes before the experiment. During the 5 minutes of each experiment, animal was placed into the central platform and then number of entries in open arm (EOA) and time spent in open arm (TOA) was determined. The complete experiment was conducted in dim light.

Marble burying behaviour (MBB)

One cubic plastic box or cage (30 cm x 30 cm x 30 cm) filled with 5 cm husk had 20 clean marbles placed on the husk randomly. The box was placed in a soundproof place to avoid disturbance to the animals. Test extracts (CSR 50 and 100 mg/kg b.w., p.o.) and standard (fluoxetine 20 mg/kg, i.p) were administered 30 minutes before the experiment started. During the experiment, each mouse was placed in an individual cage and was observed for 30 minutes. After 30 minutes, the total marbles buried (TMB) was calculated.

Mechanistic model - Reserpine induced hypothermia

A total of 24 Wistar rats (male and female) were used to evaluate the anxiolytic activity[21]. They were divided into 4 groups, namely, Reserpine control (Reserpine 1 mg/kg,, i.p), Standard (Reserpine plus fluoxetine 20 mg/kg, i.p.), CSR-50 (Reserpine + CSR 50 mg/kg b.w., p.o.) and CSR-100 (Reserpine + CSR 100 mg/kg b.w., p.o.). Reserpine was administered to all groups 30 minutes prior to test extracts and standard drug administration. The rectal temperature was recorded at various time intervals of 30, 60, 90, and 120 minutes. The difference in the temperature (TD) between the 30^{th} and 60^{th} minutes was tabulated.

Synergistic evaluation of CSR leaf extract with antidepressants

The synergistic effect of leaf extract with a selective serotonin re-uptake inhibitor (SSRI; fluoxetine) and with a tricyclic antidepressant (TCA; imipramine) at sub-therapeutic dose level was studied[22]. Six groups (n= 6) included in this study, namely control group (vehicle), fluoxetine group (20 mg/kg i.p), imipramine group (20 mg/kg ip), CSR-50 (CSR 50 mg/kg b.w., p.o.),

CSR+FLU (CSR 25 mg/kg b.w., p.o. + fluoxetine 10 mg/kg i.p), and CSR+IMP (CSR 25 mg/kg b.w., p.o. + fluoxetine 10 mg/kg i.p). Experiments were conducted as per the procedure outlined in above including SLA, FST, TST, EPM and MBB models.

Molecular docking studies

The molecular docking studies were carried on Schrödinger 2020_1 Glide Maestro work station. The drug targets used were 5HT1B (41AR), 5HT2A (6A93), β1 (2VT4), and β2 (3D4S). Seratonin, cyanopindolol, imipramine, fluoxetine, timolol were chosen as reference ligands. The phytochemical constituents of CSR leaf such as myricetin, quercetin, isorhamnetin 3-glucoside, cynarine, ellagic acid, methysergide, lysergic acid, syringaldehyde 2,3-dihydrobenzoic acid, gallic acid, thymol, ascorbic acid, and pentagalloyl glucose were test ligands. Glide ligand docking was performed to obtain glide score and binding energy as per the procedure of Schrodinger 2020_1 Glide Maestro.

Statistical analysis

Data from experiments was expressed as mean±SEM. The single treatment studies were analyzed using one-way analysis of variance and post-hoc Dunnett test. The interaction studies were analysed using a two-way analysis of variance and then by post-hoc Sidak test. *P*-values <0.05 were considered statistically significant.

Results and Discussion

The body weight of the Wistar rats, before and after administration of test extracts was measured. There was no change in the skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomous and central nervous system motor activity, and behaviour pattern. There was no sign of tremors; convulsions, salivation, diarrhoea, lethargy, sleep and coma were noted. The onset of toxicity, signs of toxicity and death were absent.

The locomotor activity was evaluated after the 7th day using Actophotometer. Compared to the control group, none of the tested doses (fluoxetine and CSR) influenced the locomotion of mice (Table 1). The CSR extract treatment at a dose of 50 and 100 mg/kg for 7 days, significantly decreased the duration of immobility as compared to vehicle treatment. The standard antidepressant, fluoxetine (10 mg/kg), significantly (P <0.01) reduced the immobility duration in FST and TST as compared to the control group (Table 1 and Fig. 1).

Analysis of EPM data showed a significantly increased percentage of entry and time spent (%) in open arms in test groups with CSR extract of 50 and 100 mg/kg (P <0.05 and P <0.01) as compared to the control group (Table 1). The MBB model for the evaluation of anxiolytic properties showed that test *CSR* extract and standard (fluoxetine) significantly reduced (P <0.05) number of marbles buried compared to the control group (Table 1). Fig. 1 and 2 show the antidepressant and anxiolytic activities of the CSR leaf.

Depletion of brain serotonin induced by Reserpine affects the central nervous system leading to hypothermia. Administration of Reserpine (1 mg/kg, i.p.) elicited a pronounced decrease in core body

Table1 — Screening of ethanolic extract of *Cochlospermum religiosum* leaves for antidepressant and anxiolytic potential in mice

Groups (n=6)	Dose (mg /kg)	Antidepressant models (in mice)			Antianxiety models (in mice)			Mechanistic model (in rats)
		SLA model	TST model	FST model	Elevated plus maze model		MBB model	RIH model
		LMS	DIM	DIM	EOA	TOA	TMB	TD
Control	VNSW	382.5± 29.92	136.7± 8.069	136.7± 8.069	34.33± 4.849	29.00± 5.762	14.33± 0.988	3.633± 0.4055
Fluoxetine	20 mg/kg	284.0± 26.32	98.1± 7.600**	97.6± 7.978*	66.67± 3.869**	55.50± 4.617**	7.50± 1.765**	0.8433± 0.3470
CSR -50	50 mg/kg	358.8± 22.34	103.8± 4.715*	105.7± 8.200*	56.00± 5.888^{ns}	43.33± 3.639*	9.16± 1.138*	1.433± 0.5590
CSR - 100	100 mg/kg	320.7± 45.22	92.6± 7.159**	102.8± 5.665*	58.00± 7.465*	52.50± 4.595**	8.83± 0.872*	1.483± 0.6036

SLA - Spontaneous locomotor activity; TST- Tail suspension test; FST-Forced swim test; LMS-Locomotor score; DIM-Duration of immobility; EOA-Number of entries in open arm; TOA-Time spent in open arm; MBB -Marble burying behaviour; TMB- Total marbles buried; RIH- Reserpine induced hypothermia; TD-Temperature difference.*P value <0.05. **P value <0.01 VNSW- Vehicle, Normal saline water.

temperature of rats. This effect was significantly (P <0.05) reversed by CSR extracts and fluoxetine (10 mg/kg) treatments (Table 1).

The effect of combined administration of CSR extracts (25 mg/kg) with fluoxetine (10 mg/kg, i.p.)/imipramine (10 mg/kg, i.p.) was evaluated. Fluoxetine with CSR extract (25 mg/kg) decreased duration of immobility in mice (Table 2). The combination of above standard drugs with CSR leaf extract showed a synergistic effect in various models like FST and TST through the increased percentage of entry into open arm and percentage time spent in open arms and the reduced number of marbles buried. The statistical two-way ANOVA analysis significantly differentiated the fluoxetine-CSR interaction from imipramine-CSR interaction (Table 2).

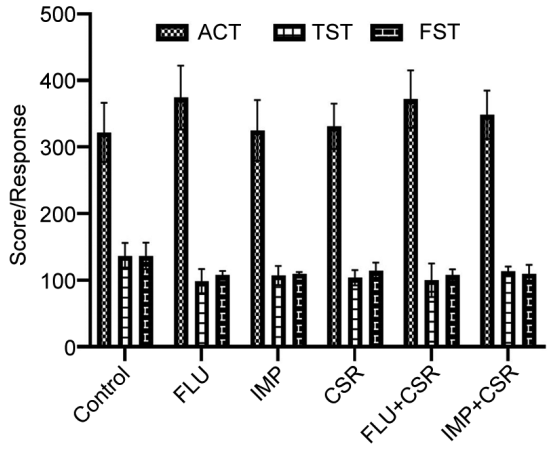

Fig. 1 — Antidepressant activity of *CSR* leaves and its synergistic effect with fluoxetine and imipramine.

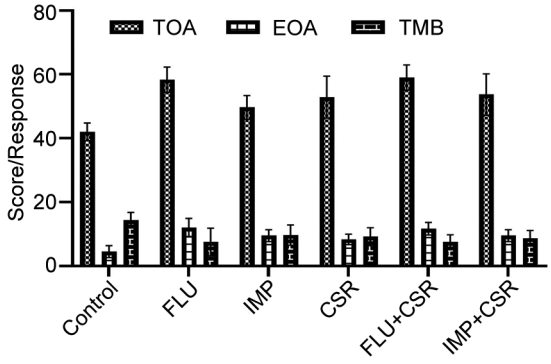

Fig. 2 — Anxiolytic activity of *CSR* leaves and its synergistic effect with fluoxetine and imipramine.

Molecular docking study for the constituents of CSR leaves was carried on 5HT1B, 5HT2B, β1 and β2 receptors. The binding site for 5HT1B and 5HT2A was identified using site map. The chemical constituents of the CSR leaf and standard ligands such as serotonin, imipramine, and fluoxetine were docked. For, β1 and β2 receptors, respective binding sites of cyanopindolol and timolol were used to dock the CSR leaf constituents. The glide score obtained in the docking studies was tabulated in Table 3. The glide score and amino acid residue interaction of constituents was compared with standard ligands to explore the possible mechanism (Fig. 3a-i).

In connection to the prevalence of depression and anxiety disorders, the authors choose a plant with proven folklore medicinal value. Accordingly, the ethanolic leaf extract of CSR was evaluated in mice using *in vivo* experiments including SLA, TST, FST, EPM, and MBB. Among them, SLA, TST, and FST tested the antidepressant activity whilst the anxiolytic activity was tested by EPM and MBB. The leaf extract was tested for oral acute toxicity studies as per OECD guidelines 423 and was found to be 2000 mg/kg.

Table 2 — Synergistic (Pharmacodynamic interaction) evaluation of ethanolic extract of *Cochlospermum religiosum* leaves in mice for antidepressant and anxiolytic potential

Groups (n=6)	Dose (mg /kg)	Antidepressant models (in mice)			Antianxiety models (in mice)		
		SLA model	TST model	FST model	Elevated plus maze model		MBB model
		LMS	DIM	DIM	EOA	TOA	TMB
Control	VNSW	382.0±18.15	136.7±8.069	136.7±8.069	4.500±0.763	34.33±4.84	14.33±0.988
Fluoxetine	20 mg/kg	274.3±7.19.61	98.17±7.600**	107.7±2.629**	12.50±0.922**	56.50±4.62**	7.500±1.765**
Imipramine	20 mg/kg	325.0±18.71	107.2±5.885	109.7±1.085**	9.50±0.763	50.00±5.145	9.667±1.282
CSR-50	50 mg/kg	331.2±13.89	103.8±4.715*	112.2±5.653*	8.33±0.666*	56.00±5.888*	9.167±1.138
FLU + *CSR*	10+25mg/kg	371.8±17.53	100.0±10.25*	107.7±3.648*	11.67±0.802*	57.33±5.346*	7.500±0.9220**
IMP +*CSR*	10+25mg/kg	349.2±15.10	113.3±3.040	109.7±5.475**	9.50±0.763	37.00±3.464	8.667±0.988*

SLA - Spontaneous locomotor activity; TST- Tail suspension test; FST- Forced swim test LMS-Locomotor score; DIM-Duration of immobility; EOA-Number of Entries in open arm; TOA-Time spent in open arm; MBB - Marble burying behaviour; TMB- Total marbles buried; FLU-Fluoxetine; IMP-Imipramine. *P value <0.05. **P value <0.01. VNSW- Vehicle, Normal saline water.

Table 3 — Molecular docking of active constituents of *Cochlospermum religiosum* leaves with 5-HT and β receptors

Compound	Glide Scores			
	5HT1B (4IAR)	5HT2A (6A93)	B 1 (2VT4)	B 2 (3D4S)
Myricetin	-7.774	-7.595	-7.827	-8.856
Quercetin	-7.958	-7.828	-7.815	-8.728
Isorhamnetin 3-glucoside	-6.843	-6.747	-7.743	-7.536
Cynarine	-8.464	-8.377	-7.645	-13.586
Ellagic acid	-7.914	-7.646	-6.904	--
Methysergide	-7.301	-8.23	-6.868	-7.95
Lysergic acid	-6.442	-6.987	-6.526	--
Syringaldehyde	-5.99	-5.915	-6.394	-6.035
2,3-dihydrobenzoic acid	-5.941	-6.109	-6.36	-6.247
Gallic acid	-5.883	-4.944	-6.007	--
Thymol	-6.4	-6.411	-5.915	-5.814
Ascorbic acid	-4.95	-4.543	-5.135	-5.562
Pentagalloyl glucose	-10.79	-10.339	--	--
Cyanopindolol	--	--	-5.68*	--
Imipramine	-5.47	-7.704	--	--
Fluoxetine	-6.913	-7.012	--	--
Timolol	--	--	-6.48	-6.452*
Serotonin	-6.941	-5.411	--	--

*co-crystal ligands

Hence, the extract was evaluated for locomotor activity using Actophotometer at 400 mg/kg. The LMS was relatively high, that might be due to the sedative property of the plant. Therefore, the dose of leaf extract was reduced to 1/4th. In the first set of experiments (Table 1), leaf extract was screened at two doses (50 and 100 mg/kg) using fluoxetine as positive control (standard; 20 mg/kg, intra-peritoneal). In antidepressant screening, the results were obtained as LMS from SLA, and as DIM from TST and FST experiments. In anxiolytic experiment, EOA and time spent in open TOA were recorded from EPM experiment whereas in MBB anxiolytic models the TMB was recorded. In addition, a mechanistic model was used to predict the mechanism by Reserpine induced hypothermia model. The TD in the test and control group was measured to determine the antagonistic potential the CSR extract. The detailed results are tabulated in Table 1.

The result from antidepressant screening revealed that none of the tested doses of fluoxetine, CSR-50 and CSR-100 have influenced the locomotion of mice in Actophotometer. The CSR extract doses (50 and 100 mg/kg) significantly (P <0.05) decreased the DIM compared to control in both FST and TST models. It means that the decreased DIM indicates a significant antidepressant potency of the CSR leaf extract. The DIM value for CSR leaf at 50 mg/kg was 103 whereas fluoxetine showed the DIM value of 98 against the normal control DIM value of 137. This indicated the considerable potency (P <0.01) of CSR leaf extract for antidepressant activity.

In FST, the mice adopted a posture of immobility with the little movements which is essential to restore head above the water. This is known as 'stress induced failure' or behavioural despair and it can be treated with antidepressants. The above observation is in agreement with earlier reports[23,24]. The TST revealed the non-observable water submersion induced hypothermia. But, the hemodynamic stress is being hanged as an uncontrollable fashion by the tail. Hence, the CSR leaf possesses considerable antidepressant like effect in mice[25].

In EPM experiments for anxiolytic activity, CSR leaf extracts significantly (P <0.05) increased the TOA and EOA at the doses of 50 and 100 mg/kg, and fluoxetine when compared to the control group (P <0.05). The increased proportion of time spent in the open arms and the increased proportion of number of entries into the open arms for CSR extracts represented the anxiolytic potential of CSR leaves (P <0.05). In MBB model, the extracts demonstrated a significant reduction (P <0.05) in the number of marbles buried as compared to control group. This MBB test indicated the anti anxiety potential of CSR leaf extract by reduced fear of CSR treated animals when compared to control group. In mechanistic model of RIH, there was significant decrease

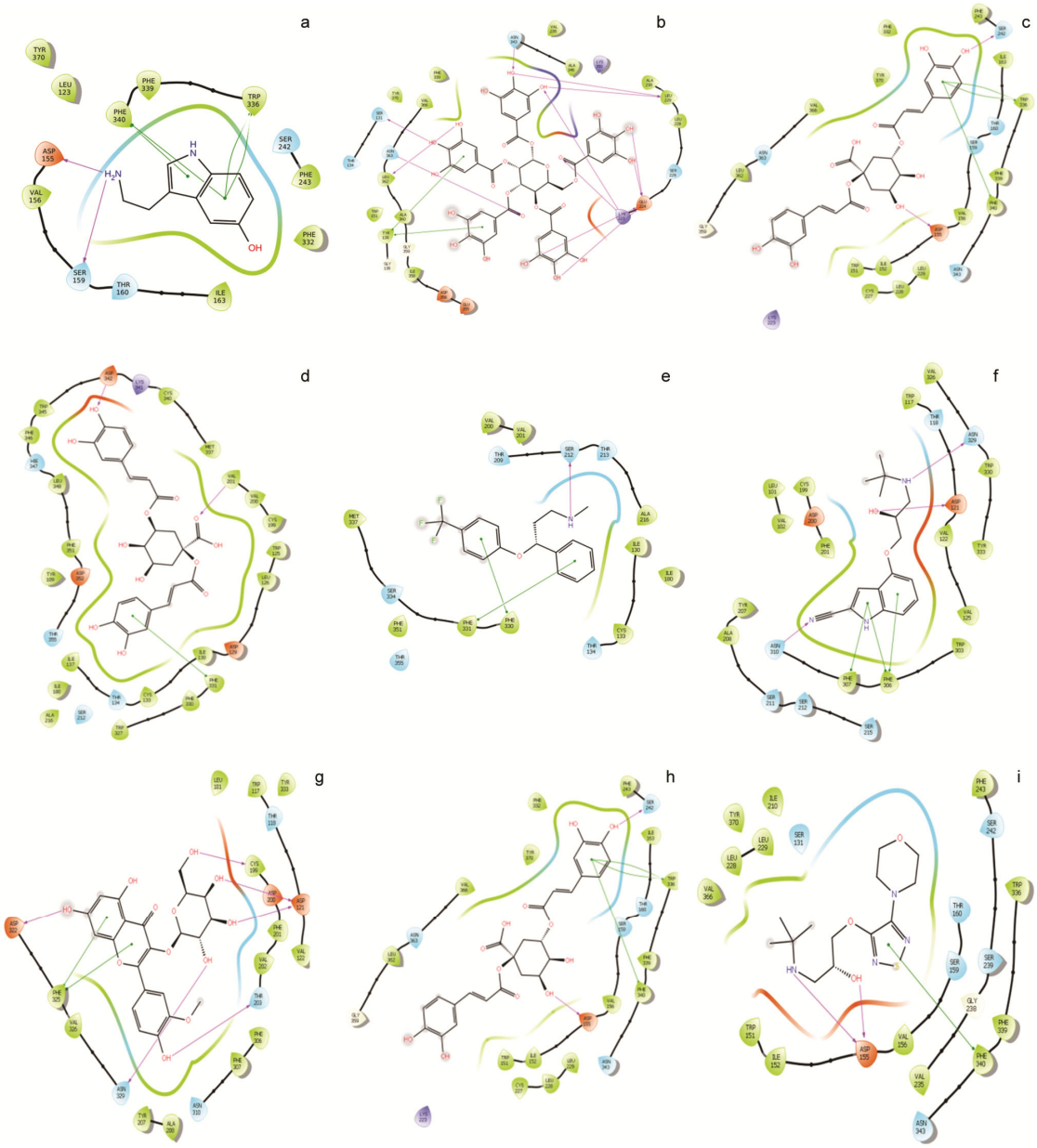

Fig. 3 — a) Serotonin binding on 5-HT2A: H-bond (ASP-155, SER-159), Hydrophobic(TRP-336, PHE-340), b) Binding of pentagalloyl glucose on 5HT2A: H-bond (SER-131, LEU-229, LYS-223, GLU-224, LEU-362, ASN-343); Hydrophobic (THR-139), c) Cynarine binding on 5HT2A at serotonin site: H-bond (ASP-155, SER-242); Hydrophobic (PHE-340), d) Binding of cynarine on 5HT1B at fluoxetine /serotonin binding site: H-bond (VAL-201, ASP-342); Hydrophobic (PHE-331), e) Binding of fluoxetine on 5HT1B: H-bond (SER-212); Hydrophobic (PHE-330, PHE-331), f) Binding of cynopindolol on B 1: H-bond (ASP-121, ASN-229, ASN-310); Hydrophobic (PHE-307, PHE-306), g) Binding of isorhamnetin on B 1 at cyanopindolol binding site: H-bond (ASP-121, CYS-199, THR-203, ASP-322, ASN-329); Hydrophobic (PHE-325), h) Binding of cynarine on B-2 at Timolol binding site: H-bond (ASP-155, SER-242); Hydrophobic (TRP-336, PHE-340), i) Binding of timolol on B 2 receptor: H-bond (ASP-155 (-OH), ASP-155 (-NH-)); Hydrophobic (PHE-340)

(P <0.05) in TD between control and CSR treated groups. Here, Reserpine served as a nonspecific monoamine depleting agent by blocking the monoamine transport into synaptic vesicles. Thus, the depletion of brain biogenic amines was characterized by hypothermia; furthermore, the decreased body temperature induced by Reserpine was antagonised by CSR and fluoxetine.

In the second set of experiments (Table 2), the pharmacodynamic (synergistic) interaction was evaluated to assess the synergistic effect of CSR leaf

extract with fluoxetine and imipramine. Here, the synergistic effect was evaluated with the experiments as mentioned in Table 1, but the combinations doses were fixed at sub-therapeutic level. Hence, test extract was evaluated at 25 mg/kg. in combination with fluoxetine and imipramine at 10 mg/kg. Overall, there was a substantial synergistic effect observed with *P* value of 0.05 while comparing the antidepressant activity of coadministration (fluoxetine/imipramine at 10 with CSR extract at 25 mg/kg) with control results from fluoxetine/ imipramine 20 mg/kg) and CSR leaf extract at 50 mg/kg.

In the present study, molecular docking[26] using standard ligands and active constituents of CSR leaf was performed to address the antagonism mechanism of CSR leaf in RIH models (Table 3). In fact, the hypothermic effect of Reserpine was mediated by both adreno β receptor and 5HT receptors (Table 1). The docking of ligands was conducted on 5HT1B, 5HT2A, β1 and β2 receptors, and the obtained docking scores were tabulated in Table 3. Amino acid interactions were shown in Fig. 3a-i.

In docking, the serotonin (5-HT) was posed on 5HT1B and 5HT2A (Fig. 3a), and it showed the respective glide score of -5.33 (SER-212, PHE-331, ASP-129) and -6.74 (ASP-155, SER-159, PHE-340, TRP-336). The molecular docking of constituents on 5HT1B and 5HT2A revealed that pentagalloyl glucose (Fig. 3b) exhibited better docking scores -10.79 and -10.33, respectively and was better than serotonin and reserpidone. It was noted that binding interaction of pentagalloyl glucose at binding site was different from fluoxetine, imipramine, and serotonin. But, cynarine (Fig. 3c-d) posed with -8.67 (VAL-201, ASP-242, PHE-331) and -8.37 (ASP-155, SER-242, PHE-340) respectively, on 5HT1B and 5HT2A. They posed better than imipramine and fluoxetine (Fig. 3e) at serotonin binding site. Docking on β1 with cyanopindolol (Fig. 3f) revealed the glide score of -5.68 (ASP-121, ASN-229, ASN-310, PHE-306, PHE-307). It was noted that the isorhamnetin (Fig. 3g) glide score better than standard ligand (Table 3). Accordingly, myrecetin and quercetin showed glide score of -7.82 and -7.81 respectively. The docking results from β 2, revealed the high binding affinity of cynarine (Fig. 3h) with a score of -13.586 (ASP-155, ASP-343, PHE-339) at timolol (Fig. 3i) binding site (ASP-155, PHE-34).

On 5HT1B, both cynarine and pentagalloyl glucose shared the common binding residue of VAL 201, but they differed from the binding residue interaction of fluoxetine (SER212, PHE330, PHE331) and imipramine (PHE330). However, cynarine binding at PHE 331 was the common residue with fluoxetine. The cynarine with 5HT2A, the interaction with PHE 340 has been notified as common residue as compared to fluoxetine and imipramine. Notably, pentagalloyl glucose exhibited typical interaction with both 5HT1B and 5HT2A and its binding site was differed from standard ligands. It inferred that cynarine and pentagalloyl glucose exhibits fluoxetine and imipramine like action at 5HT receptors, and they probably showed synergism through alternate binding site. Similarly, cynarine interact with ASP 121, ASN 310, PHE 201 and PHE 306 was the important common feature to cyanopindolol and reserpidone. Thus, it justified the antagonistic effect of Reserpine induced hypothermia in rats. Thus cynarine and pentagalloyl glucose are the most interacting chemical constituents of CSR leaf on the 5HT and β receptors.

Conclusion

Based on the present study, it is concluded that ethanolic extract of the *Cochlospermum religiosum* leaf possesses significant antidepressant and anxiolytic activities in animals. The extract showed considerable synergistic effect with fluoxetine and imipramine for both antidepressant and anxiolytic activities. The *in silico* studies indicated cynarine and pentagalloyl glucose as promising interacting molecules that bind to 5HT and β receptors.

Conflict of interest

The authors declare no conflict of interest.

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