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Phytochemical analytical and antidepressant activity screening of flowers of *spathodea campanulata*

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Abstract
The antidepressant activity of *Spathodea campanulata* flowers was evaluated in mice and in silico. When 33 tested at doses of 200 and 400 mg/kg, the methanol extract of *S. campanulata* (MESC) showed dose dependent antidepressant activity in the force swim test (FST), tail suspension test (TST), lithium induced head twitches test, Haloperidol Induced Catalepsy and the open field test. In FST and TST, animals treated with MESC demonstrated a significant decrease in the immobility period compared to the control group. These data suggest that *S. campanulata* flowers warrants further investigation as a source of novel templates for antidepressive drugs.

Keywords: Antidepressant activity, depression, monoamines, molecular docking, *Spathodea campanulata*

Introduction

Depression is a common and serious psychiatric disorder that is a major contributor to the global burden of disease. According to the World Health Organisation, an estimated 322 million people worldwide suffer from depression [1]. It has been reported that reactive oxygen species (ROS) and nitrogen species play an important part in the pathogenesis of depression by regulating the levels and activity of noradrenaline (norepinephrine), serotonin, dopamine and glutamate, the principal neurotransmitters involved in the neurobiology of depression [2]. Several classes of antidepressant drugs (i.e. tricyclic antidepressants, selective serotonin reuptake inhibitors, selective reversible inhibitors of monoamine oxidase A, and specific serotonin–norepinephrine reuptake inhibitors) are used currently to treat depression However, a high number of side effects (e.g. sexual dysfunction) have been reported with these drugs and there is a need to discover an effective alternative treatment for depression that is better tolerated [3]. A variety of medicinal plants are used as antidepressants in traditional medicine [4] and/or contain some chemical constituents that have already demonstrated antidepressant activity [5]. *Spathodea campanulata* Beauv. (*Bignoniaceae*), known as the African tulip tree, is native to Africa but commonly distributed throughout South America, the Caribbean and the Pacific Isles [6]. Traditionally, *S. campanulata* is useful for the treatment of fever, dysentery, constipation, malaria, diabetes and skin diseases [7]. The species is reported to contain some iridoids, flavonoids, tannins, saponins, alkaloids, terpenoids and steroids [8-10]. Previous reports have suggested that phytochemicals like iridoids, flavonoids, alkaloids and tannins have antidepressant activity [11-12]. To the best of our knowledge, the antidepressant activity of *S. campanulata* and its constituents as yet to be evaluated. The present study was performed to evaluate the antidepressant activity of *S. campanulata* flowers in different mice models.

Materials and Methods

Plant collection and identification

Spathodea campanulata flowers were collected in - Ranga Reddy District & Hyderabad, Telangana, India in the month of July and August 2022. The plant was identified and authenticated by Dr. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateshwara academia, Tirupathi, Andhra Pradesh, India, where a voucher specimen number (SJCBOT 1566/2022) has been deposited.

Extraction of plant material

The flowers were air dried in the shade for 10-15 days and the dried material (246 g) was ground to a fine powder and stored in air tight bottles. Extraction was performed by maceration at room temperature with petroleum ether at temp. of 60-80°C, chloroform, ethyl acetate and then methanol (10% w/v) for 72 h with intermittent shaking. Following filtration, the extracts were concentrated under reduced pressure at < 40 °C and were stored in desiccators.

Preliminary phytochemical investigation

Extracts were screened for presence of flavonoids, tannins, saponins, alkaloids, terpenoids and steroids using standard methods [13-15].

Experimental Animals

Entire animal study was executed according to CPCSEA guidelines & NIH Publications no. 8023, revised on 1978). Every procedure carried in the study was authorized by IAEC & CPCSEA committee (Ref no PBRI/IAEC/PN-15014) study. 28-35g wt of swiss albino mice of either sex were selected for the present study. Four mice per cage were sheltered at conventional laboratory conditions of temperature (25 ± 2 0C) with 45-55% of RH (relative humidity) and light/dark cycle of 12/12 hour. Sterile husk paddy was used as bedding material. Standard pellets (golden feeds, New Delhi, India) were used as standard diet with the availability of water ad libitum in a firmly strict hygienic condition. Pellets were withdrawn (but not water) 4 h prior to administration of extracts and/or drug till completion of experiment on the day. Animal were transferred 1h prior to experimental room for adaptation.

The ethyl acetate and methanolic extracts were selected and further undergo acute toxicity studies and antidepressant activity.

Acute Oral Toxicity Studies (as per OECD 423 guidelines)

The acute oral toxicity was performed in albino mice. Four dose levels (5, 50, 500, 1000, 2000 mg/kg) were selected for acute oral toxicity. As per annexure 2a of OECD 423 guidelines, following methodologies were adopted [16-18].

Behavioral study

Forced swim test (FST)

The FST was developed to investigate the potency of antidepressant drugs in rodents. The mice were casually selected and further divided into 6 groups; each group had 6 mice [19-21].

Group 1: Administered vehicle
Group 2: Administered impiramine at dose of 10 mg/kg oral
Group 3: Administered EASC at dose of 200 mg/kg oral
Group 4: Administered EASC at dose of 400 mg/kg oral
Group 5: Administered MESC at dose of 200 mg/kg oral
Group 6: Administered MESC at dose of 400 mg/kg oral

(EASC: Ethyl acetate extract of *S. Campanulata;* MESC: Methanolic extract of *S. Campanulata*)

The animals were placed in a barrel shaped compartment filled with water where most of the creatures attempt to escape by effective swimming. When the animal quit swimming and floats on the surface of the water it will be considered to have "surrendered" and attributed like human depression behaviour.

Tail suspension test (TST)

The assessment of potential antidepressants was done by TST. The mice were casually selected & further divided into 6 groups; each group contained 6 mice:

Group 1: Administered vehicle
Group 2: Administered impiramine at dose of 10 mg/kg oral
Group 3: Administered EASC at dose of 200 mg/kg oral
Group 4: Administered EASC at dose of 400 mg/kg oral
Group 5: Administered MESC at dose of 200 mg/kg oral
Group 6: Administered MESC at dose of 400 mg/kg oral

Above 58 cm from the floor the mice was being hanged distinctly with the help of sticky tape that was around 1 centimeter from the tip of the tail. The duration for the immobility displayed by the mice was noted over the period of 6 minutes and the initial 2 min of immobility was discarded [22-24].

Evaluation of antidepressant activity

Lithium induced head twitches in mice

The mice were selected casually and divided into 4 groups, each group had 6 mice:

Group 1: Administered vehicle
Group 2: Administered imipramine at dose of 10mg/kg oral
Group 3: Administered MESC at dose of 200 mg/kg oral
Group 4: Administered MESC at dose of 400 mg/kg oral

The mice were treated with imipramine at dose of 10mg/kg ip at beginning of the study. After administration of imipramine, the frequency of head twitches was counted for 1 hr [25-26].

Haloperidol induced catalepsy in mice

The mice were separated into 4 groups; each group had 6 mice:

Group 1: Administered vehicle
Group 2: Administered imipramine at dose of 10 mg/kg oral
Group 3: Administered MESC at dose of 200 mg/kg oral
Group 4: Administered MESC at dose of 400 mg/kg oral

The process was used to measure the time during which the mice maintained its forced position with both of its front appendages which were extended and resting on 4.5 cm high glass bar (0.9 cm diameter). After the administration of haloperidol, duration of catalepsy (measured in seconds) was accounted at 30min, 60min, 90min, 120min using bar test [27].

Open Field Test

The mice were selected randomly and separated into 4 groups; each group contained 6 mice:

Group 1: Administered vehicle

Group 2: Administered chlorpromazine at dose of 2 mg/kg oral

Group 3: Administered MESC at dose of 200 mg/kg oral

Group 4: Administered MESC at dose of 400 mg/kg oral

To determine the efficacy of MESC on the locomotor activity, mice were explored in wooden box. The mice were placed in 40-60 centimeter of wooden box & its floor was partitioned into twelve squares. The investigation was preceded for 6 min and the frequency of locomotion displayed by the mice was observed [28, 29].

Statistical evaluations

The results were indicated as mean ± SEM. Measurable variability between groups was expressed using Dunet's test by ANOVA. Significant value i.e. $P < 0.05$ was contemplated as factually critical.

Result

Phytochemical Screening of *S. campanulata*

Preliminary investigation of phytochemicals from *S. campanulata* flower extracts uncovered the presence of flavonoids, tannins, phenolics, alkaloids, glycosides, fats, proteins, saponin and starches in various plant extracts.

Acute Oral Toxicity Studies (OECD 423)

The EASC and MESC were administered orally at different doses (5 - 2000 mg/kg) to the albino mice. After administration of extract no lethality was seen. The extracts upto the dose of 2000 mg/kg body weight were found for use. However, at 2000 mg/kg few animals showed symptoms of somnolence and fatigue. 200mg/kg and 400 mg/kg doses of extracts were used for the screening of antidepressant activity.

Assessment of Antidepressant Activity

FST and TST

The behavioral score of immobility in control, standard and extract-treated groups has been depicted in [Fig. 1]. In both FST and TST, the mice treated with MESC showed significant ($p<0.001$) reduction in immobility period in dose dependent manner when compared to control group (204.66±7.04 in FST, 231.33± 11.31 in TST). MESC demonstrated antidepressant potential (152.66 ± 9.47 at 200mg/kg and 120± 9.50 at 400 mg /kg) in FST and (168.66 ±16.39 at 200 mg/kg and 132.00 ± 15.30 at 400 mg/kg) in TST assays respectively and thus it was selected for further study. While, the animals treated with EASC also showed reduction in immobility period at the both the doses (200 mg/kg and 400 mg/kg body weight) when compared to control group. Behavioral score of immobility in control, standard and extract-treated groups has been depicted in Table 1.

Table 1: Efficacy of Extracts on Immobility Period Displayed by the Mice in FST and TST Assay

Treatment	Immobility	
	FST	TST
Vehicle	204.66 ± 7.04	231.33 ± 11.44
Impiramine (10mg/kg)	95.16 ± 9.08*	115.83 ± 10.94*
EASC (200mg/kg)	187.33 ± 8.28	213.33 ± 9.62
EASC (400mg/kg)	180.83 ± 8.47	201.66 ± 11.82
MESC (200mg/kg)	152.66 ± 9.47*	168.66 ± 16.39*
MESC (400mg/kg)	120.00 ± .50*	132. 00 ± 15.03*

Values are expressed as mean ± SEM, *p < 0.05 compared to vehicle

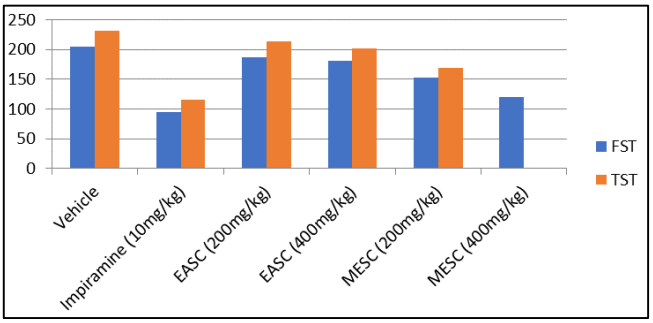

Fig 1: FST and TST assay

Lithium Induced Head Twitches in Mice

The findings of head-twitches responses after treatment with extract and standard drug are demonstrated in Table 2.The mice treated with MESC at a dose of200 mg/kg and 400 mg/kg significantly reduced the head twitches as compared to vehicle group mice. A marked decrease in head twitches was shown by imipramine at a dose of10mg/kg when compared with vehicle group mice.

Table 2: Efficacy of extract on lithium induced head twitches

Treatment	Head Twitches
Vehicle	26.66±2.16
Imipramine-10 mg/kg	4.66±1.21*
MESC(200mg/kg)	13.66±1.36*
MESC(400mg/kg)	8.83±1.47*

Values are expressed as mean ± SEM, *p < 0.05 compared to vehicle

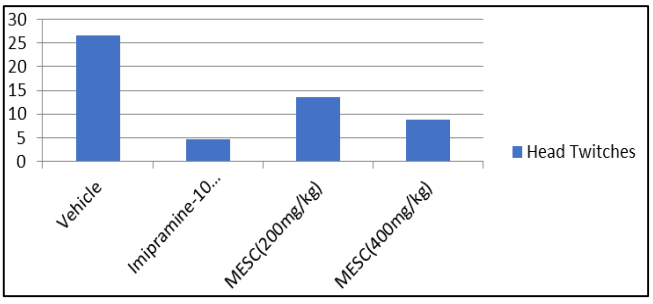

Fig 2: Lithium induced head twitches

Haloperidol Induced Catalepsy in Mice

The mice treated with haloperidol produces peak of catalepsy at 120 minutes. The mice treated with MESC caused effective reduction in catalepsy at a dose of 200 mg/kg and 400 mg/kg when compared with vehicle group. Likewise, the mice treated with imipramine at dose of 30 mg/kg have also demonstrated significant decrease in catalepsy as shown in table 3. These finding clearly indicates antidepressant potential of test extract in mice.

Table 3: Effect of Extract on Haloperidol-Induced Catalepsy

Treatment	Duration of catalepsy at different timeinterval					
	30 min	60 min	90 min	120 min	150 min	180 min
Vehicle	82.83± 12.155	129.16± 15.967	203.16± 19.853	210.16± 11.513	195.66± 11.483	186.50± 12.708
Imipramine (10 mg/kg)	25.50± 4.847*	50.00± 7.099*	33.50± 5.890*	28.00± 5.403*	20.83± 5.845*	14.33± 6.439*
MESC (200mg/kg)	47.33± 7.146*	83.50± 7.739*	74.00± 5.477*	64.00± 5.366*	56.16± 5.528*	49.33± 5.715*
MESC (400mg/kg)	35.50± 7.092*	67.66± 11.165*	47.66± 8.286*	40.16± 7.678*	33.50± 7.713*	26.66± 7.420*

Values are expressed as mean ± SEM, *p < 0.05 compared to vehicle

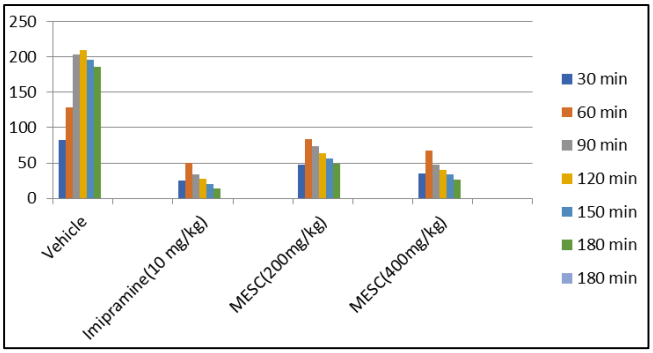

Fig 3: Haloperidol-Induced Catalepsy

Open Field Test

The prominent elevation in locomotors activity was observed after the oral administration of MESC at a dose of 200mg/kg (13.83± 1.83) and 400mg/kg (17.66±2.42) in mice as compared to control group (3.16±1.21). The imipramine treated group produced a significant (p<0.001) increase in locomotor activity as depicted in (Fig. 3).

Table 4: Effect of Extract on Locomotor Activity

Treatment	Activity count
Vehicle	17.16±4.26
Imipramine-10mg/kg	1.33±1.21*
MESC(200mg/kg)	13.83±1.83*
MESC(400mg/kg)	17.66±2.42*

Values are expressed as mean ± SEM, *p < 0.05 compared to vehicle

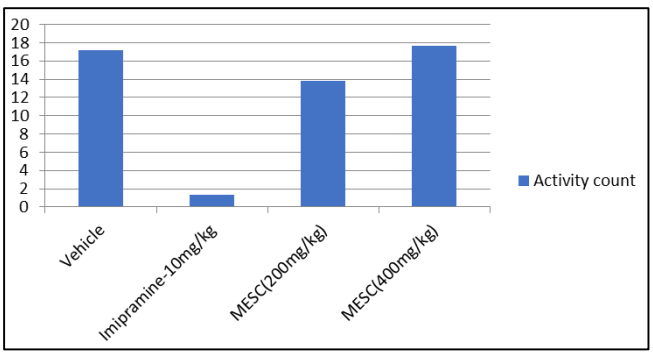

Fig 4: Locomotor Activity

In this present investigation, the antidepressant activity of orally administered EASC and MESC was evaluated in albino mice. Being rich in flavonoids and polyphenols, MESC was selected for the evaluation of antidepressant activity using some robust models like TST, FST, lithium induced head twitches, haloperidol induced catalepsy, open field model.

The FST and TST are social depression tests which are used for the estimation of antidepressant drugs. Characteristic behavior scored by the rodents in both models including immobility reflects behavioral state of despair [30]. The animals treated with MESC at a dose of 200 & 400 mg/kg decreased immobility period as compared with the vehicle group. While EASC failed to decrease immobility period in animals. The findings suggested that MESC produced significant antidepressant activity when evaluated in FST and TST. Thus for further investigations, MESC was being selected.

In FST and TST, antidepressants can be recognized from stimulants, since stimulants cause marked motor stimulation, rather than antidepressants, which do not [31]. The MESC was screened for the locomotion counts to avoid the excitatory or inhibitory stimulus so as to decide whether MESC holds a stimulant like antidepressant activity. The noticeable decrease in locomotor activity was observed after the administration of MESC. The locomotor activity clearly indicated the antidepressant potential of MESC on the immobility time displayed by the rodents in FST and TST.

Serotonergic neurotransmission in CNS is involved in the treatment of depression. The animals and the patients experiences stress and depression have decrease level of 5-HT and 5- HIAA (the major metabolite of 5-HT) in brain, thus exhibit dysfunctioning of serotonergic system [32]. The results of lithium induced head twitches demonstrated that the mice treated with MESC at a dose of 200 mg/kg and 400 mg/kg and imipramine at dose of 30 mg/kg significantly decreased the head twitches as compared with the vehicle group. These findings suggested the prompt serotonergic activation of extract treated mice in 5-HTP-induced head-twitches test. Catalepsy is characterized by the failure to correct an abnormal posture. The inhibitory activity of the neuroleptics in the nigrostriatal dopaminergic system is said to instigate catalepsy [33] and the cataleptic behaviour is suppressed by the antidepressants which was clearly reflected in our findings.

The result of haloperidol-induced catalepsy revealed that the mice treated with MESC demonstrated significant reduction

in duration of catalepsy at 120 minutes compared to the vehicle group. The lower cataleptic score induced by haloperidol indicated higher antidepressant activity. The cataleptic score of mice group treated with extract was lower than that of vehicle group, which signifies the antidepressant activity of *S. campanulata***.**

Discussion

Depression is a neurological disorder that prompts changes in mind-set, considerations, behaviour, and physical wellbeing. It is accounted that 21% of the worldwide population suffering from depression. Sadness can occur at any age from adolescence to older life. It is estimated that more than 20% of the grown-up population experiences these problems sometime in their lifetime. The WHO expects that depression will turn into the second driving reason for sudden passing or inability everywhere throughout the world till 2020. At present, currently available medications used for the treatment of depression are costly and are associated with various symptoms including increase in body weight, cardiovascular lethality and sexual dysfunction etc. There is a necessity for utilization of herbal drugs in the effective treatment of depression because of easy accessibility and affordability.

Subsequently, alternative treatment for depression is frequently looked by medicinal services experts. Presently the demands for natural products have been increased for the effective management of depression because of lesser symptoms, simple accessibility and lower cost. It has been reported that various herbal plant extracts demonstrated prominent antidepressant activity due to the presence of rich antioxidants and flavonoids. The flavonoids have been accounted for their neuroprotective effects as they decrease the oxidative stress in CNS.

On the basis of literature and traditional of uses of *S. campanulata flowers*, an effort has been made to establish the scientific validity to investigate antidepressant activity.

Phytochemical tests were performed to investigate the existence of various classes of secondary metabolites which are responsible for imparting pharmacological activity.

The findings from phytochemical screening of *S. campanulata* flowers depicted the presence of polyphenols in all extracts, while flavonoids were present in CESC, EASC and MESC**.**

After the administration of EASC and MESC no lethality was observed. The results of antidepressant activity in FST and TST revealed the significant antidepressant potential by MESC. Therefore MESC was selected for further evaluations. The mice treated with MESC potentially reduced the head twitches as compared to vehicle group mice. The mice treated with MESC also showed a significant decrease in term of catalepsy compared with vehicle group. Moreover a marked increase was observed in locomotor activity of rats after the administration of MESC. The lower cataleptic score and locomotor activity of mice treated with extract signifies the antidepressant potential of *S. campanulata* flower**.** Findings of the animal behavioral studies revealed that MESC has the most effective antidepressant.

Conclusion

The flower of *S. campanulata* is rich in flavonoids and polyphenolic has been found effective against various pharmacological activities. The MESC demonstrated significant antidepressant activity in albino mice. The present study confirmed the antidepressant activity *S. campanulata* flower, yet further studies are required to elucidate its mechanism of action. Hence it would be a wide opportunity for the researchers in future.

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