

**PHYTOCHEMICAL ANALYSIS AND IN VIVO ANTIDEPRESSANT ACTIVITY OF
HYDROALCOHOLIC EXTRACT OF *CASCABELA THEVETIA*****Adeeba Shaikh*, Balweer Singh Kirar, Pratyush Jain****RKDF College of Pharmacy, SRK University, Bhopal (M.P.)***Corresponding Author's E mail: Adeebashaikh01@gmail.com

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ABSTRACT

Depression is a common mental condition that is characterised by low self-esteem, diminished energy, lack of interest or pleasure, guilt feelings, disturbed sleep, difficulty concentrating, and melancholy mood. Today, a wide range of synthetic drugs are used as the standard treatment for patients who are clinically depressed. However, these drugs have side effects that can interfere with therapeutic treatment. There is a need to find novel medications derived from medicinal plants. Thus, this study deals with phytochemical analysis and in vivo antidepressant activity of hydroalcoholic extract of *Cascabela thevetia*. The plant material was collected and subject to extraction followed by quantitative & in vivo study. Results showed that plant contain almost all phytochemicals except phenol & diterpene. Total flavonoid & alkaloid content was found to be 0.863 mg/100mg & 0.756 mg/100mg. In FST, extract of *Cascabela thevetia* leaf 100 and 200 mg/kg, p.o. produced significant reduction ($p < 0.01$) in the immobility period when compared with that of control group animals that received only the vehicle. In TST, Extract of *Cascabela thevetia* leaf 100 and 200 mg/kg, p.o. produced significant reduction ($p < 0.01$) in the immobility period when compared with that of control group animals that received only the vehicle. It is clear from the results that extract of *Cascabela thevetia* leaf at 200 mg/kg showed potent antidepressant activity compared to that of standard drug.

Keywords: *Cascabela thevetia*, antidepressant, Medicinal plants, Phytochemicals

INTRODUCTION

According to world health report, about 450 million people suffer from a mental or behavioral disorder. By the year 2020, depression is expected to constitute the second largest source of global burden of disease after heart disease. Depression is whole body illness which involves not only mood or emotion but also the physical body and thought process. The symptoms of depression are intense feelings of sadness, hopelessness, and despair, as well as the inability to experience pleasure in usual activities, changes in sleep patterns and appetite, loss of energy, and suicidal thoughts^{1,2}.

Unipolar depression, which is prevalent (occurs in about 75% of cases), non-familial, obviously linked to stressful life events, and accompanied by symptoms of anxiety and agitation, is one of two forms of mental depression. Bipolar depression, which accounts for roughly 25% of cases and is occasionally referred to as endogenous depression, exhibits a well-known pattern. Unrelated to outside influences, it typically manifests in early adulthood and causes oscillating despair and mania over a few weeks. Depression patients have symptoms that are related to decreased levels of the monoamine neurotransmitters norepinephrine, serotonin, and dopamine in the brain^{3,4}.

Today, a wide range of synthetic drugs are used as the standard treatment for patients who are clinically depressed. However, these drugs have side effects that can interfere with therapeutic treatment. These side effects include dry mouth, nausea, gastrointestinal or respiratory issues, drowsiness, anxiety, and cardiac arrhythmias. Because of these conditions, alternative depression treatments using medicinal plants are now possible. Currently, researchers are looking for more specialised medications with high therapeutic effectiveness, few adverse effects, and affordable price⁵⁻⁶.

In tropical Asia and America, the *Cascabela thevetia* plant is frequently used in home medicine. It has historically been used to cure conditions like amenorrhea, malaria, jaundice, haemorrhoids, constipation, migraines, and skin diseases. Jaundice, fever, and intestinal worms are all treated with a decoction of the leaves. To treat severe headaches, the leaf sap is utilised as eye and nose drops. The seeds can be used as an abortifacient and as a purgative to treat rheumatism and dropsy. Sometimes the ground seeds are used as a component of suppositories to treat haemorrhoids. To treat skin issues, the oil from the kernel is administered topically^{7,8}. Thus considering its medicinal properties this study deals with evaluation of antidepressant activity of hydroalcoholic extract of *Cascabela thevetia*.

Materials & Methods

Plant material collection

The leaves of *Cascabela thevetia* were collected from Bhimbetka Bhojpur, Raisen (Madhya Pradesh) in the month of November, 2019.

Extraction by maceration process

Dried powdered leaves of *Cascabela thevetia* has been extracted with hydroalcoholic solvent (ethanol: water: 70:30) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40°C⁹.

Total alkaloid content

In dimethylsulphoxide, 1 mg of the plant extract was dissolved. 1 ml of 2N HCl was then added, and the mixture was then filtered. This solution was transferred to a separator funnel, and then 5ml of the

phosphate buffer and 5ml of the bromocresol green solution were added. A 10ml volumetric flask was used to collect the mixture after it had been vigorously agitated with 1, 2, 3, and 4ml of chloroform. The mixture was then diluted to the required volume with the chloroform. In the same way as previously described, a set of reference standard solutions of atropine (20, 40, 60, 80, and 100 g/ ml) were made. An UV/Visible spectrophotometer was used to measure the absorbance for standard solutions and test solutions at 470 nm on the reagent blank. Alkaloids were expressed as mg of AE/g of plant extract for the content ¹⁰.

Total flavonoid content

The total phenol concentration was measured using a spectrophotometric technique. The stock solution (1 mg/ml, w/v) was made by combining 10 mg of the separate solidified leaves extracts with 10 ml of methanol. The 3 ml of extract was then transferred to a test tube. The 2% AlCl₃(1 ml) added to the test tube. After 15 minutes at room temperature, the sample solutions' absorptions were measured at 420 nm with methanol as a reference. same procedure is followed for standard quercetin. Using the standard curve, the total flavonoid content was calculated and expressed as equivalent in µg/mg of extract ¹¹.

In vivo Antidepressant activity

Animals: -

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Toxicity study

Healthy adult male albino rats were fasted overnight prior to the experiment. Different doses (50-2000 mg/kg, P.O) of the extract of *Cascabela thevetia* leaf were administered to each group of rats (Each group carries 6 rats) and they were observed continuously for 1 hour and then at half-hourly intervals for 4 hour, for any gross behavioural changes and further up to 72 hour, followed 14 days for any mortality as per the OECD (Organization for Economic Co-operation and Development) Guideline 425 (OECD guidelines,2001). The extract of *Cascabela thevetia* leaf was found to be non-toxic up to the maximum dose of 2000 mg/kg body weight. Dose selected for antiulcer evaluation was 100 and 200 mg/kg respectively.

Experiential

Group 1: Received Normal saline

Group 2: Received 15 mg/kg imipramine orally (Standard)

Group 3: Received 100 mg/kg of extract of *Cascabela thevetia* leaf orally

Group 4: Received 200 mg/kg of extract of *Cascabela thevetia* leaf orally

Forced swimming test (FST)

Principle: “Behavioral despair” is a standard proposed model to test for antidepressant activity. It is suggested that rats or mice forced to swim in a restricted space from which they cannot escape are induced to a characteristic behaviour of immobility. This behaviour reflects a state of despair which can be reduced by agents which are therapeutically effective in human depression.

Procedure: The animals were forced to swim in a glass cylinder measuring 25cm height, 12cm diameter containing water at room temperature to a depth of 15cm. After an initial 2-minute period of vigorous activity, each animal assumed a typical immobile posture. The mouse was considered immobile when it remained floating in the water without struggling, making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during next 4 minutes of total 6-minute test. After 6 min mouse was taken out, dried with a towel. The water is changed after each test because urine and the other chemicals released by the first mouse will affect the swimming pattern of the next mouse. Each animal was used only once ¹².

Tail suspension test (TST)

Principle: This test has been a facile means of evaluating potential antidepressants. The immobility displayed by rats when subjected to an unavoidable and inescapable stress has been hypothesized to reflect “behavioral despair” which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that rats display after active and unsuccessful attempts to escape when suspended by the tail.

Procedure: Animals were suspended upside down on a metal rod at a height of 55 cm from the ground with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. Initially the animals tried to escape by making vigorous movements but when unable to escape became immobile. The animal was considered immobile when it did not show any movement of body and hanged passively. The total duration of immobility was noted during last 4 minutes of 6-minute period. Each animal was used only once ¹³.

Statistical analysis

The results are presented as mean \pm SEM. The statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett’s post hoc test as appropriate using graph pad

software. Differences between groups were considered significant at a level of $p < 0.001$.

RESULTS & DISCUSSION

The phytochemical test revealed the almost all phytochemicals except phenol & diterpene. Total flavonoid & alkaloid content was found to be 0.863 mg/100mg & 0.756 mg/100mg. In FST, extract of *Cascabela thevetia* leaf 100 and 200 mg/kg, p.o. produced significant reduction ($p < 0.01$) in the immobility period when compared with that of control group animals that received only the vehicle. In TST, Extract of *Cascabela thevetia* leaf 100 and 200 mg/kg, p.o. produced significant reduction ($p < 0.01$) in the immobility period when compared with that of control group animals that received only the vehicle. It is clear from the results that extract of *Cascabela thevetia* leaf at 200 mg/kg showed potent antidepressant activity upto that of standard drug.

Table 1: Phytochemical screening of extract of *Cascabela thevetia*

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	
	Dragendroff's test	-ve
	Hager's test	+ve
2.	Glycosides	
	Legal's test	+ve
3.	Flavonoids	
	Lead acetate	+ve
	Alkaline test	+ve
4.	Phenol	
	Ferric chloride test	-ve
5.	Proteins	
	Xanthoproteic test	+ve
6.	Carbohydrates	
	Fehling's test	+ve
7.	Saponins	
	Foam test	+ve
8.	Diterpenes	
	Copper acetate test	-ve

Table 2: Estimation of total flavonoids and alkaloid content of *Cascabela thevetia*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.863	0.756

Table 3: Effect of extract of *Cascabela thevetia* leaf on immobility time in Forced swim test

Group	Treatment	Dose (mg/Kg)	Forced Swim test Duration of Immobility (Sec)
Group 1	Normal saline	-	150.30±6.6
Group 2	Imipramine	15 mg/kg orally	48.8±4.5***
Group 3	Extract of <i>Cascabela thevetia</i> leaf	100 mg/kg of orally	65.5±4.5**
Group 4	Extract of <i>Cascabela thevetia</i> leaf	200 mg/kg of orally	58.5±4.5***

Table 4: Effect of extract of *Cascabela thevetia* leaf on immobility time in Tail Suspension test

Group	Treatment	Dose (mg/Kg)	Tail Suspension test Duration of Immobility (Sec)
Group 1	Normal saline	-	148.5±5.6
Group 2	Imipramine	15 mg/kg orally	91±4.56***
Group 3	Extract of <i>Cascabela thevetia</i> leaf	100 mg/kg of orally	105.5±4.2**
Group 4	Extract of <i>Cascabela thevetia</i> leaf	200 mg/kg of orally	98.5±4.0***

CONCLUSION

The current study offers the first proof that *Cascabela thevetia* hydroalcoholic extract significantly reduced depressive symptoms in the TST and FST models of depression. To understand how it works & mechanism of action behind it at molecular level, more investigation is needed.

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