



PHYTOCHEMICAL SCREENING AND ANTIDEPRESSANT ACTIVITY OF LEAVES EXTRACT OF DESMODIUM GANGETICUM

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Abstract

According to Biological Conservation Letter, more than 7,000 species of plants found in various ecosystems are said to be medicinal in the country. So, India is one of the world's richest sources of medicinal and aromatic plants. *Desmodium gangeticum* is an important medicinal plant. It is commonly used in ayurvedic formulations for the treatment of various disorders. Phytochemical evaluations, pharmacogonostic evaluation, organoleptic characters, TLC profile was carried out to set them as diagnostic indices for the identification/validation of the raw material and standardization of the formulations. Preliminary phytochemical analysis showed the presence of active constituents which is necessary for the pharmacological activity. Organoleptic properties, phyto-chemical studies, powder analysis, showed the presence of adulteration in the powder. Majority of the antidepressant drugs improve depressive symptoms, but they exert multiple undesirable side effects. The search for more productive and well tolerated drugs is in progress. Phytochemical analysis of *Desmodium gangeticum* revealed the presence of alkaloids, phenols, flavonoids, Saponins, Steroids. *Desmodium gangeticum* is a well known medicinal plant as anti-inflammatory, antimicrobial and nephroprotective etc. It is a very good drug for urinogenital problems, hepatic problems, oxidative stress etc. The present study was depict to evaluate the antidepressant activity of hydroalcoholic extract of *Desmodium gangeticum* in mice. It was evaluated using the Tail Suspension Test (TST) and Forced Swimming Test (FST) in mice. *Desmodium gangeticum* (200 and 400 mg/kg) was administered orally in separate groups of Swiss albino mice weighing 20-25 for 14 days in TST and FST tests.. The Leaves extract of *Desmodium gangeticum* showed a dose dependant reduction in duration of immobility in mice. The dose of 400 mg/kg of Leaves extract of *Desmodium gangeticum* significantly reduced the immobility time of mice in both FST and TST. The effectual of extract was found to be similar to fluoxetine (20 mg mg/kg, po). It was found to be toxicologically safe with no deaths of mice when administered orally at the dose of 2000 mg/kg. From the current study, it can be concluded that the Leaves extract of *Desmodium gangeticum* possess dominant antidepressant activity as reveal by the TST and FST tests and is toxicologically safe.

Keywords: *Desmodium gangeticum*, Organoleptic properties, phytochemical studies, TLC, Depression, Tail suspension test, Forced swimming test, Fluoxetine.

Introduction

Depression as “a mental condition define by severe feelings of despondency and inadequacy, typically go along with by a lack of energy and interest in life.” Depression is a common mental disorganization that presents with depressed mood, loss of interest or rapture, feelings of guilt or low self-worth, disturbed sleep or appetite, low vitality, and poor attentiveness. It usually occurs as a result of unfavourable life occasion, such as: losses of a significant person, object, relationship or health, but it can also occur due to no apparent cause. These problems can become immedicable or recurrent and lead to substantial destruction in an individual's ability to take care of his or her every day responsibilities. Depression is the leading cause of disability and the 4th leading contributor to the global essence of disease in 2000. Today, depression is already the 2nd cause in the age category 15-44 years for both sexes combined. The lifetime risk of depression varies from 5% to 12% in men and 10% to 25% in women. Suicide is the major consequences in most of the depressive illnesses. About 60% deaths are due to depression and related disorders psychological depression is a incurable illness that affects a person's mood, thoughts, actual well-being and behavior. Symptoms of depression include biological and emotional components. Biological symptoms include retardation of thought and action, loss of sensuality, sleep disturbance and loss of appetite. Emotional symptoms include misery, apathy and pessimism, low amour proper consisting of feeling of guilt, inadequacy and ugliness, indecisiveness and loss of inspiration. There are two types of mental depression, namely unipolar depression, in which mood swings are always in the same direction and is common (about 75% of cases), non-familial, clearly associated with stressful life events, and escort by symptoms of disquietude and agitation. The second type is bipolar depression (about 25% of cases), sometimes also called as endogenous depression, shows a familiar pattern, unrelated to external stresses and

usually appears in early adult life, and is much less common, results in wigwag depression and mania over a period of a few weeks. Patients with depression have symptoms that reflect decrease in brain monoamine neurotransmitters, specifically nor epinephrine, serotonin and dopamine.

Manic-depressive or bipolar is not almost as usual as other forms of depressive illnesses. It involves cycles of depression and elation or mania. Sometimes the mood switches are considerable and rapid, but most often they are restrained. When in the depressed cycle, one can have any or all other the symptoms of a depressive illness. When in the manic cycle, any or all symptoms listed under mania may be experienced. Mania often affects thinking, judgment, and social deportment in ways that may cause serious problems and embarrassment *Desmodium gangeticum* is an annual plant widely allocate around the world. It is alter to grow in dry climate locations in which few other plants can hold on. It is domestic to warm temperate and tropical regions in southern Eurasia and Africa. It has been unintentionally introduced to North America and Australia. An aggressive and hardy presumptuous species, the plant is an erect, diffusely branched under shrub, 90-120 cm in height with a short woody stem and numerous prostrate branches provided with soft grey hairs. Leaves simple, ovate lanceolate and membranous. Flowers white or purple or lilac in elongate lax terminal or axillary racemes. Fruits moniliform 6-8 glabrescent, pods sparsely pubescent with hooked hairs, joints separating when ripe into indehiscent one seeded segments, seeds compressed and reniform. It is distributed in all parts of India in dry conditions. Although in traditional medicine genus *Desmodium* is very well known, but still *Desmodium gangeticum* invites attention of researchers worldwide for its ethnomedicinal uses, phytochemistry and pharmacological activities ranging from antidiabetic to antiviral. To the best of our knowledge, very little information is available on phytochemical profile of *Desmodium*

gangeticum. Hence, the present investigation was overviewed to explore the phytochemical profile and ethnomedicinal uses of valued endangered medicinal plant – *Desmodium gangeticum*. Preliminary phytochemical analysis showed the presence of active constituents which is necessary for the pharmacological activity. Organoleptic properties, phyto-chemical studies, powder analysis, showed the presence of adulteration in the powder.

2. Methodology

2.1 Preparation of plant extract

Dried Leaves of plant of *D. gangeticum* were collected during the month of September 2020 from local herbal garden of Bhopal (M.P.). Leaves of plant of *D. gangeticum* were washed and shade dried than coarsely powdered in a grinder. Powder dried plant were extracted with water and ethanol (30:70) by the maceration Process.

2.2 Phytochemical screening

Phytochemical screening for the identification of various phytoconstituents such as alkaloids, carbohydrates, steroids, cardiac glycosides, flavonoids, carbohydrates, amino acids, phenolics and tannins according to standard methods were performed.

Test for carbohydrate

Molisch test

A small quantity of the extracts was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to Molisch's reagent and formation of brick red colour confirmed the presence of reducing sugar.

Fehling's test

Equal volume of Fehling A and Fehling B reagents were mixed with few drops of crude extract is added and boiled, a brick red precipitate of cuprous oxide forms, if reducing sugar are present.

Test for glycosides

Borntrager's test

100 mg crude extract was mixed with 2 ml of dilute sulphuric acid and 2 ml of 5% aqueous ferric chloride solution, boiled for 5 minutes which lead to oxidation to anthraquinones, indicating the presence of glycosides.

Test for Alkaloids

Mayer's test

Crude extract was mixed with Mayer's reagent (potassium mercuric iodide solution). Cream colour precipitate was formed, indicating the presence of alkaloids.

Dragendroff's test

Crude extract was mixed with Dragendroff's reagent (potassium bismuth iodide solution). Reddish brown precipitate was formed which suggested the presence of alkaloids.

Wagner's test

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Test for Flavanoids

Alkaline reagent test

Crude extract was mixed with few drops of sodium hydroxide solution. An intense yellow colour was formed. Yellow colour turned to colorless on addition of few drops of diluted acid, marked the presence of flavanoids.

Lead acetate test

To a solution of 0.5 g extract in water, about 1ml of 10% lead acetate solution was added. Production of yellow precipitate is considered as positive for flavonoids.

Test for Saponins

Froth test

0.5g extracts were dissolved in 10ml of distilled water for about 30 seconds. The test tube was stoppered and shaken vigorously for about 30 seconds. The test tube was allowed to stand in a vertical position and observed over 30 minutes period of time. If a "honeycomb" froth above

the surface of liquid persists after 30 minutes the sample is suspected to contain saponin.

Test for Tannins

Ferric chloride test

Crude extract was mixed with ferric chloride. Blue green colour appeared, suggested the presence of tannins.

2.3 Determination of extractive value

5 grams of the coarse drug was macerated with 100ml of alcohol for 24 hours with occasional shaking for the first 6 hours and then left aside for 18 hours. It is filtered taking precautions to avoid loss of solvent. It is evaporated in a flat container at 105 degrees until constant weight. the percentage of water soluble extractive value was calculated with reference to the air dried drug.

2.4 Thin layer chromatography

100 gram of silica gel G was dissolved in sufficient amount of water and was coated on the glass plate. Solvent system chosen was toluene: chloroform :methanol (5:8:3) aqueous extract was dissolved in sufficient water to make up a concentration of 1mg/ml. similarly ethanolic extract and chloroform extract was dissolved in ethanol and chloroform.

The spots were made 1 cm from the bottom of the glass slide. The glass plate was kept in to the chamber after chamber saturation and allowed to run 2-3rd of the glass plate. R_f value was calculated.

$$R_f \text{ value} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front}}$$

2.5 Antidepressant activity of desmodium gangeticum

2.5.1 Animals

Swiss albino mice weighing 20-25 of either sex were used for this study. The experimental animals were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25±3°C and 35-60% humidity). Standard pelletized feed and tap

water were provided *ad libitum*. Study protocol was approved by the Institutional Animal Ethical Committee (IAEC).

2.5.2 Acute toxicity studies

Acute toxicity studies were performed according to OECD (Organization for Economic Co-operation and Development) 425 guideline. Animals were acclimatized to laboratory condition five days prior to the experiment. Body weight of animals was recorded and individual identification was done, fixed dose method: Test procedure with a starting dose of 2000 mg/Kg Body weight of hydroalcoholic leave extract of *Desmodium gangeticum*. Starting dose of extract was administered orally to 5 animals and animals were observed for behavioral changes and death. No animals were found dead after 14 days. The study was repeated with same dose and again no death was observed.

A. Evaluation of antidepressant activity

1. Forced swimming test (FST)

The FST is the most widely used pharmacological *in vivo* model for assessing antidepressant activity. Mice were individually placed in cylinder (45×20 cm) containing 15 cm water (25±2°C), so that it could not touch the bottom of the cylinder with its hind limb or tail, or climb over the edge of the chamber. Mice were divided into groups of five and received the hydroalcoholic leave extract of *Desmodium gangeticum* at different doses *viz.* 200 and 400 mg/kg and Floxutine (20mg/kg) was used as standard drug. One hour post administration each mice were placed individually in a tank. Period of immobility during the 6 min test period was measured.

2. Tail suspension method

Each mice in the group was suspended individually by the end of tail with adhesive tape placed approximately 1cm from the tip of the tail. mice were divided into groups of five and received hydroalcoholic leave extract of *Desmodium gangeticum* at different doses *viz.* 200 and 400 mg/kg control and Floxutine (20mg/kg) was used as standard drug on the test day after 60 mins of the administration of last dose. The Duration of immobility was observed for a period of 8 minutes. After the

early escape oriented actions, the mice rapidly turns out to be immobile and immobility (when it did not show any movement of body and hanged passively) was recorded during last 5 mins of observation period.

Statistical Analysis

The data were expressed as mean \pm standard error mean (SEM). The significance of differences among the groups was assessed

using one way analysis of variance (ANOVA). The test was followed by Dunnett's'-test, p values less than 0.05 were considered as significance.

3. Results and Discussion

3.1 Result of % Yield of hydroalcoholic leaves extract of *Desmodium gangeticum*

Table 1: % Yield of hydroalcoholic extract of *Desmodium gangeticum*

S. No.	Plant	% Yield (w/w)
1.	<i>Desmodium gangeticum</i>	1.20 %

3.2 Result of Phytochemical Screening of hydroalcoholic extract of *Desmodium gangeticum*

Table 2;

S. no.	Constituents	Hydro alcoholic extracts
1.	Alkaloids	
	Dragendroff's test	+ve
	Wagner's test	+ve
	Mayer's test	+ve
2.	Glycosides	
	Libermann's test	-ve
	Keller Kilani Test	-ve
3.	Flavonoids	
	Shinoda test	+ve
	Lead Acetate test	+ve
4.	Phenolics	
	FeCl ₃ test	+ve
5.	Carbohydrates	
	Molichs test	+ve
	Benedict;s test	-ve
6.	Tannins	
	Ferric chloride test	-ve
7.	Saponins	
	Foam Test	+ve
8.	Phytosterol	
	Liberman burchard test	-ve
	Salkowski reaction	+ve
9.	Proteins and amino acids	
	Million's test	-ve
	Biuret test	-ve
	Ninhydrin test	-ve

3.3 Results of Comparative Thin Layer Chromatography of hydroalcoholic extract of *Desmodium gangeticum*

From the R_f value it was confirmed the presence of Quercetin as Flavanoids in the extract.

Result of TLC of extracts, Table No. 3

S. No.	Toluene: Ethyl acetate: Formic acid (5:4:1) Quercetin (R _f value)
1.	0.71

3.4 Results of *in vivo* Antidepressant activity

3.4.1 Acute toxicity study

2000 mg/kg dose of hydroalcoholic extract aerial parts of *Desmodium gangeticum* employed for acute oral toxicity studies were found to be non toxic. Hydroalcoholic Leaves extract of *Desmodium gangeticum* did not produce any mortality even at the highest dose (2000 mg/kg) employed. Two sub maximal doses (200 and 400 mg/kg), were found to be safe, were employed for further pharmacological investigations.

A. Evaluation of Anti-stress activity

1. Forced swimming test (FST)

The forced swimming test is the most widely used method for the evaluation of Antidepressant property of a novel compound. mice when forced to swimming in a restricted space become immobile after an initial period of vigorous activity, indicating the stress. This method is based on the observation that animals forced to swim in water eventually assumed a characteristic immobile posture, devoid of any activity. The appearance of immobility,

therefore indicate a state of tiredness, fatigue, reduced stamina or a lowered mood (hopelessness). These signs represent the core symptoms observed in individuals under intense stress.

In forced swim test, the immobility time of control, test (200 and 400 mg/kg) and standard was 130 ± 1.50 , 95 ± 1.32 **, 72 ± 2.18 ** and 64 ± 0.28 *** respectively. The immobility time of test and standard was significant (** $p < 0.01$) and more significant (***) $p < 0.001$) respectively. The immobility time decreases with increase in dose of the extract. The immobility time of test was gradually decreases when compared to control. Mice pretreated with hydroalcoholic leaves extract of *Desmodium gangeticum* show significant improvement in the swimming time as compared to standard control. The antidepressant effect of the *Desmodium gangeticum* was prominent at 400mg/kg. In the forced swimming test all the doses administered were able to reduce immobility time and simultaneously enhance swimming.

Table 4: Effect of hydroalcoholic extract of *Desmodium gangeticum* on animal in Forced Swim Test

Group	Treatment	Immobility time (in Seconds)
Group 1	Control-Water	130 ± 1.50
Group 2	Hydroalcoholic leaves extract of <i>Desmodium gangeticum</i> (200 mg/kg, p.o.)	95 ± 1.32 **
Group 3	Hydroalcoholic leaves extract of <i>Desmodium gangeticum</i> (400 mg/kg, p.o.)	72 ± 2.18 **
Group 4	Fluoxetine (20 mg/kg, p.o.)	64 ± 0.28 ***

Each values represents the mean \pm SEM; (n=6), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ respectively when compared with control group (one-way ANOVA followed by Dunnett's test).

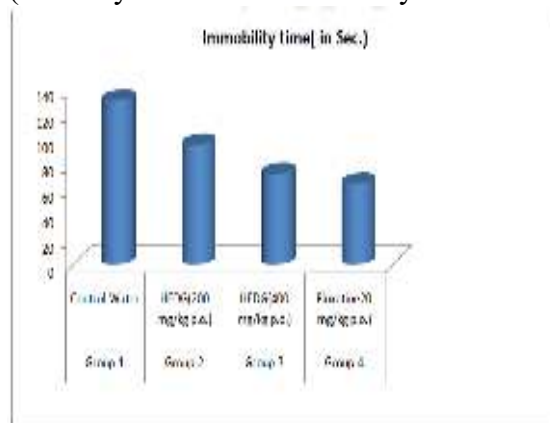


Figure 1: Effect of hydroalcoholic extract of *Desmodium gangeticum* on animal in Forced Swim Test

2. Tail suspension method

In the tail suspension test, the mice shown immediate sign of struggles or escape like behaviors when they were suspended in the air followed by temporary increasing periods of immobility. The tail suspension method revealed in the present study that anti stress activity increases with decrease in immobility time compared to control mice of *Desmodium gangeticum*. In tail suspension test, the

immobility time of control, test (200 and 400 mg/kg) and standard was 215 ± 2.64 , 189 ± 3.24 , 171 ± 9.15 and 160 ± 7.52 respectively. The immobility time of test and standard was significant (** $p < 0.01$) and more significant (***) $p < 0.001$) respectively. The immobility time decreases with increase in dose of the extract. The immobility time of test was gradually decreases when compared to control.

Table 5: Effect of hydroalcoholic extract of *Desmodium gangeticum* on animal in Tail suspension test

Group	Treatment	Immobility time (in Seconds)
Group 1	Control-Water	215 ± 2.64
Group 2	Hydroalcoholic Leaves extract of <i>Desmodium gangeticum</i> (200 mg/kg, p.o.)	189 ± 3.24 **
Group 3	Hydroalcoholic Leaves extract of <i>Desmodium gangeticum</i> (400 mg/kg, p.o.)	171 ± 9.15 **
Group 4	Fluoxetine-(20 mg/kg. p.o.)	160 ± 7.52 **

Each values represents the mean \pm SEM; (n=6), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ respectively when compared with control group (one-way ANOVA followed by Dunnett's test).

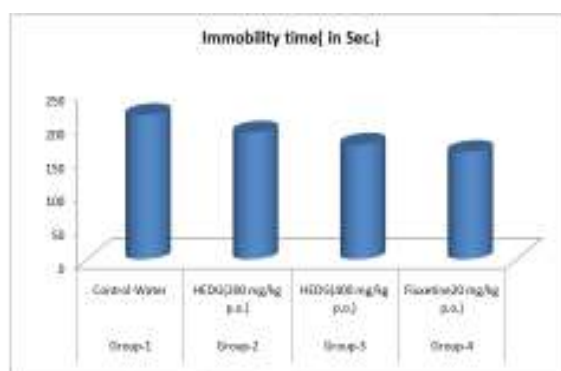


Figure 2: Effect of hydroalcoholic extract of *Desmodium gangeticum* on animal in Tail suspension test

Conclusions

Preliminary phytochemical screening showed the presence of active constituents necessary for the pharmacological activity. Depression is a common mental disorder that presents with depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, and poor concentration. In the present study phytochemical analysis showed the presence of Flavonoids compound have been reported to have multiple biological effects such as Central

nervous system disorders. In this study, *Desmodium gangeticum* Leaves extracts revealed dose-dependent antidepressant activity in mice, and the higher dose of extracts was found to have the highest effect in depression. The extracts of *Desmodium gangeticum* and significantly inhibited the activity of MAO-A *in vivo* in the mice brain in a dose-dependent manner. But, just 400 mg/kg b.wt of *Desmodium gangeticum* extract and inhibited MAO-B activity. These results pointed out that the antidepressant activity of extracts in mice

of immobility testings may be correlated to the inhibition of MAO activity, particularly to MAO-A activity. The oral administration *Desmodium gangeticum* extract to the mice had antidepressant activity, possibly by regulating the central neurochemical axis and HPA, in response to FST-induced stress. Consequently, this work recommends the application of administration *Desmodium gangeticum* extracts as a meaningful botanical supplement for medicating the depression. In the coming study, the elaborate study is required to entirely clarify the action mechanisms of the bioactive compounds presenting in the administration *Desmodium gangeticum* extracts at the cellular level.

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