



Evaluation of Antidepressant Activity of *Plocama pendula* root extract

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Abstract:

Plocama is a flowering plant genus belonging to Rubiaceae family. The natural products are soxlated from the dried powder of Plocama root with various solvents like *n*-Hexane, Ethyl acetate, Chloroform, Water and Ethanol. Phytochemical screening revealed that Alkaloids are present in chloroform extract where as Anthocyanins are present in ethanol extract. Terpenoids, Flavonoids, Alkaloids and Phenolic compounds are present in water extract.

In the present study, Plocama pendula root water extract (PPRWE) (400 mg/kg) produced significant antidepressant effect in Forced swim test & Tail Suspension Test. Ecliptin alkaloid & Culumbin, a flavonoid present in PPRWE may be facilitating mono aminergic transmission there by producing antidepressant effects.

In this work, it was demonstrated that the administration of different doses of the water extract of Plocama pendula root in mice was able to induce antidepressant effects. On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess stress-precipitated behaviors. Results of this study showed that the administration of the PPRWE produced a diminution of immobility time of mice exposed to the both forced swimming and tail suspension tests.

Harmaline alkaloids present in Plocama pendula act as reversible monoamine oxidase inhibitors and in common with other beta carboline binds to 5-Hydroxy Tryptamine receptors. Inhibition of this enzyme causes a reduction in metabolism and subsequent increase in the concentration of biogenic amines. The flavonoid components of PPRWE might be interacting with adrenergic and serotonergic systems in mediating the antidepressant effects of PPRWE. However, the precise mechanisms by which the extract produced antidepressant-like effect are not completely understood. Further studies would be necessary to evaluate the contribution of active chemical constituents for the observed antidepressant activity.

Keywords: Plocama pendula, Antidepressant Activity, Forced Swim Test, Tail Suspension Test.

Introduction:

Plocama is a genus of flowering plants in the family Rubiaceae. It was described by William Aiton in 1789. It is distributed from the Canary Islands to northwestern India [1]. The genus was expanded in 2007 when several other Rubiaceae genera were merged into it. There are now about 34 species.[2]. Plants of this genus are herbs or shrubs. They generally have an offensive scent when crushed. The leaves are oppositely arranged. The corolla is funnel shaped with a short to long tubular base. The fruit is either a drupe or a body that splits into two parts when ripe[2].



Image.1 Plocamapendula

Kingdom:	<u>Plantae</u>
Order:	Asterids
Family:	Rubiaceae
Genus:	Plocama
Species:	P. pendula
Binomial name:	PlocamaPendula

Table.2 Scientific data of Plocamapendula

Plocamapendula is a species of flowering plant in the coffee family Rubiaceae, endemic to the Canary islands.[1][2]:260 It is a shrub growing up to 2 m (6 ft 7 in), with fili form (very narrow leaves) and minute flowers grouped near the tips of slender, pendulous branches. The fruit is a black berry.[2]

This species is a shrub or small tree that is endemic to the Canary Islands, where it can be frequently found across its range. Its habitat is mainly xerophytic scrubs over coastal areas and mid-altitude slopes. Although suitable habitat for this species has been negatively affected by urban development, invasive species and other impacts, the situation has not reached a point where the conservation status of this species is threatened. The species is thus assessed as Least Concern. The introduction and rapid spread of the exotic species *Acacia farnesiana* could become a factor to be considered in the future. Confirmation of its current presence and distribution on some of the islands is required.

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This species is endemic to the Canary Islands, where it is found on all the islands except Lanzarote (Gobierno de Canarias 2017), although its presence in El Hierro and La Palma requires confirmation. The extent of occurrence (EOO) of the wild populations of the species is estimated to be about 39,046 km², including El Hierro and La Palma (Gobierno de Canarias 2017) and the area of occupancy (AOO) has been calculated to be close to 1,700 km² (Gobierno de Canarias 2017). It occurs from 50 to 500 m above sea level (Dirección General de Protección de la Naturaleza 2011), and even up to 700 m a.s.l.

There is little information regarding the population of this species although it is considered abundant throughout its distribution and the trend is assumed to be stable.

This is a shrub or small tree that can grow up to 4 to 5 m in height. It is found in xerophytic scrubs in coastal areas, in cliffs and hill slopes with rocky soils and plenty of sun exposure, and along the margins and channels of ravines (Kunkel 1981). Although suitable habitat for this species has been negatively affected by urban development, the situation has not reach a point where the conservation status of this species is threatened [3]. The fruit of this species is an important part of the summer diet of birds and reptiles such as sylviid warblers (*Sylvia* spp.) and lacertid lizards (*Gallotia* spp.) (Valido and Nogales, cited by Dennis *et al.* 2007), which act as seed dispersers. Invasive species are also related to *P. pendula*. In Tenerife by the European rabbit (*Oryctogaluscuniculus*) (Nogales *et al.* 1995, 1996); and in all of the islands by the feral cat (*Felis catus*) (Nogales *et al.* 1996).

Given its resistance to drought, this species can be used in the re-vegetation of lower and dryer regions of the Canary Islands (Dirección General de Protección de la Naturaleza 2011). It has been traditionally used as a natural coloring element (Pérez de Paz and Hernández Padrón 1999).

Some of the known locations are found in protected areas in the Canary Islands (Protected Planet 2017). According to BGCI (2017) there are no *ex situ* collections of this species in botanic gardens worldwide. No other conservation actions needed have been identified [3].

3. Materials and Methods:

Preparation of plant root extract:

The collected *PlocamaPendula* roots were dried under shade, powdered and sieved through 40mesh. About 100g of powdered materials were extracted with petroleum ether (60°-80°C) using soxhlet apparatus. The extraction was carried out until the extractive becomes colorless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in tween 80 and used for the experiment.

The list of chemicals and equipment with make are showed in Table.1 and Table.2. All reagents are analytical grade. Required animals are purchased from Raaj Farms, Shastri Nagar, Chennai, India.

S.No	Equipment	Make
1	Electronic balance	Denver
2	Spectrophotometer	TECH CHOMP
3	1. Tail Suspension Test apparatus	HA 76-0783
4	Forced swim test	2. ANY-MAZE

Table.2 List of Equipment

S.No	Equipment	Make
1	Sulphuric acid	SD Fine (Analytical Grade)
2	Ferrous chloride	Merck (Analytical Grade)
3	Ammonia	Merck (Analytical Grade)
4	Lead Acetate	SD Fine (Analytical Grade)
5	Ferric chloride	SD Fine (Analytical Grade)
6	Hydrochloric acid	Merck (Analytical Grade)
7	Barfoed's reagent	Merck (Analytical Grade)
8	Molisch's reagent	Merck (Analytical Grade)
9	Fehling-B reagent	Merck (Analytical Grade)
10	Fehling -A reagent	Merck (Analytical Grade)
11	Pricric acid	SD Fine (Analytical Grade)
12	Potassium iodide	SD Fine (Analytical Grade)
13	Potassium bismuth iodide	Merck (Analytical Grade)
14	Chloroform	Merck (Analytical Grade)
15	Sodium chloride	SD Fine (Analytical Grade)
16	Glacial acetic acid	SD Fine (Analytical Grade)
17	Chlorosulfonic acid	Merck (Analytical Grade)
18	Zinc chloride	SD Fine (Analytical Grade)
19	Acetic anhydride	Merck (Analytical Grade)
20	Alloxan	Sigma-Aldrich
21	EDTA -	SD Fine (Analytical Grade)
22	KCl -	SD Fine (Analytical Grade)
23	HCl	SD Fine (Analytical Grade)
24	Fluoxetine hydrochloride	Cadila Pharmaceuticals Ltd.
25	Imipramine hydrochloride	Lupin Ltd
26	Diazepam	Biomed Pharma

Table.3 List of chemicals

Preparation of plant root extract:

The collected *Plocama Pendula* roots were dried under shade and powdered by a mechanical grinder, sieved through 40mesh. About 100g of powdered materials were extracted with petroleum ether (60°-80°C) using soxhlet apparatus. The extraction was carried out until the extractive becomes colorless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in tween 80 and used for the experiment.

Preliminary phytochemical screening:**1. Test for steroids:**

Salkowski Test: Few drops of concentrated sulphuric acid are added to the plant extract, shaken and on standing; lower layer turns red in color.

Liebermann Burchard's Test: To the extract, few drops of acetic anhydride is added and mixed well. 1 ml of concentrated sulphuric acid is added from the sides of test tube, a reddish brown ring is formed at the junction of two layers.

2. Tests for triterpenoids:

Salkowski Test: Few drops of concentrated sulphuric acid is added to the extract, shaken and on standing, lower part turns golden yellow colour.

Lieberman Burchard's Test: To the extract, few drops of acetic anhydride is added and mixed well. 1 ml of concentrated sulphuric acid is added from the sides of test tube, a red ring indicates triterpenes.

Ischugajiu Test: Excess of acetylchloride and pinch of zinc chloride are added to the extract solution, Kept aside for reaction to subside and warmed on water bath, cosin red colour is produced.

Brickorn and Brinar Test: To the extract, few drops of chloro sulfonic acid in glacial acetic acid (7:3) are added, red colour is produced.

3. Test for Saponins:

Foam Test: Small amount of extract is shaken with little quantity of water, the foam produced persists for 10 minutes. It confirms the presence of saponins.

Haemolysis Test: To 2ml of 1.8% Sodium chloride solution in two test tubes, 2ml distilled water is added to one and 2ml of 1% extract to the other, 5 drops of blood is added to each tube and gently mixed with the contents. Haemolysis observed under the microscope in the tube containing the extract indicates the presence of saponins

4. Test for Steroidal Saponin:

The extract is hydrolysed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for steroids.

5. Tests for Triterpenoidal Saponin:

The extract is hydrolyzed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for triterpenoids

6. Tests for Alkaloids

Mayer's Test: The acid layer when mixed with Mayer's reagent (Potassium mercuric iodide solution) gives creamy white precipitate

Dragendroff's Test: The acid layer with few drops of Dragendroff's reagent (Potassium bismuth iodide) gives reddish brown precipitate.

Wagner's Test: The acid layer when mixed with few drops of Wagner's reagent (solution of iodide in potassium iodide) gives brown to red precipitate

Hager's Test: The acid layer when mixed with few drops of Hager's reagent (Saturated solution of picric acid) gives yellow colored precipitate.

7 Tests for Carbohydrates:

Fehlings's Test: The extract when heated with Fehling's A and B solutions gives an orange red precipitate showing the presence of reducing sugar.

Molisch's Test: The extract is treated with Molisch's reagent and conc .sulphuric acid along the sides of the test tube, a reddish violet ring shows the presence of carbohydrate.

Benedict's test: The extract on heating with Benedict's reagent, brown precipitate indicates the presence of sugar.

Barfoed's Test: Barfoed's reagent is added and boiled on water bath for few minutes, reddish precipitate is observed for the presence of carbohydrate.

8. Test for Flavonoids:

Shinoda Test: The extract solution with few fragments of magnesium ribbon and concentrated hydrochloric acid produced magenta color after few minutes.

Ferric chloride test: Alcoholic solution of extract reacts with freshly prepared ferric chloride solution and given blackfish green color

Lead Acetate Test: Alcoholic solution of extract reacts with 10% lead acetate solution and given yellow precipitate.

9. Test for Glycosides:

Anthraquinone test: Drug is powdered and extracted with either ammonia or caustic soda. The aqueous layer shows pink color

Keller-killiani test: This is for cardiac glycosides. The extract and 0.4 glacial acetic acid are mixed with ferrous chloride and 0.5 ml of concentrated sulphuric acid. The acetic acid layer shows blue color

10. Test for Phenolic Compounds:-

Ferric chloride test:-Treat the extract with ferric chloride solution then blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.

Gelatin test: - To the test solution add 1% gelatin solution containing 10% NaCl, and then ppt is formed.

Test for chlorogenic acid:-Treat the test solution with aqueous ammonia and expose to air gradually, green colour is developed.

Antidepressant Activity of Plocama Pendulaplant root extract:

Acute toxicity study:

Animals were observed for four hours hourly for behavior changes and daily for six days. The extract was devoid of any toxicity in rats when given in dose up to 500 mg/kg by oral route. Hence, for further studies 100,200,400 mg/kg doses of extract were used.

Acute toxicity study was done according to OECD (Organization for Economic Co-operation and Development) Guideline, fixed dose method; with starting dose of 2000mg/kg body weight was adopted. Starting dose of 2000mg/kg (per oral) of each was given to 5 animals, animals were kept for observation of behavioral change and death up to 72h.

Animals

Male Swiss albino mice weighing between 25 – 30 g were used for the present study. The experiment animals were maintained under standard environmental conditions i.e 25°-27° C and relative humidity. Standard pellet diet provided and water and libitum.

Experimental protocols

Overnight fasted 42 mice were selected randomly, 42 animals are divide in to Seven groups i.e A,B,C,D,E,F,G Each group having six animals. On the day of experiment for administration of vehicle, standard drug and study drug i.e PPRWE (**Plocama Pendula root water extract**). The animals were acclimatized one hour before for behavioral tests. Thirty minutes and 1 hour time interval between drug administration and behavioral tests were maintained in case of intra peritoneal and oral administrations respectively.

Group No (n=6)	Drug and Dose
Group A	Control, received distilled water
Group B	Imipramine 15mg/kg
Group C	Fluoxetine 20 mg/kg
Group D	Diazepam 1mg/kg
Group E	PPRWE 100 mg/kg
Group F	PPRWE 200 mg/kg
Group G	PPRWE 400 mg/kg

Table. 4 Drug and dosage

The antidepressant activity was carried out using two different models. Further the effect of drugs was evaluated in open field test.

Forced Swim Test:

Forced Swim Test (FST): FST or behavior despair was proposed as a model to test for antidepressant activity by Porsolt et al[5]. Depression was produced by forcing the animal to swim individually in a glass jar containing fresh water of 15cm height and maintained at 25°C. This constituted pretest session. Twenty-four hour later each animal was again forced to swim. After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. The total duration of immobility was recorded in next 4 min of a total 6 min test. The change in the immobility period was calculated after administering drugs to the groups as mentioned in the above table.

For the forced swim test (FST), Rats of either sex were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm) containing 19 cm of water at $25\pm 1^\circ\text{C}$. Treatment was given 60min prior to study as described by study design. All animals were forced to swim for 6 min and the duration of immobility was observed and measured during the final 4 min interval of the test. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant like effect. Results are showed in Table.6

Tail Suspension Test:

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al [6] Depression was produced by suspending the animal from the edge of a table 50 cm above the floor by an adhesive tape placed approx. 1cm. from the tip of the tail. Immobility time was recorded during a 6 min. period. Changes in the immobility duration were studied after administering drugs in separate groups of animals.

The antidepressant activity was expressed as reduction in the immobility duration between the control, standard and animals treated with test drug.

Treatment was given 60 min prior to study as described by study design. Mice were suspended on the edge of the table, 50 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded during a 6 min of the 10 min period. Animal was considered to be immobile when it did not show any movement of the body, hanged passively and completely motionless. Results are showed in Table.7

Results and Discussion:

Plocama pendula roots are dried under shade and roots were dried and powdered. 250 grms of leaves powdered packed for soxhlet extraction. The chemical constituents are collected in Hexane, Ethyl acetate, Chloroform, Water and Ethanol. The Yield of extract was showed in Table.5. The collected extract was screened for the identification of phyto chemicals. The results are showed in Table.5

Compound	Hexane Extraction	Ethyl Acetate Extraction	Chloroform Extract	Water Extraction	Ethanol Extract
Steroids	-	-	-	-	-
Terpenoids	-	-	-	+	-
Flavonoids	-	-	-	+	-
Alkaloids	-	-	+	+	-
Fatty Acids	-	-	-	-	-
Phenolic compounds	-	-	-	+	-
Carotinoids	-	-	-	-	-
Saponins	-	-	-	-	-
Tanis	-	-	-	-	-
Polyuronides	-	-	-	-	-
Chlorogenic acid	-	-	-	-	-
Anthocyanins	-	-	-	-	+
Anthocyanin glycosides	-	-	-	-	-
Amino acids	-	-	-	-	-

Weight of collected plant extract (grms)	7.25	2.62	3.74	6.45	1.26
% of yield	2.9	1.04	1.49	2.58	0.5

Table.5Phyto chemical screening results

In phyto chemical screening most of the chemical constituents are present in water and Methonlic extract. Alkaloids are present in chloroform extract and Anthocyanins are present in methanol extract. Terpenoids, Flavonoids, Alkaloids, Phenolic compounds are present in water extract.

Antidepressant Activity:

In the present study, PPRWE (400 mg/kg) produced significant antidepressant effect in in Forced swim test & Tail Suspension Test. These models of depression are widely used to screen new antidepressant drugs. The tests are quite sensitive and relatively specific to all major classes of antidepressant drugs including TCAs, SSRIs, MAOI, and A typical antidepressants. The forced swimming test is the most widely used tool for assessing antidepressant activity pre-clinically. The widespread use of this simple model is mainly due to its ability to detect a broad spectrum of antidepressant agents. It has been argued that Tail Suspension Test is less stressful than Forced swim test and has greater pharmacological sensitivity. Environmental factors and hereditary factors play a major role in producing deficient monoaminergic transmission in central nervous system thereby producing symptoms of depression. Ecliptin alkaloid &Culumbin, a flavnoid present in PPRWE may be facilitating monoaminergic transmission there by producing antidepressant effects.

Forced Swim Test:

Group No (n=6)	Drug and Dose	Immobility Period (Secs)		
		Pre Treatment	Post Treatment (7 Days)	Post Treatment (14 Days)
Group A	Control, received distilled water	190.2 ± 0.6	189.5±0.4	189.2 ± 0.2
Group B	Imipramine 15mg/kg	196.4 ± 1.4	145.2 ± 1.46	140.2 ± 0.45
Group C	Fluoxetine 20 mg/kg	190.2± 0.56	125.7 ± 0.49	119.5 ± 0.36
Group D	Diazepam 1mg/kg	188.5±1.6	124.55±1.2	115 ± 0.42
Group E	PPRWE 100 mg/kg,	194.8 ± 1.24	185.2 ± 1.64	179.4 ± 1.22
Group F	PPRWE 200 mg/kg	190.6 ± 1.96	162.5 ± 0.68	155.7 ± 1.32
Group G	PPRWE 400 mg/kg	193.3 ± 0.42	159.5 ± 1.43	145.6 ± 0.20

Table 6: Effect of PPRWE on Immobility Period (Seconds) of rats using Forced Swim Test

Group No (n=6)	Drug and Dose	Immobility Period (Seconds)			
		Pre Treatment	Post Treatment (4 Days)	Post Treatment (7 Days)	Post Treatment (14 Days)
Group A	Control, received distilled water	191.4± 1.45	189.8± 0.28	188.2±0.65	186.4± 0.49
Group B	Imipramine 15mg/kg	189.6± 0.24	188.2± 1.23	120.4± 0.54	115.6± 0.53
Group C	Fluoxetine 20 mg/kg	190.2± 0.62	189.3±1.45	125.2± 0.56	120.9± 1.66
Group D	Diazepam (DZP) 1mg/kg	191.6± 0.25	190.5± 1.62	130.6± 0.29	128.4± 1.42
Group E	PPRWE 100 mg/kg,	189.2± 0.86	188.6± 1.8	150.2± 0.41	148.6± 1.09
Group F	PPRWE 200 mg/kg	190.2± 0.93	189.4± 1.05	145.7± 0.69	140.2± 0.36
Group G	PPRWE 400 mg/kg	191.1± 0.19	190.2± 1.33	140.4± 0.85	135.6± 0.44

Table 7: Effect of PPRWE on immobility period (seconds) of rats using tail suspension test

The incidence of anxiety and depression in the community is very high and is associated with lot of morbidity. Hence, it is very important to address these problems and find effective remedies.

Though several drugs are available, all are associated with some limitations and there is an urgent need for alternative medications for these disorders. The use of *Plocama pendula* for treating nervous disorders, there is an absence of scientific reports about the evaluation of its pharmacological effects.

In this work, it was demonstrated that the administration of different doses of the water extract of *Plocamapendula* root in mice was able to induce antidepressant effects. On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess stress-precipitated behaviors.

The two most widely used animal models for antidepressant screening are the forced swimming and tail suspension tests. These tests are quite sensitive and relatively specific to all major classes of antidepressants. In Tail Suspension Test, immobility reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression.

Similarly in the FST, mice are forced to swim in restricted space from which they cannot escape. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression. It has been seen that the Tail Suspension Test is less stressful and has higher pharmacological sensitivity than Forced swim test. Results showed that the administration of the PPRWE produced a diminution of immobility time of mice exposed to the both forced swimming and tail suspension tests.

In the present study, PPRWE (100, 200 and 400 mg/kg) administered to mice, produced significant antidepressant-like effect in both Tail Suspension Test and Forced swim test and their efficacies were found to be comparable to Imipramine (15 mg/kg) and Fluoxetine (20 mg/kg).

Data in the literature demonstrated that drugs that alter general motor activity may give false-positive/negative results in the forced swimming test. The effects produced by PPRWE and DZP (1.0mg/kg) upon the open field test demonstrated that these products do not modify the spontaneous locomotor activity of mice, which indicates that the plant extract exerts antidepressant effects without modifying significantly this parameter. Therefore, it is probable that these effects are not related to the stimulation of general motor activity. It has been established that the shortening of immobility time in the forced swimming and the tail suspension tests depends mainly on the enhancement of central 5-HT and catecholamine neurotransmission.

Early evidence of a role for noradrenaline in depression came from the discovery that drugs, either causing or alleviating depression, acted to alter the noradrenaline metabolism. Furthermore, depletion studies carried out in treated and untreated patients indicated a role for serotonin and noradrenaline in depression. Harmaline alkaloids present in *Plocamapendula* act as reversible monoamine oxidase inhibitors and in common with other beta carboline binds to 5-Hydroxy Tryptaminereceptors. MAO regulates the metabolic degradation of catecholamines, serotonin and other endogenous amines in central nervous system. Inhibition of this enzyme causes a reduction in metabolism and subsequent increase in the concentration of biogenic amines. Also the flavonoid components of PPRWE might be interacting with adrenergic and serotonergic systems in mediating the antidepressant effects of PPRWE. However, the precise mechanisms by which the extract produced antidepressant-like effect are not completely understood. Further studies would be necessary to evaluate the contribution of active chemical constituents for the observed antidepressant activity as it still remains to be determined which components were responsible for these effects.

Conclusion:

Hence *Plocama pendula* roots possesses antidepressant effect in animal models of depression which was comparable to that of Imipramine and Fluoxetine as demonstrated in this study. The phyto chemical analysis, separation of active ingredients and further investigation in this line is essential to establish its therapeutic benefits.

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