

Investigation of *In-Vivo* Neuropharmacological and *In-Vitro* Thrombolytic Activity & Phytochemical Analysis of Ethanolic Extract of *Argyria Captiformis* Leaves

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Abstract: Objectives: Investigation with the crude ethanolic extract of *Argyria captiformis* leaves was carried out to evaluate its possible thrombolysis and to analyze in *-vivo* neuro-pharmacological effects as anxiety is a particular form of behavioral inhibition that occurs in response to novel environment events and also phytochemical screening of plant extract. Method: Ethanolic extract of *Argyria captiformis* Leaves was assessed for sedative and anxiolytic activity on Swiss albino mice and Thrombolytic activity was assessed with human blood and also phytochemical screening test was done by various chemical reagents. Sedative activity was evaluated by using hole cross, open field, thiopental sodium-induced sleeping time and anxiolytic property was evaluated by elevated-plus maze (EPM) tests at 400mg/kg while the peripheral and thrombolytic activity determined by percentage of clot lysis. Result: In anxiolytic study, the extract displayed increased percentage of entry into open arm at the dose of 400mg/kg. The extract produced a significant ($P < 0.01$) increase in sleeping duration and reduction of onset of sleep compared to sodium thiopental at doses (400 mg/kg). The extract (400 mg/kg) also showed suppression of motor activity and exploratory activity of the mice in both open field and hole cross test. *Argyria captiformis* alone & *Argyria captiformis* in combination with Streptokinase demonstrated $38.19 \pm 4.76\%$ & $77.45 \pm 2.97\%$ clot lysis effect respectively & revealed significant with comparison to both the control agent. The presence of tannins, glycosides, saponins, flavonoids, cardiac glycosides, and phytosterols was determined. Conclusion: The pharmacological profiles of the present investigation of the ethanol extract of *A. captiformis* indicate that the extract possess good CNS depressant and exhibited considerable thrombolytic as it significantly reduced locomotion, onset of sleep, increased duration of sleep and also presence of, glycosides, cardiac glycosides, saponins, flavonoids, tannins and phytosterols.

Keywords: Sedative and Anxiolytic Activity, Phytochemical, *Argyria Captiformis*, Thrombolytic Activity, % Lysis of Clot

1. Introduction

Anxiety and depression are the most common psychiatric disorders. Over 20% of the adult population suffers from these illnesses at some time during their lives^[1-3]. It has become an important area of research interest in psychopharmacology during this decade^[4].

Benzodiazepines are among the most prescribed and effective anti-anxiety drugs used worldwide^[5].

But these are being slowly replaced by antidepressants,

which are not only efficacious in depression, but also in the acute and long-term Treatment of several anxiety disorders^[6].

Consumption of these drugs is believed to double every five years^[7]. Most of these drugs, however, have an unfavorable risk and benefit ratio, and their prominent side effects still represent a barrier to long-term treatment with these drugs^[8]. In addition, the risk of interaction with other substances is high, particularly with alcohol^[9]. Hence, there is an urgent need to search for newer, better-tolerated, and more efficacious therapeutic agents, for better management of anxiety and depression.

However, so far, its effect on central nervous activity has not been studied. Therefore; we undertook the study to evaluate the anxiolytic potential of *A. Captiformis* by using different animal models and studying the effect of the plant on their exploratory behavior.

One of the major causes of blood circulation problem is the formation of blood clots. Thrombi or emboli can lodge in a blood vessel and block the flow of blood in that location depriving tissues of normal blood flow and oxygen. This can result in damage, destruction (infarction), or even death of the tissues (necrosis) in that area^[10]. A blood clot (thrombus) is formed from fibrinogen by thrombin and is lysed by plasmin, which is activated from plasminogen by tissue plasminogen activator (tPA). Fibrinolytic drugs have been used to dissolve thrombi in acutely occluded coronary arteries there by to restore blood supply to ischaemic myocardium, to limit necrosis and to improve prognosis^[11].

Streptokinase is an antigenic thrombolytic agent used for the treatment of acute myocardial infarction. It reduces mortality as effectively as the nonantigenic alteplase in most infarct patients while having the advantages of being much less expensive. Tissue-type Plasminogen activator (tPA) is generally preferred as being effective and safer than either urokinase or streptokinase type activators. All available thrombolytic agents still have significant shortcomings, including the need for large doses to be maximally effective, limited fibrin specificity and a significant associated bleeding tendency. Because of the shortcomings of the available thrombolytic drugs, attempts are underway to develop improved recombinant variants of these drugs^[12-16].

The plant kingdom represents an enormous reservoir of biologically active compounds with various chemical structures and protective/disease preventive properties (phytochemicals). Nearly 50% of drugs used in medicine are of plant origin, and only a small fraction of plants with medicinal activity has been assayed. Therefore much current research devoted to the phytochemical investigation of higher plants which have ethnobotanical information associated with them. The phytochemicals isolated are then screened for different types of biological activity like thrombolytic potentials^[17]. Herbal preparations are used potential source of medicine since ancient times to maintain health and regain healthy state of mind. Herbs showing thrombolytic activity have been studied and some significant observations have been reported^[18].

Argyria captiformis (Poir.) Ooster is a medicinal plants group is Dicot, Family is Convolvulaceae-Morning-glory family and growth habit is climber.

It is a large climber with milky juice, branches hispid. Leaves 7-13 cm long, ovate to orbicular, rarely oblong-lanceolate, base shallowly to deeply cordate, suddenly short acuminate, sparsely hispid on both sides. Inflorescence of dense, capitate cymes in the axils of the leaves, on long peduncles. Bracts and sepals bristle-hispid. Flowers 3-4 cm long, redfish-purple or pink. Fruit globose, orange to reddish. It is widely distributed in Chittagong, Chittagong Hill Tracts, Cox's Bazar, Mymensingh, Moulvi bazaar, Rangpur,

Noakhali, Sylhet, Dhaka, in marginal forests and village thickets of Bangladesh^[19]. A leaf paste is applied to affected areas to treat bruising on the legs (Chakma)^[20]. Leaf-ash is used in eruption at the junction of ear pinnae.

2. Material and Methods

2.1. Collection of Plant Material

For investigation of *Argyria captiformis* leaves were collected from Naekangchori Upozilla, Bandarban District, Chittagong Division, Bangladesh during the month of April 2013. After that taxonomy confirmed by an expert of botany and plant Herbarium specialist Dr. Sheikh Bokhtear Uddin, Associate professor, Department of Botany, University of Chittagong, Chittagong, Bangladesh. The leaves of *Argyria captiformis* were collected at their fully mature form, from Chittagong hill Tract. After cleaning, the collected plant leaves were shade dried for twenty days in the low temperature and pulverized into a coarse powder using a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark, and dry place until further analysis.

2.2. Animals

Swiss-albino mice (both sexes) weighing between (18-25 g) was used for the present study collected from International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR, B), Dhaka, Bangladesh. After their purchase, the mice's were kept in standard environmental conditions (24.0 ± 0°C & 55-65% relative humidity and 12 h light/dark cycle) for one week to acclimate and fed ICDDR formulated rodent food and water ad libitum. Guidelines of Institutional Animal Ethics Committee were followed to carry out this study.

2.3. Drug and Chemicals

The drugs and chemicals used for the experiments were diazepam (Square Pharmaceutical Ltd; Bangladesh), thiopental sodium (Gonoshastho Pharmaceuticals Ltd; Bangladesh), ethanol (Sigma Chemicals Co; USA). For Thrombolytic activity commercially available lyophilized Streptokinase (SK) vials (Polamin Werk GmbH, Herdecke, Germany) of 1500000 IU was used as positive control. All other chemicals were of analytical grade.

2.4. Extraction Process

Approximately (620 gm.) powdered material was placed in clean flat bottomed glass container and soaked in 1.5 liter ethanol. The container with its content was sealed and kept for 15 days accompanied with occasional shaking and stirring for maximum wetting and extraction. The entire mixture was coarse filtration by piece of clean white cotton materials. Then the extract was filtered through whatman filter paper (Bibby RE200, Sterilin Ltd; UK) and was concentrated to obtain ethanol crude extract and evaporated to dry using

water bath. Then the extract was stored at 4°C until used.

The study was performed to find out if the extract had any effect on central nervous system. Elevated plus maze test was conducted for determination of anxiolytic activity whereas thiopental sodium induced sleeping time test was for sedative activity. Effect on exploratory behavior of mice was evaluated by hole cross test and open field test.

2.5. Anxiolytic Activity

In elevated plus maze test, the apparatus was made of wood with two open and two closed arms across each other respectively forming a plus-sign figure. The elevated plus maze (EPM; 30 cm×6 cm×6 cm, each arm) was situated 50 cm above the floor. After administration of the drug, each animal was placed at the center of the maze facing one of the closed arms. The number of open and closed arm entries, plus time spent in open and closed arms was recorded for 5 min at 0, 30, 60, 90, 120 min after administration of the extract (400 mg/kg), diazepam (1 mg/kg) and vehicle (1% Tween 80 in water). The whole test was carried out in a sound attenuated room^[21]. Entry into an arm was defined as the point when the animal placed all four paws onto the arm.

$$\text{Percent of time spent in arm} = \frac{\text{Time in open arm}}{\text{Time in open arm} + \text{Time in closed arm}}$$

This test has been widely validated for measuring anxiolytic- and anxiogenic-like activities in rodents^[22, 23].

2.6. Sedative Activity

For the experiment, the animals were randomly assigned to four groups, each with 5 mice. The test groups were given the leaf extract of *A. captiformis* at doses of 400 mg/kg body weight, while the positive control was treated with diazepam (1 mg/kg) and control group with vehicle (1% Tween 80 in water). Thirty minutes later, thiopental sodium (40mg/kg) was administered to each mouse to induce sleep. The animals were observed by placing them on separate chambers for the latent period (time between thiopental administrations to loss of righting reflex) and duration of sleep *i.e.* time between the loss and recovery of righting reflex. The onset of sleep and total sleeping time were recorded for control, positive control and test groups^[24].

2.7. Exploratory Activity

2.7.1. Open Field Test

The method was adopted as described by Gupta et al [24]. In open field test, the animals were divided into control, positive control and test groups containing 5 mice each. The test groups received extract of *A. captiformis* at the doses of 400 mg/kg body weight orally whereas control group received vehicle (1% Tween 80 in water). The floor of half square meter open field was divided into a series of squares each alternatively colored black and white. The apparatus had a 40 cm height wall. The number of squares traveled by the

animals was counted for 5 min at 0, 30, 60, 90, 120 min after oral administration of doses of the extract.

2.7.2. Hole Cross Test

The apparatus was a cage of 30 cm×20 cm×14 cm with a steel partition fixed in the middle, dividing the cage into two chambers. A hole of 3.5 cm diameter was made at a height of 7.5 cm in the center of the cage. Animals were randomly divided into control, positive control and test groups containing 5 mice each. The test groups were treated with extract of *A. captiformis* at the dose of 400 mg/kg body weight orally whereas positive control group with diazepam (1 mg/kg) and control group with vehicle (1% Tween 80 in water). Number of passages of the animals through the hole from one chamber to the other was counted for 5 min at 0, 30, 60, 90 and 120 min after oral administration of the extract as well as diazepam and vehicle^[25]. The apparatus was thoroughly cleaned after each trial.

2.8. Statistical Analysis

The data were expressed as mean±standard error of mean (S.E.M.). Statistical comparisons were performed using One way ANNOVA followed by Dennett's multiple comparison tests. The values obtained were compared with the vehicle control group and were considered statistically significant when P<0.05.

2.9. Preparation of Extract Solution for Thrombolytic Test

10 mg of the extract was suspended in 10ml distilled water and shaken vigorously on a vortex mixer. Then the suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a filter paper (Whatman No. 1). The solution was then ready for *in vitro* evaluation of clot lysis activity^[26].

2.10. Streptokinase (SK) Solution Preparation

To the commercially available lyophilized SK vial (Polamin Werk GmbH, Herdecke, Germany) of 15, 00,000 I.U., 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 µl (30,000 I.U) was used for *in vitro* thrombolysis^[26].

2.11. Specimen for Thrombolytic Test

Whole blood (5 ml) was drawn from healthy human volunteers (n = 10) without a history of oral contraceptive or anticoagulant therapy (using a protocol approved by the Ethics Committee of Pharmacy Department, International Islamic University Chittagong (approval no. ECPDIUC2013/03)500 µl of blood was transferred to each of the ten previously weighed Eppendorf tubes to form clots^[26].

2.12. Test Procedure for Thrombolytic Test

Experiments for clot lysis were carried as reported earlier^[26]. Venous blood drawn from healthy volunteers was transferred in different pre-weighed sterile Eppendorf tube

(500 μ l/tube) and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed (aspirated out without disturbing the clot formed). Each tube having clot was again weighed to determine the clot weight (Clot weight = weight of clot containing tube – weight of tube alone).

Each eppendorf tube containing clot was properly labeled and 100 μ l of plant extract was added to the tubes. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, fluid obtained was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. Streptokinase and water were used as a positive and negative (non thrombolytic) control respectively. The experiment was repeated several times with the blood samples of different volunteers.

% clot lysis = (Weight of the lysis clot / Weight of clot before lysis) \times 10

2.13. Statistical Analysis

The significance between % clot lysis by herbal extract by means of weight difference was tested by the paired t-test analysis. Data are expressed as mean \pm standard deviation.

2.14. Phytochemical Identification Tests Procedure

The tests were done to find the presence of the active chemical constituents such as alkaloids, glycosides, resin, flavonoids, carbohydrates etc.

Phytochemical screening: Phytochemical examinations were carried out for all the extracts as per the standard methods [27].

2.15. Detection of Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtrate [27].

A) *Mayer's Test*: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

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

C) *Dragendroff's Test*: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

D) *Hager's Test*: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

2.16. Detection of Carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates [27].

A) *Molisch's Test*: Filtrates were treated with 2 drops of alcoholic α -naphthol solution in a test tube. Formation of the

violet ring at the junction indicates the presence of Carbohydrates.

B) *Benedict's Test*: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

C) *Fehling's Test*: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

2.17. Detection of Glycosides

Extracts were hydrolysed with dil. HCl, and then subjected to test glycosides [27].

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides.

2.18. Detection of Saponins [27]

A) *Froth Test*: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

B) *Foam Test*: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

2.19. Detection of Phytosterols [27]

A) *Salkowski's Test*: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

B) *Liebermann Burchard's test*: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of brown ring at the junction indicates the presence of phytosterols.

2.20. Detection of Phenols [27]

Ferric Chloride Test: Extracts were treated with 3 -4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

2.21. Detection of Tannins [27]

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

2.22. Detection of Flavonoids [27]

A) *Alkaline Reagent Test*: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

B) *Lead acetate Test*: Extracts were treated with few drops of lead acetate solution. Formation of yellow color

precipitate indicates the presence of flavonoids.

2.23. Detection of Proteins and Amino Acids [27]

A) *Xanthoproteic Test*: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins

B) *Ninhydrin Test*: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

2.24. Test for Terpenoids (Salkoski Test)

Five (1mg/ml) of each extract was mixed in 2ml of chloroform, and then 3ml concentrated H₂SO₄ was carefully added to form a layer. A reddish brown coloration of the interface was formed which showed positive results for the presence of terpenoids [28].

2.25. Anthraquinone Detection

200 mg of each plant fraction was boiled with 6 ml of 1% HCl and filtered. The filtrate was shaken with 5ml of benzene. The layer was removed and then 10 % NH₄OH was added. Formation of pink, violet or red color in alkaline phase was observed for the presence of anthraquinone [28].

2.26. Identification of Phlobatinins

For identification of Phlobatinins 80mg of each plant extract was boiled in 1 % aqueous Hydrochloric acid, the deposition of a red precipitate indicates the presence of Phlobatinins [28].

2.27. Cardiac Glycosides Determination (Keller-Killani Test)

Five ml of each extracts was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was under with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form gradually through thin layer [28].

3. Result

In the EPM, the behavior of mice model, as observed, confirmed the anxiolytic activity of diazepam as reported previously. The ethanol extract. Of *A. captiformis* at the dose of 400 mg/kg (P<0.05), significantly increased the percentage of entries of mice into the open arms, and the percentage of time spent in the open arms of the EPM as shown in Table1. The number of closed arm entries and time spent in the closed arms were decreased significantly in the extract treated groups which was comparable with the standard diazepam.

Table 1. Effect of ethanolic extract of *A. Captiformis* on EPM test during 5 min test session.

Group	Treatment	Dose, Route	% Entry into open arm	%of time (in seconds) spent in open arm
Control	1% tween 80 in water	10 ml/kg, p.o	54.61 ± 5.044	58.33± 1.527
Standard	Diazepam	10 mg/kg, p.o	77.14 ± 4.95**	79.33± 1.52*
Test	Extract	10 mg/kg, p.o	60.6 1± 2.15*	62.67± 0.577*

Values are expressed as mean ± S.E.M., (n=5); *P<0.05, **P<0.01, Dunnet test as compared to control (Vehicle=0.4 mL/mouse)

Table 2. The effect of the ethanolic extract of *A. captiformis* on sodium thiopental induced hypnosis in mice .

Groups	Onset of Sleep (min)	Duration of Sleep (min)
Control	40.53 ± 0.577	47.00 ± 1.000
Diazepam	14.82 ± 0.025**	149.87 ± 0.115**
Extract 400mg/kg	25.33 ± 1.52**	127.67 ± 8.621**

Values are expressed as mean ± S.E.M., (n=5); *P<0.05, **P<0.01, Dunnet test as compared to control (Vehicle=0.4 mL/mouse).

In the thiopental induced hypnosis test, the extract at dose, 400 mg/kg showed a significant reduction in the time of onset of sleep and increased the duration of sleep. (Table 2). The effect of the extract (400 mg/kg) on the onset of sleep and the duration of sleep were comparable to that of standard. Doses of the extract potentiated the duration of thiopental sodium induced sleeping time in test animals compared to controls (Table 2).

Open field test of *A. captiformis* treated groups (400 mg/kg body weight) showed significant reduction of movement from its initial value at 0 to 120 min (Figure 1). The number of squares traveled by the mice was decreased significantly from its initial value at 0 to 90 min at the dose level of 400 mg/kg body weight (P<0.01) of the ethanol extract from the

leaves of *A. captiformis* (Figure 1).

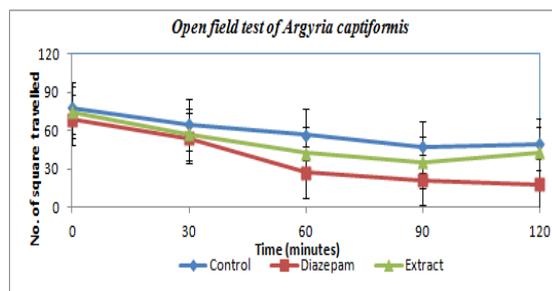


Figure 1. Effect of ethanolic extract of *A. captiformis* on exploratory behaviour Open field test in mice.

Values are mean±S.E.M, (n=5); *P< 0.05, **P< 0.01, Dunnet test as compared to Control (Vehicle=0.4 mL/mouse).

The number of hole crossed from one chamber to another by mice of the control group was similar from 30 to 120 min (Figure 2). Hole cross test of *A. captiformis* treated groups showed decrease of movement from its initial value at 0 to 90 min. But, at doses of 400 mg/kg (P<0.01), maximum suppression of locomotors activity was displayed which was comparable to the reference drug diazepam (Figure 2).

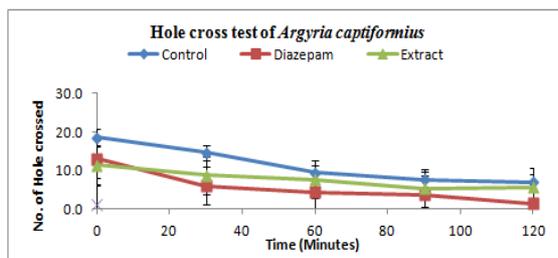


Figure 2. Effect of ethanolic extract of *A. captiformis* on exploratory behaviour (hole cross test).

Values are mean±S.E.M, (n=5); *P< 0.05, **P< 0.01, Dunnet test as compared to control (Vehicle=0.4 mL/mouse).

Addition of 100 µl SK, a positive control (30,000 I.U.) to the clots along with 90 minutes of incubation at 37°C, showed 75.01± 3.20 % clot lysis. Clots when treated with 100 µl sterile distilled water (negative control) showed only negligible clot lysis (6.42%). The mean difference in clot lysis percentage between positive and negative control was very significant (p value < 0.0001). The *in vitro* thrombolytic activity study revealed that *Argyria captiformis* showed 38.19± 4.76 % clot lysis and *Argyria captiformis* in combination with Streptokinase showed 77.45 ± 2.97% clot lysis activity. Percent clot lysis obtained after treating clots with herb and appropriate controls is shown in Figure 3. Statistical representation of the effective clot lysis percentage

by our herbal preparation, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) is tabulated in Table 3.

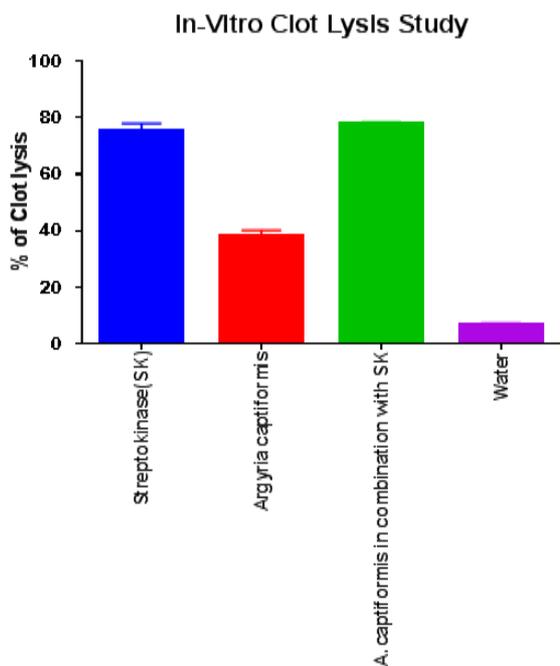


Figure 3. Clot lysis by Streptokinase, *Argyria captiformis* in combination with Streptokinase and Water.

Table 3. Effect of ethanolic extract of *Argyria captiformis* leaves & Streptokinase (Positive control) on *in vitro* clot lysis.

Herb/Drug	Mean± S.D. (Clot Lysis %)	t- value	P value(Two-tailed) when compared to negative control (Water)
Streptokinase	75.01 ± 3.20	68.86	< 0.0001
<i>Argyria captiformis</i>	38.19 ± 4.76	22.24	< 0.0001
Combination of <i>Argyria captiformis</i> & Streptokinase	77.45 ± 2.97	71.04	< 0.0001

Statistical representation of the effective clot lysis percentage by herbal preparation's, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) were done by paired t-test analysis; clot lysis % is represented as mean ± S.D. and p values of Herbal preparation (*Argyria captiformis*) was < 0.05 ; which was considered as significant.

Table 4. Phytochemical constituents identified in the ethanolic plant extracts of *Argyria Captiformis*.

Test for	Ethanolic extract of <i>Argyria captiformis</i> .
Tannins	(+ve)
Phlobatannins	(-ve)
Terpenoids	(-ve)
Glycosides	(+ve)
Steroids	(-ve)
Alkaloids	(-ve)
Carbohydrates	(-ve)
Saponins	(+ve)
Phytosterols	(+ve)
Phenols	(-ve)
Flavonoids	(+ve)
Protein & Amino acid	(-ve)
Antraquinone	(-ve)
Cardiac glycosides	(+ve)

4. Discussion

This study examined some neuropharmacological effects of *A. captiformis* and established that it has anxiolytic and Antidepressant-like activities. The EPM is one of the most widely validated tests and is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the gamma amino butyric acid type A (GABAA) - benzodiazepine complex [29].

In EPM, normal mice will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms that is generated by the fears of the open spaces. Drug like diazepam that increases open arm exploration are considered as anxiolytic and the reverse holds true for anxiogenics [30]. In this study, we observed that the administration of dose (400 mg/ kg body weight) of ethanolic extract of *A. captiformis* induced an anxiolytic-like effect in mice, as it increased open arm entries and the time spent in the open arms of the EPM when compared to the control animals.

Earlier reports showed a significant decrease in the locomotor score for diazepam when compared to the control

animals. Locomotor activity is considered as an index of alertness and a decrease in that indicates sedative effects^[31]. The doses (400 mg/kg body weight) of the *A. captiformis* showed a decrease in the locomotor score and produced a significant increase in the hypnotic effect induced by the thiopental sodium, thus suggesting a profound sedative activity. Thiopental is basically a hypnotic agent, given at appropriate dose, induced hypnosis by potentiating GABA mediated post synaptic inhibition through allosteric modification of GABAA receptors. Substances which possess CNS depressant activity either decrease the time for onset of sleep or prolong the duration of sleep or both^[32, 33]. In addition, the study on locomotor activity, as measured by hole cross and open field tests, showed that dose of ethanol extract from the leaves of *A. captiformis* decreased the frequency and the amplitude of movements.

The method employed for this assay is considered as a very sensitive way to detect agents with CNS depressant activity^[34]. The sedative effect recorded here may be related to an interaction with benzodiazepines and related compounds that bind to receptors in the CNS and have already been identified in certain plant extracts. Phytochemical analysis of the plant extract reveals that *A. captiformis* contains glycosides, cardiac-glycosides, flavonoids, tannins, Phytosterols and saponins. Many flavonoids and neuro-active steroids were found to be ligands for the GABAA receptors in the CNS; which led to the hypothesis that they act as benzodiazepine-like molecules^[35-37]. This is supported by the present study on the behavioral effects in animal models of anxiety and sedation.

It may be possible that the mechanism of anxiolytic action of *A. captiformis* ethanol extract could be due to the binding of any of the phyto-constituents to the GABAA-BZD complex. In support of this, it has been found that flavones bind with high affinity BZD site of the GABAA receptor^[38].

The locomotor activity is a measure of the level of excitability of the CNS and sedation resulting from depression of the central nervous system^[39]. The result indicated that the extract significantly decreased the locomotor activity as shown by the results of the open field and hole cross tests. The results were also statistically significant. Therefore, the use of *A. captiformis* in folkloric medicine may be due to its CNS action validated by our findings. However, further investigation is necessary to determine the exact phyto-constituents and mechanism of action that are responsible for the biological activities of the ethanol extract of *A. captiformis*.

There are several thrombolytic drugs obtained from various sources. Some are modified further with the use of recombinant technology in order to make these thrombolytic drugs more site specific and effective. Side effects related to these drugs have been reported that lead to further complications. Sometimes the patients die due to bleeding and embolism^[40-42].

In the clot lysis study, the % of clot lysis by positive & negative controls differs significantly as the p value was < 0.0001. The comparison among the extract of *Argyria*

captiformis & *Argyria captiformis* in combination with Streptokinase, water (negative control) & streptokinase (positive control) concluded by the significant thrombolytic activity of the ethanolic extract of *Argyria captiformis* leaves.

The preliminary Phytochemical screening of different extracts was done to ascertain the Presence of bioactive components. The Presence of Tannins, Glycosides, Saponins, Phytosterols, Flavonoids, and Cardiac glycosides was determined.

5. Conclusion

From this experiment, it can be concluded that *Argyria captiformis* has got the very good potential as a candidate for future thrombolytic agent and also interested to find out the active component(s) responsible for anxiolytic effect of this plant. This is only a preliminary study and to make final comment the extract should be thoroughly investigated phytochemically and pharmacologically to exploit their medicinal and pharmaceutical potentialities.

6. Contest

All persons in this study were willingly volunteered and no one was forced to do this. Before withdrawal of blood sample from them they were alert about the procedure. We also maintained and ensured the personal hygiene and safety procedure before starting with them, thus they feel safe with us. So there is no chance of violation with patient consent or ethical rules in case of using human volunteers.

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