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Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND ACUTE ORAL TOXICITY STUDY OF THE FLOWER OF *PHLOGACANTHUS THYRSIFLORUS* NEES IN ALBINO MICE

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ABSTRACT
Medicinal plants contains a variety of chemical substances with important therapeutic properties that can be utilised in the treatment of human diseases. *Phlogacanthus thyrsiflorus* Nees of family Acanthaceae is used in folklore remedies for treatment of Cough, Bronchitis, Fever, Asthama, Cancer and many other ailments. The present investigation was carried out to assess the qualitative phytochemical analysis of aqueous extract of the flower of *Phlogacanthus thyrsiflorus* Nees. The phytochemical screening reveals the presence of Tannin, Saponin, Flavonoid, Steroid, Triterpenoid, Phenol. For Acute Oral Toxicity study aqueous extract of the flower was used. The Acute Oral Toxicity test showed no mortality upto 1000 mg/kg body weight. The presence of these phytochemicals reveals its medicinal properties and non toxic nature of the plant indicated the value of the plant as medicine. This result suggests that the flower of *Phlogacanthus thyrsiflorus* can be used to cure various ailments.
KEY WORDS: *Phlogacanthus thyrsiflorus*, Phytochemical screening, Acute Oral Toxicity, Aqueous extract, Herbal remedies.

INTRODUCTION

Phlogacanthus thyrsiflorus Nees is found in the sub tropical Himalayas, upper Gangetic plain, Bihar, North Bengal and Assam[1]. *Phlogacanthus thyrsiflorus* Nees is a medicinal herb which belongs to Acanthaceae family. It is known as Vasaka in Hindi. An evergreen shrub upto 2.4 m high, branchlets quadrangular, leaves are 13-35 cm long, oblanceolate, elliptic oblong, acute or acuminate, entire. Flowers are in terminal elongated, thyrsoid panicles, upto 30cm long. Capsule is 3.8 cm long, linear clavate. In early spring the plant becomes showy with its dense cylindrical spikes of brick red velvety flower. Calyx lobe is 6.8 mm, bristly haired. Bracts are 6 to 12 mm long. Seeds are disc like. Flowering occurs in the month of February to April[2]. Whole plant is used like Adhatoda vasica in Whooping cough and Menorrhagia. Fruits and leaves are burnt and it is prescribed for fever. The leaves are reported to contain diterpene lactone, Phlogantholide A. A decoction of leaves is also beneficial in liver and spleen diseases[1]. Jaintia tribe of Meghalaya uses fruit and leaf ash of *Phlogacanthus thyrsiflorus* Nees and use it to treat fever[3]. Ethanolic extract of *Phlogacanthus thyrsiflorus* Nees has analgesic activity on experimental mice[4]. *Phlogacanthus thyrsiflorus* Nees has antimicrobial activity also[5]. The generation of free radicals has been implicated in the causation of several diseases of known and unknown etiologies such as Rheumatoid Arthritis, Cancer etc., and compounds that can scavenge free radicals have great potential in ameliorating these disease processes. *Phlogacanthus thyrsiflora* Nees has prominent free radical scavenging property so it may prove as a very good medicinal herb[6].

MATERIALS AND METHOD

Collection of the plant material

The flowers of *Phlogacanthus thrysiflorus* Nees were collected from the local market in the month of March, 2011 and herbarium was prepared. The herbarium was identified for authenticity by the experts of Department of Botany, Gauhati University, Guwahati, Assam, India. The flowers were washed thoroughly and shade dried.

Preparation of plant extract

After shade drying the dried flowers were powdered in mixer grinder. The dried powder is soaked in distilled water for 72 hours with occassional stirring. Then the mixture was filtered and the filtrate was taken for the experiments wherever applicable. For dose preparation the powdered flower was Soxhlet extracted with water and was kept in vaccum which gave a deep red semisolid residue (yield:10%w/w)

Animals

Swiss albino mice used in the present study were obtained from the Animal House of Department of Zoology, Gauhati University, Assam, India. The animals were bred in our laboratory. They were maintained under uniform condition of natural photoperiod. They were supplied with nutritious food, water on a regular basis. Hygiene is properly maintained. Healthy albino mice of both sexes of 3 months of age and weighing 22-25 gm were taken for the experiment.

Preliminary phytochemical screening

The powdered sample or the filtrate was taken for the experiments wherever applicable using standard protocols to test the presence of bioactive compounds:[7,8,9,10,11]

Test for Tannins

1 g of powdered sample was boiled with 20 ml distilled water for 5 minutes in a water bath and was filtered while hot. 1ml of cool filtrate was mixed with 5 ml distilled water and few drops (2-3) of 10% Ferric chloride and observed for any formation of bluish black or brownish green colour.

Test for Saponins

Froth test

I g of powdered sample was boiled with 10 ml of distilled water in a water bath for 10 minutes. The mixture was filtered while hot and allowed to cool then 2.5 ml of filtrate was diluted to 10 ml with distilled water and shaken vigorously for 2 minutes. Frothing indicated the presence of saponin in the filtrate.

Test for Alkaloids

1. Hager's test

1 ml of filtrate was taken and 3 ml of Hager's reagent (Saturated solution of Picric acid) was mixed in it and observed for the formation of a yellow precipitate.

2. 1 g of powdered sample was boiled with water and 10 ml HCl was dissolved in it. A very small quantity was mixed with picric acid. Coloured precipitate or turbidity indicated the presence of Alkaloids.

Test for Flavonoids

1. 1ml filtrate was mixed with few fragments of Magnesium ribbon and Concentrated HCl was added dropwise. Pink scarlet colour indicated the presence of flavonoids.

2. 1 g of the powdered sample was boiled with 10ml of distilled water for 5 minutes and filtered while hot. Few drops of 20% NaOH solution was added to 1 ml of the cool filtrate. A change to yellow colour which on addition of acid changes to colourless solution depicted the presence of flavonoids.

Test for Phenol

2 ml of filtrate was taken then freshly prepared 1% Ferric chloride and 1ml of Pottasium ferrocyanide was added to it. Formation of bluish green colour indicated the presence of phenol.

Test for Steroids and Terpenoids

1. Salkowski test

1 ml of filtrate was mixed with chloroform and few drops of concentrated Sulphuric acid then shaked and allowed to stand for some time. Red colour appearence in the lower layer indicated the presence of steroids and formation of yellow coloured upper layer indicated the presence of triterpenoids.

2. 1ml of filtrate dissolved in 1 ml of chloroform and filtered. To the filtrate 1ml of acetic acid was added and then few drops of concentrated Sulphuric acid was run down the side of the test tube. The appearence of pink or pinkish brown ring/colour indicated the presence of terpenoids. The appearence of blue colour indicated the presence of steroids.

Test for Carbohydrates

Benedict's test

1 ml of filtrate was mixed with few drops of Benedict's reagent and boiled in water bath. The appearence of reddish brown precipitate indicated the presence of sugar.

Acute Oral Toxicity Study

Acute oral toxicity studies were performed as per OECD 423 guidelines. Albino mice of both sexes of body weight 22-25 gm were taken for this experiment. All the animals were randomly divided into five groups one control group and four treated group containing five animals in each group. Group 1, 2, 3, 4 were orally administered 100, 500, 800, 1000 mg/kg body weight aqueous extract following the method of Lorke *et al*.,1983[13]. The contol group received vehicle alone. The animals were observed for first 72 hours and then 7 days for any sign of behavioral change, mortality and body weight.

RESULTS

Preliminary Phytochemical Analysis

The results of the phytochemical tests of the flower of *P.thyrsiflorus* Nees are shown in Table 1.

Acute Oral Toxicity Study

In the acute toxicity test of the aqueous extract of *Phlogacanthus thyrsiflorus* Nees, there was no mortality and no sign of behavioural change or toxicity observed after the oral administration of the aqueous extract upto 1000 mg/kg body weight in mice. There was no significant differences in the body weight of the control and treated animals.

DISCUSSION

The present study carried out in the plant sample reveals the presence of many bioactive compounds. The qualitative analysis is shown in Table 1. The curative properties of medicinal plants are perhaps due to the presence of flavonoids, phenols, sterols, terpenoids etc. Thus the preliminary screening tests may be useful in the detection of many bioactive components which may subsequently lead to the drug discovery and development. Medicinal herbs has comparatively less side effects than the chemical ones. *Phlogacanthus thrysiflorus* Nees was reported to have anti oxidant and free radical scavenging property which predicts that this plant may be used as a remedy for many diseases. As mentioned above this plant has many medicinal property but it is essential that the plant should be non toxic. The aqueous extract does not show any mortality upto 1000 mg/kg body weight. For any plant related studies in animals it is essential that the plant should not have any toxic effect so acute toxicity study is very important to determine the safety level of the plant.

CONCLUSION

In the present study we have found that many bioactive components are present in the flowers of *Phlogacanthus thrysiflorus* Nees mainly tannins, flavonoids, phenol, steroid, terpenoids. The curative properties of this plant may depend mainly on these phytochemicals mentioned above. Further studies are in progress in our laboratory to isolate the active components. Acute Oral Toxicity studies helps in the determination of LD_{50}. It is very important to determine the safety of the plant material to be used in the animals.

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TABLE 1:PHYTOCHEMICAL SCREENING OF THE FLOWER OF *PHLOGACANTHUS THYRSIFLORUS* NEES

PHYTOCHEMICALS	RESULTS
1.Tannin	++
2.Saponin	+
3.Alkaloid	-
4.Flavonoid	++
5.Phenol	++
6.Steroid	++
7.Terpenoid	++
8.Carbohydrate	-

++ = Presence ,+ = Trace, - = Absence

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