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(RESEARCH ARTICLE)

Screening of aerial parts of the plant *Clerodendrum paniculatum* Linn for anti-anxiety activity

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Abstract

The present study was undertaken to investigate and validate the traditional claims of *Clerodendrum paniculatum Linn* for its anti-anxiety activity. *C. paniculatum linn* belongs to Verbenacae family. Ethnomedical importance of various species of *Clerodendrum* genus has been reported in various indigenous systems of medicines and folk medicines. It is used as a medicine for the treatment of sore eyes, gonorrhoea, urinary tract infection and kidney problems. Till date there are no reports available regarding its pharmacological properties of hence, it is therefore worthwhile to study the unrevealed properties of this plant. The shade dried aerial parts of plant were extracted by extracted using ethyl acetate, petroleum ether and aqueous as a solvent. Obtained extracts were subjected for phytochemical screening and antianxiety activity was studied using elevated plus maze and light and dark models. Results of the phytochemical screening revealed the presence of flavonoids, tannins, glycosides, saponins, and terpenoid like compounds. In addition, among the extracts studied for anti-anxiety activity of ethyl acetate extract shown significant activity comparable with standard drug, diazepam. Petroleum ether and aqueous extracts shown least anti-anxiety activity in comparison with the standard.

Keywords: *Clerodendrum paniculatum* Linn; Verbenacae; Anti-anxiety activity; Ethylacetate; Flavonoids; Diazepam

1. Introduction

Clerodendrum paniculatum Linn. (Family Verbenacea) commonly known as 'Red Pagoda plant' is a semi woody shrub of 1-2 m height growing naturally in shady places throughout India. It is used traditionally in India, China and Japan in the treatment of rheumatism, neuralgia, ulcer, inflammation, and for healing wounds [1, 2]. Preliminary phytochemical screening showed the presence of terpenes, flavonoids, tannins, alkaloids, phenolic acid, sterols, glycosides, phenolic acid, sterols, and glycosides. The plant has got immense medical importance which is used for treatment for inflammation ulcer, vitrated vata, pitta, wound and skin disease. A decoction of root is used as tonic for aches and pains. It is used as a medicine for the treatment of sore eyes, urinary tract, gonorrhoea and kidney problems [3, 4]. The objective of this work is to evaluate the phytochemical analysis and anti-anxiety properties of ethyl acetate, petroleum ether and aqueous extract of aerial parts of *Clerodendrum paniculatum* Linn as a part of exploration of new and novel bio-active compounds.

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2. Material and methods

2.1. Experimental details

2.1.1. Plant materials

Clerodendrum paniculatum linn plant was collected from the local areas of Shivamogga and it has been authenticated by Dr. Rudrappa, Dean and Botanist, Department of Biological sciences, S.R.N.M. College, Shivamogga. The aerial parts of *C. paniculatum linn* were shade dried and reduced to a coarse powder in a Pulverizer (Sunbeam, Munger, India) using mesh No. 3 and passed through a sieve No. 40 to obtain about 2 kg of powder for further analysis.

2.1.2. Preparation of plant extracts

The powdered plant material of *C. paniculatum linn* was extracted with ethyl acetate and petroleum ether using soxhlet extractor for 48 hrs in 8 batches of 50g each. Whereas the water extract was prepared using maceration method. The extract was concentrated in vacuum using rotary flash evaporator (Buchi, Flawil, Switzerland). The solvent was removed completely over the water bath and finally dried in a desiccator. The extract, so obtained was labelled, weighed and the yield was calculated in terms of grams percent of the weight of the powdered aerial parts of the plant. These extracts are then used for the phytochemical study and antianxiety activity.

2.1.3. Preliminary screening

The presence of bio- active compounds was screened for saponins, flavanoids, terpenoids, saponins, glycosides and tannins [5,6].

2.2. Animals

Healthy young adult male and non-pregnant female Swiss albino mice (20 – 30g) and rats (150 – 200 g) of Wistar strain of either sex were used for the acute toxicity and pharmacological studies (anti-anxiety activity) using ethyl acetate, petroleum ether and aqueous extracts of the aerial parts of the plant *Clerodendrum paniculatum linn*. The animals were procured from Central Animal House, National College of Pharmacy, Shivamogga, Karnataka. After randomization into various groups, animals were acclimatized for period of 10 days under standard husbandry conditions. Room temperature $27^0 \pm 30^0$C, relative humidity 65 ± 10% and 12 hours – light/dark cycle.

All the animals were fed with rodent pellet diet (Gold Mohr, Lipton India Ltd.,) and water was allowed *ad-libitum* under strict hygienic condition. Ethical Clearance (NCP/IAEC/CL/04/2016-17) for performing experiments on animals was obtained from Institutional Animal Ethical Committee (IAEC).

2.3. Statistical analysis

All the values of animal studies were expressed as mean ± S.E.M. Statistical analysis was carried out by performing one-way ANOVA followed by Pair wise comparisons of Tukey's HSD (honestly significant difference) test. A probability level of $P<0.05$ was considered significant, $P<0.01$ is considered as moderately significant and $P<0.001$ is considered as highly significant.

2.4. Acute toxicity study [7]

Acute oral toxicity study was carried out using OECD guideline 425 (modified, adopted 23 rd march 2006). Tween-80 (1%) was used as a vehicle to suspend the extracts and was administered orally and the first animal receives a dose step below the level of the best estimate of the LD_{50} and dose progression factor should be chosen to be the antilog of 1/ (the estimated slope of the dose-response curve) and should remain constant throughout testing (a progression of 3.2 corresponds to a slope of 4). The testing samples were prepared by suspending the different extracts (ethyl acetate, petroleum ether and aqueous) in distilled water using tween 80 (1%) as suspending agent. The initial dose in this experiment was 200 mg/kg body weight and there was no mortality or toxicity in animals hence this dose is considered as lethal. To study pharmacological activities the fraction was administered in the dose of 200 mg/kg body weight which is equal to $1/10^{th}$ of 2000 mg/kg body weight.

Ethyl acetate, petroleum ether and aqueous extracts of *Clerodendrum paniculatum linn* were used to investigate the following anti-anxiety activity at the dose of 200 mg/kg body weight.

2.5. Anti-anxiety activity

Anxiety is a negative emotion that occurs in response to perceived threats that can come from internal or external sources and can be real or imagined. Anti-anxiety activity was evaluated by using in-vivo using total aerial parts extract of *C.paniculatum linn.* Anti-anxiety activity was performed by elevated plus maze and light and dark model. In both model, the Ethyl acetate extract showed more significantly entries in light chamber and decreased the time spent and entries in the dark chamber as compared to control, petroleum ether and Aqueous extracts. Diazepam (4 mg/kg b.w) is used as standard drug. In more recent literature, flavonoids are considered the most likely ingredient because some flavonoids have anxiolytic properties in mice similar to benzodiazepines and modulate or inhibit GABAA and GABAC receptor currents. GABA is the most potent inhibitory transmitter in the CNS controls the state of neuronal excitability.

2.5.1. Elevated plus maze method [8]

Anxiolytic compounds acts by decreasing anxiety and increase the open arm exploration time; in contrast anxiogenic compounds have the opposite effect. The anti-anxiety activity was evaluated by using elevated plus maze (EPM) model in albino mice. The elevated plus-maze model is a well-established animal model for testing anxiolytic drugs. The elevated plus-maze apparatus consists of two open arms (16 x 5 cm for mice and 50 x 10 cm for rats), two closed arms (16 x 5 x 12 cm for mice and 50 x 10 x 40 cm for rats), and an open roof with the entire maze elevated (25 cm for mice and 50 cm for rats) from the floor. Groups consist of 6 rats for each dose. Thirty min after *i.p*. administration of the Group I served as control received distilled water (1 ml/100gm b.w); Group II received Diazepam (4 mg/kg b.w), Group III, IV, V received the ethyl acetate, petroleum ether and aqueous extract at the dose of 200 mg/kg b.w. respectively. The animals were placed individually in the center of the maze, head facing towards open arms and the stop watch was started and the parameters were noted for the period of 5 min. a) First preference of mice to open and closed arm. b) Number of entries in open and closed arms (an arm entry defined as the entry of four paws into the arm) c) Average time each animal spends in each arm (average time = total duration in the arm/number of entries). In this method, motor activity and open arm exploratory time are registered. The values of treated groups are expressed as percentage of controls.

2.5.2. Light-dark model [9]

The instruments consists of a light and a dark chamber divided by a photocell-equipped zone. A polypropylene animal cage, 44 × 21 × 21 cm, is darkened with black spray over one-third of its surface. A partition containing a 13 cm long × 5 cm high opening separates the dark one third from the bright two thirds of the cage. An electronic system using four sets of photocells across the partition automatically counts movements through the partition and clocks the time spent in the light and dark compartments. The groups consist of 6 rats for each dose. Thirty min after *i.p*. administration of the Group I served as control received distilled water (1 ml/100gm b.w); Group II received Diazepam (4 mg/kg b.w), Group III, IV, V received the ethyl acetate, petroleum ether and aqueous extract at the dose of 200 mg/kg b.w. respectively.

3. Results and discussion

3.1. Anti-anxiety activity of various extracts

3.1.1. Elevated plus maze

Table 1 Effect of ethyl acetate, petroleum ether and aqueous extract of aerial parts of plant *C. paniculatum linn* on mean no. of entries and mean time spent in open arm and closed arm in elevated plus maze model**.**

Group	Dose mg/kg	Mean no. of entries in (sec/min)		Mean time spent in (sec/min)	
		Open arm	Closed arm	Open arm	Closed arm
Control	-	4.5±0.6196	14.16±0.61	78.33±5.63	218.83±4.96
Standard (Diazepam)	4	24.16±0.70***	4.83±0.70*	207.83±1.40*	92.5±1.384***
Ethyl acetate extract	200	12.83±0.7493***	5.33±0.42*	176±2.30***	90.33±2.028*
PE extract	200	7.16±0.4724*	4.33±0.42*	156.33±2.95*	86.66±2.95*
Aqueous extract	200	6.16±0.47*	4.83±0.63*	142.33±1.22*	80.66±1.22*

Data was analysed using one way ANOVA followed by pairwise comparision.Values are expressed as mean ± S.E.M. *n*=6, ***P < 0.001(highly significant), **P < 0.01(Moderately significant) and *P < 0.05(significant).

3.1.2. Light –dark test

Table 2 Effect of ethyl acetate, petroleum ether and aqueous extract of aerial parts of plant *Clerodendrum paniculatum linn* on Mean No. of entries and Mean time spent in Light and Dark area in Light and Dark model**.**

Group	Dose (mg/kg)	Time spent in each area in (sec/min)		No. of entries in each area (sec/min)	
		Light	Dark	Light	Dark
Control	-	99.83 ±2.81	191.16± 2.81	5.5±0.61	15.16±1.16
Standard (Diazepam)	4	186.5± 2.09***	98.83±3.851*	25±0.68***	3.33±0.66*
Ethyl acetate extract	200	173.33±2.39***	95.66±2.39*	14.83±0.74***	4.33±0.42*
Petroleum ether extract	200	148.16±1.16*	96.83±1.16*	8.16±00.47*	3.33±0.42*
Aqueous extract	200	135.5±0.76*	94.5±0.76*	7.16±0.47*	3.83 ±0.60*

Note: Data was analysed using one way ANOVA followed by pairwise comparision.Values are expressed as mean ± S.E.M. *n*=6, ***P < 0.001(highly significant), **P < 0.01(Moderately significant) and *P < 0.05(significant).

4. Discussion

The present study was designed to evaluate the anti-anxiety effect of the aerial parts of *C. paniculatum* Linn in mice using elevated plus maze and light and dark models and the results compared with standard drug (Diazepam).

Ethyl acetate extract of *Clerodendrum paniculatum* Linn has shown significant anti-anxiety activity in both elevated plus maze and light and dark models. Plant extract administration at the dose of 200 mg/kg body weight have shown dose dependent significantly increased open arm activity in elevated plus maze model by increasing time spent and number of entries into open arms and decreased the number of entries into closed arm as compared to those of control. In light and dark model, results have shown significantly increased open area activity by dose dependent increase in time spent and number of entries to open area and decrease in time spent and number of entries to dark area compared to control and standard. Various extracts of *Clerodendrum paniculatum* Linn showed anti-anxiety activity as shown by standard drug Diazepam and results are comparable in both elevated plus maze and light and dark models. Anxiolytic activity of diazepam is due to its GABA facilitatory action through GABA-A receptors [10,11] as pharmacological profile of ethyl acetate extract of *C. paniculatum* Linn was similar to that of benzodiazepines in our study, it is possible that ethyl acetate extract of *C. paniculatum* Linn might possess similar mechanism of action.

5. Conclusion

In conclusion ethylacetate extract of *Clerodendrum paniculatum* Linn showed significant anti-anxiety activity probably due to GABA facilitatory action of phytoconstituents such as flavonoids, phenols, saponin etc. Hence *Clerodendrum paniculatum* Linn may become potential resource for natural psychotherapeutic agent against various anxiety related disorders with fewer side effects compared to current therapy. Further, extensive studies are required to determine its active ingredient responsible for antianxiety activity.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that they have no conflict of interest.

Statement of ethical approval

The experiments were conducted according to the ethical norms approved by the Institutional Animal Ethical Committee (IAEC) (No:NCP/IAEC/CL/04/2016-17).

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