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Phytochemical Screening and Evaluation of Anthelmintic Activity of *Euphorbia tithymaloidus*

Tarun Kumar, Anshita Gupta, Bina Gidwani and Chanchal Deep Kaur
Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg, Chhattisgarh, India

Corresponding Author: Chanchal Deep Kaur, Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg, Chhattisgarh, India

ABSTRACT

Euphorbia tithymaloidus is a shrub having a range of activities like anti-inflammatory, antioxidant, anti-malarial, anti-tuberculosis, antifungal, antibacterial, antidaibetic and wound healing etc., but the anthelmintic activity of this plant is still under consideration. In this study carried out the phytochemical and anthelmintic activity of *Euphorbia tithymaloidus* in order to estimate its potential in treating the intestinal worms. The *in vitro* anthelmintic activity was performed on ethanolic leaf extract using adult earthworms (*Pheretima posthuma*). The standard drug used was albendazole in the concentration of 15-60 mg mL⁻¹ to note the paralysis time and death time. The result of the study showed that the ethanolic leaf extract shows the presence of certain phytoconstituents like flavonoids, carbohydrates, steroids and alkaloids. The reaction time was noted as paralysis time and death time for all the concentrations and compared with standard albendazole. The extract exhibited remarkable activity at a concentration of 45 mg mL⁻¹ as compared to standard drug albendazole within a shorter time span of less than 10 min.

Key words: Phytoconstituents, anthelmintic, pedilanthain, albendazole

INTRODUCTION

Herbal medication generally referred to as Herbalism or Botanical medicine. Is the usage of herbs for their therapeutic or medicinal value. An herbs is a plant or plant part valued for its medicinal, fragrant or savory qualities. *Euphorbia tithymaloidus* is an erect shrub, the plant is also known by the scientific name *Pedilanthus tithymaloidus*. The roots, stems and leaves of the plant are known as toxic. These parts of the plant contain euphorbol (a complex terpene) and other diterpene esters (Houghton and Mukherjee, 2009). These are also known carcinogens. The plant's leaves and stems also contain beta-sitosterol, cycloartenone, octacosanol and oxime, all of which have known medicinal as well as toxic properties. It is known as a powerful emetic. *Euphorbia tithymaloidus* contain a proteolytic enzyme known as pedilanthain can be extracted from the plant's latex and shows effectiveness against intestinal worms and to reduce inflammation when ingested. A galactose-specific lectin was purified from the plant's latex and indications are that it might be useful in combating diabetes mellitus (Surana *et al.*, 2011). It has been also used to treat asthma, persistent coughing, laryngitis, mouth ulcers and venereal disease. The latex has been used topically to treat calluses, ear ache, insect stings, ringworm, skin cancer, toothache, umbilical hernias and warts (Fig. 1).

Various studies have been coined on *Euphorbia tithymaloidus* stating its important role in curing different diseases. Helminthic infection is a term which is very common in Asian countries which is mainly persisting due to unhygienic conditions, impure water system and low sanitary



Fig. 1: Ethanolic extract of the plant

situations. Multiple drug regimen are available in order to cure this disease which provide only symptomatic relief, leading to relapsation of the problem (Parkash *et al.*, 2009; Priyadarshani *et al.*, 2007).

Herbal drugs provides a solution on this type of problem as various drugs have been proven to be anthelmintic in nature. It has been reported that the latex of *Euphorbia tithymaloidus* have certain degree of anthelmintic property but have been not proved yet. Hence, in our study we analyzed the ethanolic leaf extract of the plant for its phytochemical properties and its anthelmintic potential against standard drug albendazole (Jain *et al.*, 2009).

MATERIALS AND METHODS

Plant material: The plants were collected from local farm house at Kumhari, district Durg and was authenticated by Department of Botany, Govt. K.L. Arts, Science and Commerce Collage of Mahasamund. The voucher specimen were submitted for future reference.

Preparation of extract

Method: The fresh leaves of the plant were cut into fine pieces. A 50 g of fresh coarsely leaves of *Euphorbia tithymeloids* were extracted with 250 mL of ethanol by using Soxhlet apparatus for 24 h (Khadse and Kakde, 2010). Than the extract was place in soxhlet assembly for solvent recovery upto 65%, than after the recovery of solvent the concentrated extract was obtained. The concentrated mass was evaporated till dryness at 40-60°C (Fig. 1).

Physicochemical parameters: The determination of various physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value, swelling index, foaming index, moisture content, ash value, pH were calculated as per Indian Pharmacopoeia (Anonymous, 1966).

Phytochemical screening of crude extracts: In this process, it is possible to remove some secondary metabolites present in the plants, which may produce undesirable side effects. Preliminary phytochemical studies of the ethanolic extract of *Euphorbia tithymaloidus* was performed for major classes of constituents like alkaloids, carbohydrates, protein and amino acid,

Saponins, glycosides, steroids, tannins, flavonoid and phenolic compounds according to published standard methods (Raut *et al.*, 2009; Harborne, 1998).

Evaluation of Anthelmintic activity of *Euphorbia tithymaloidus*

Worm collection and authentication: Adult earth worm (*Pheretima posthuma*) were used to evaluate anthelmintic activity *in vitro*. The Indian earth worm *Pheretima posthuma* (family-annelida) was collected from the Patel Krishi form Kumhari, Durg (CG). The average size of earthworm 8-10 cm.

Drug and chemicals: Albendazole was obtained as gift sample from Glaxo Smithkline, Mumbai, India.

Sample and standard preparation

- **Standard preparation:** The albendazole having strength of 15 mg mL⁻¹ was prepared
- **Sample preparation:** For the ethanolic extract of *Euphorbia tithymaloidus*, different test concentration were prepared i.e., 15, 30 and 45 mg mL⁻¹

Evaluation of anthelmintic activity: The anthelmintic experiment was carried as per the method described by Mali *et al.* (2005) with minor modifications using adult earthworm (*Pheretima posthuma*) owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings for preliminary evaluation anthelmintic activity. Test sample of the extract of different concentrations (15, 30 and 45 mg mL⁻¹) were prepared in ethanol and 7-8 worms of *Pheretima posthuma* of 8-10 cm were placed petri dish containing 25 mL of above test solution of extract (Kosalge and Fursule, 2009). Albendazole (15 mg mL⁻¹) was used as reference standard and normal saline (0.9% NaCl) as control and 25 mL of ethanol was also taken for consideration as all the extracts were prepared in same solvent and the effect of it should be analyzed. All the test solution and standard drug solution were prepared freshly before starting the experiment. Observation were made for the time taken for paralysis noted when no movement or loss of movement. Time for death of worms were recorded after controlling that worms neither moved when shaken vigorously nor when dipped in warm water and fading away the color of worms. All the result were shown in table and expressed as a mean of selected worms in each group.

Statistical analysis: The calculation of the experimental data is done by using One-Way analysis of variance (ANOVA). Later on the calculation is done by various non parametric test. The mean and standard deviation is obtained. The results were presented as Mean±SEM. Differences between means of treatment and control groups were accepted significant at p<0.001.

RESULT

Physico-chemical parameters: The determination of physico-chemical parameter is important in determination of adulterants and improper handling of drugs. Table 2 shows the result of various physico-chemical parameter of powdered drug carried out using standard methods. Moisture content of drugs could be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. Ash values used to determine quality and purity of crude drug. It indicates the presence of various impurities like carbonate, oxalate and silicate (Chaudhari *et al.*, 2009).

Table 1: Physico-chemical parameters of *Euphorbia tithymaloides*

Parameters	Values (%) (w/v)
Loss on drying	0.53±0.025
Ash values	
Total ash	6.20±0.15
Acid insoluble ash	0.15±0.32
Water soluble ash	6.32±0.28
Swelling index	2.50±0.41

Table 2: Extractive value of *Euphorbia tithymaloides*

Solvent	Extractive value (%)
Ethanol	56.4
Benzene	32.0
Acetone	35.6
Water	12.4

Table 3: Phytochemical analysis of *Euphorbia tithymaloides*

Tests	Result
Carbohydrate	+
Alkaloids	-
Anthraquinones	-
Saponins	-
Steroid	+
Coumarins	-
Cardiac Glycoside	+
Flavonoids	+
Phenolic compound	+
Fixed oil	-
Terpenoids	-
Tannins	+
Proteins	+
Vitamin A	+
Vitamin C	-
Vitamin D	-
Volatile oil	-
Cadinolides	-
Gums	-
Mucilage	-

+: Presence of that phytoconstituents, -: Absence of that phytoconstituents

The acid insoluble ash consist mainly silica and indicate contamination with earthy material. The water soluble ash is used to estimate the amount of inorganic elements present in drugs (Anshita *et al.*, 2014). The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent (Table 1 and 2).

Phytochemical screen ethanolic extract of *Euphorbia tithymaloides*: The ethanolic extract were taken and standard methods were used to detect the nature of phytoconstituents present in them and to analyze the chief phytoactive present in *Euphorbia tithymaloides*. Table 3 shown summarizes the data.

Evaluation of anthelmintic activity: Evaluation of anthelmintic activity was compared with the standard drug albendazole (Table 4).

Anthelmintic activity of ethanolic extract of *Euphorbia tithymaloides*: From the results of the experiment it can be observed that, higher concentration of extract produced paralytic effect

Table 4: Dose determination of standard albendazole

Concentration (mg mL ⁻¹)	Time of paralysis (min)	Time of death (min)
15	09:03±0.12*	13:15±0.11*
30	07:38±0.35*	11:29±0.41*
45	06:52±0.12*	08:38±0.10*
Normal saline	Not observed	Not observed
25 mL Ethanol	Not observed	Not observed

Values are expressed as Mean±SEM, Values were find out by using one way ANOVA followed by Dunnett's t-test. *Values are significantly different from control at (p<0.001)

Table 5: Determination of anthelmintic activity of ethanolic extract of *Euphorbia tithymeloids*

Concentration (mg mL ⁻¹)	Time of paralysis (min)	Time of death (min)
15	04:58±0.25*	05:35±0.32*
30	03:44±0.39*	04:19±0.37*
45	02:27±0.21*	02:58±0.14*
Normal saline	Not observed	Not observed
25 mL Ethanol	Not observed	Not observed

Values are expressed as Mean±SEM, Values were find out by using one way ANOVA followed by Dunnett's t-test. *Values are significantly different from control at (p<0.001)

much earlier and the time to death was shorter for all worms. Ethanolic extract of the dried leaves of *Euphorbia tithymeloids* showed anthelmintic activity in dose dependent manner giving shortest time of paralysis and death with 45 mg mL⁻¹ concentration for all three types of worms (Table 5). The effect of 25 mL of ethanol as solvent was also determined by taking pure solvent in the study. There was no effect observed on *Euphorbia tithymeloids* activity in the form of paralysis or death. The activity showed during the experiment may be the result of alkaloids, flavonoids, tannins etc., phytoconstituents present in the ethanolic extract.

DISCUSSION

The aim of the present study was to evaluate the of anthelmintic effect of ethanolic extract of the plant and to analyze the physiochemical and phytochemical activity profile of the plant. The plant is blessed with immense potent activities in combating different types of diseases the requirement is to explore it the most for its active constituents and further more regarding its mode of action and structural analysis so that a better and more advanced formulation can be prepared for the main stream administration of the drug. The function of the anthelmintic drugs like albendazole is to cause paralysis of worms so that they are expelled in the faeces of man and animals. In the other genus of the same family the anthelmintic activity exhibited by the plants are some what similar. In a study by Kane *et al.* (2009), aqueous and methanolic extracts of *Euphorbia thymifolia* was evaluated for anthelmintic activity. They found that the leaf extract of *Euphorbia thymifolia* Linn. not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 100 mg mL⁻¹ in shorter time as compared to reference drug Piperazine citrate. In a previous study, the anthelmintic potentials of the aqueous and methanol extracts of *Euphorbia helioscopia* were investigated and they found that *E. helioscopia* possesses significant anthelmintic activity and could be a potential alternative for treating cases of helminth infections in ruminants. Similarly, in another study by Kumar *et al.* (2014), in which evaluation of the anthelmintic activity of *Euphorbia prostrata* plant's decoction was investigated for the same and found that the plant showed potent anthelmintic activity when compared to standard drug. The time to paralysis (vermifuge) and death (vermicide) was found to be lesser in higher concentration of extracts. It showed shortest time of paralysis and death with 100 mg mL⁻¹ of ethanolic and aqueous concentration. The extracts not only demonstrated this property during the

anthelmintic activity for *Euphorbia tithymaloides* leaves, the extract also showed a positive result in the form of death of the worms, especially at 45 mg mL⁻¹ as compared with the albendazole. The drug showed dose dependent activity, i.e., on increasing the dose the activity also increases when compared to the standard drug albendazole at a range of 15-45 mg mL⁻¹. In a comparative study by Wath *et al.* (2014), on the anthelmintic activities of aqueous, methanolic and acetone extract of *Acalypha indica* L. and *Euphorbia hirta* L. belonging to the family Euphorbiaceae, a significant relation in the results were observed. Each extract were analyzed for anthelmintic activity at various concentrations (10, 25 and 50 mg mL⁻¹). The activities were well comparable with standard drug albendazole and piperazine citrate as positive control, distilled water (for aqueous extract) was used as negative control did not show any anthelmintic activity. The methanolic extract of both plant exhibited dose dependent anthelmintic activity showing maximum efficacy at 50 mg mL⁻¹ concentrations. Methanolic extract of *Acalypha indica* L. showed profound anthelmintic activity.

CONCLUSION

The *Euphorbia tithymaloides* are used to treat intestinal worms infection, showed significant anthelmintic activity at 45 mg mL⁻¹ concentration measured by time taken for paralyze and death of the earth worms. The experimental evidence lead to conclusion that the leaves of have potent anthelmintic activity when compared with the conventionally used drug. The result did not, however exclude the possibility that what doses of extract might be efficacious against other species of helminthes. Further studies using *In vivo* models and to isolate active constituent from extract are required to be carried out in support to get a established data.

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