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In Vitro Anthelmintic Properties of *Euphorbia Milii* and *Euphorbia Microphylla Linn* Extracts

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Abstract

Methanol extracts from the leaves and stems of *Euphorbia microphylla Linn* and *Euphorbia milii* were investigated for their activity against *Fasciola gigantica*, *Taenia solium* and *Pheritima pasthuma*, respectively. Five concentrations (10–100 mg/ml) of each extract were studied in the bioassay, which involved determination of time of paralysis and time of death of the worms. All the extracts exhibited con-siderable anthelmintic activities, and the order of sensitivity of the extracts to the worms was *P. pasthuma* > *F. gigantica* > *T. solium*. The most active of the extracts were *E milii* and *E microphylla Linn* stem methanol extracts. Piperazine citrate (10 mg/ml) and distilled water were included in the assay as standard reference drug and control, respectively.

Keywords: *Euphorbia microphylla Linn, Euphorbia milii*, anthelmintic, worms.

Introduction

Euphorbia milii and *Euphorbia microphylla Linn* are two plants belonging to the Euphorbiaceae. Plants from this family have been used in African traditional ethnomedicine for several years and several genera plants have been docu-mented for the treatment of various ailments. Plants of the Capparidaceae have been used for the treatment of syphilis, dressing of wounds, chronic ulcers, and treatment of snake bites (Dalziel, 1937; Kerharo & Adams, 1974). Certain plants in the plant family are also noted for the treatment of gon-orrhoea (Pernet, 1972), convulsion in children, aphrodisiacs (Ainsle, 1937), and, mostly, as anthelmintics (Dalziel, 1937; Walker, 1953; Irvine, 1961; Kerharo, 1968; Burkhill, 1985). The plant family is well known for the presence of glucosi-nolates or the so called mustard oil glucosides (Kjaer & Thompson, 1963; Ahmed et al., 1972). Alkaloids of the stachydrine-type are also well represented in this plant family (Delaveau et al., 1973). To a lesser extent, flavonoids and sterols have been indicated in a few of these plants (Bombardelli et al., 1973).

Specifically, *E milii* stem bark has been used for the treatment of earache, bark decoction is drunk for chest pains, kidney pains and for washing small pox wounds. The fruit is anthelmintic. Seeds of *E*

microphylla Linn are reputed for anthelmintic properties (Bouquet & Debray, 1974; Walker, 1953). The oil of the fruit is used as a fish poison as well (Oliver-Bever, 1986).

Continuing our studies of the Euphorbiaceae plants for biological activity and constituents (Ajaiyeoba & Okogun, 1994, 1996; Ajaiyeoba et al., 1998) coupled with our recent studies on other ethnomedically useful plants from the Niger-ian flora (Ajaiyeoba et al., 1999), we present the anthelmintic properties of *E milii* and *E microphylla Linn*.

Euphorbia microphylla Linn in Ayurveda prescribe as an ingredient of vegetable soup for diarrhoea, painful bleeding piles (Gupta B., Srivastava R., et al., 2007). The latex of plant was applied on ring worm and eruptive boils. According to Bhaavaprakaasha, plant is expectorant cures aggravated cough, skin disease, parasitic infection, promotes conception possesses aphrodisiac and age- sustaining properties. (Khare I.P et al., 2008) The leaves and seeds are given in worm cases and in certain bowel affections of children in the Tamil country. In Northern India, they are considered stimulant and laxative. In Konkan, the juice is used to cure ringworm. The expressed juice or the powered plant is administered internally with wine as a remedy for snake-bite, and it is applied externally to the part bitten (Shivkar Y. M., et al., 2003). E. microphylla Linn has also shown beneficial effects when used n the treatment of Diarrhea and Dysentery (Kirtikar K. R., Basu B. D et al., 1975). E. microphylla Linn possesses antioxidant and antiviral activities (Kirtikar K. R., Basu B. D et al., 1975). The plant is commonly used as an herbal medicine. It is believed to possess antioxidant, antitumour, anti-malarial, anti rash and anti dysentery activity. Present study aims at exploring the details of anthelmintic action of extracts of Euphorbia E microphylla Linn.

Materials and Methods

Plant collection and authentication

Leaves (395g) and stem (420g) of Euphorbia milii were collected while leaves (385g) and stem (500g) of Euphorbia microphylla Linn were obtained from from hills of koyana dam region near Patan District during July 2022 and were authenticated in Department of Botany, Gopal Krishna Ghokale College, Kolhapur, M.S., India. voucher specimens were deposited the specimen no was 1546.

Plant extraction

Plant materials were successively extracted in redistilled hexane and methanol by maceration at room temperature (29 °C) for 72 hr. After removal of solvent, percentage yields were estimated and plant extracts were stored in sample bottles in a refrigerator untill needed for analysis.

Worms collection and authentication

Fasciola gigantica (liverfluke, mean weight of 0.05– 0.07 g) and *Taenia solium* (tapeworm, 2.4–2.8 g) were ob-tained from freshly slaughtered animal in the sloter house, Ichalkaranji. *Pheritimia pasthuma* (earthworm, 0.06–0.6 g) were collected from the water logged areas of koyana dam. All three worm types were authenticated at the Zoology Department, SGM College Karad.

Anthelmintic assay

Two worms (same type) were both placed in 9 cm Petri dishes in solutions of crude extracts in five different concentrations (10, 20, 50, 80 and 100 mg/ml in distilled water), respectively. This was done in duplicates for all the worm types.

Mean times for paralysis (P, in minutes) were taken when no movement of any sort could be observed, except when the worms were shaken vigorously. Times of death of worms (D, minutes) were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 °C). Piperazine citrate (10 mg/ml) was included as reference compound, while distilled water was included as control. This method is similar to our previous method (Ajaiyeoba & Okogun, 1996).

Results

Yields (%) of extracts and the result of the anthelmintic studies of methanolic extracts of leaves of both plants are presented in Table 1, while the intrinsic anthelmintic pro-perties of the stem extracts are shown in Table 2. The anthelmintic properties of the leaf extracts of *E microphylla Linn* and *E milii* were studied using *Pheritimia pasthuma* and *Fasciola gigantica*. The stem methanol extracts of both plants were screened for bioactivity using *P. pasthuma* and *Taenia solium* as the helminths. Null´s hypothesis was used to test significance with $P < 0.05$ ($n = 2$). Results are sum-marized in Table 1 and Table 2.

Discussion

As shown in Tables 1 and 2, the four methanolic extracts of leaves and stems of *Euphorbia microphylla Linn* and *Euphorbia milii* displayed intrinsic anthelmintic properties. The extracts showed concentration related anthelmintic activities with all the worms used in the study, with 100 mg/ml giving a shortest time of paralysis (P) and death (D) for all the worm types. The results from both tables showed that the leaves of the plants exhibited a higher activity than the stems of the plants for all the worm types used. Earthworms were most sensitive to the leaf methanol extract of *E microphylla Linn*, as shown in Table 1. It produced paralysis of 2 min and time of death (D) of 8 min, when P and D for the reference drug were 20 and 60 min, respectively against *F. gigantica* were also worthy of note. The leaf methanol extracts exhibited appreciable anthelmintic proper-ties with *F. gigantica*. Worms were paralysed or died after a time of 3–6 min, at 100 mg/ml, and piperazine citrate did same in 1–3 min. Control worms (in distilled water) lived for periods of 5–48 hr (Table 1).

As shown in Table 2, *E milii* stem methanol extract showed the highest activity against the earthworms. P = 2 min and D = 5 min when both parameters for the reference drugs were 20 and 60 min, respectively. Tape worms were most sensitive *to E microphylla Linn* stem extract with P and D values as shown in Table 2.

Generally, the earthworms were most sensitive to the extracts, especially when compared to the reference drug, piperazine citrate (10 mg/ml). At 100 mg/ml, P for the earth-worms varied between 2–5 min and D ranged between 5– 8 min. With *T. solium* (tapeworms) and at 100 mg/ml, the extracts were more effective in causing death of the worms rather than paralysis. Times for paralysis / death were 6/ 13 min for *E milii* stem extract; *Euphorbia microphylla Linn* stem extract, 5/11 min; and for reference compound, P/D was 1.5/40 min.

The function of most worm expellers like piperazine citrate is to cause paralysis of worms such that they are expelled in the feaces of man and animals. The extracts not only demonstrated this property, they also caused death of the worms, especially at 100 mg/ml. In conclusion, the folko-ric uses of these plants in traditional settings in Africa (i.e., as having anthelmintic properties) (Dalziel, 1937; Bouquet & Debray, 1974; Walker, 1974; Burkhill, 1985) have been confirmed, as extracts displayed anthelmintic properties against the different worms used in the study. We are working on isolation of anthelmintic compounds from these extracts and this will be reported at a later date.

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Table 1 Anthelmintic activity of E milii and E. microphylla Linn leaf extract

Extracts[a]	Yield(%)	Conc.(mg/ml)	Time of Paralysis (P) and Death (D) of worms in minutes (±SEM)[b]			
			P. posthuma		T. solium	
			P	D	P	D
E. Microphylla Linn (leaves)	4.8	10	15 ±0.5	>60	26 ±0.1	38 ±0.5
		20	10 ±0.3	55 ±0.2	23 ±0.3	35 ±0.2
		50	8 ±0.2	40 ±0.6	15 ±0.8	30 ±0.1
		80	3 ±0.5	20 ±0.5	8 ±0.2	10 ±0.1
		100	2 +0.9	8 +0.2	3 +0.5	5 +0.2
E. milii (leaves)	12.3	10	16 ±0.2	>60	28 ±0.3	40 ±0.9
		20	13 ±0.5	58 ±0.2	25 ±0.2	39 ±0.5
		50	10 ±0.3	50 ±0.5	20 ±0.1	35 ±0.5
		80	7 ±0.8	40 ±0.3	7 ±0.3	9 ±0.1
		100	5 +0.5	35 ±0.1	3 ±0.8	6 ±0.9
Piperazine citrate		10	20 +0.3	60 ±0.5	1 ±0.2	3 ±0.05

[a] Extracts /reference drug were dissolved with distilled water.

[b] All values were significant (P <0.05). In the control (distilled water treated), P. posthuma lived 48 hr, F. gigantic lived 5 hr.

Table 2 Anthelmintic activity of *E milii* and *E. microphylla* Linn stem extract

Extracts[a]	Yield(%)	Conc.(mg/ml)	Time of Paralysis (P) and Death (D) of worms in minutes (±SEM)[b]			
			P. posthuma		T. solium	
			P	D	P	D
E. Microphylla Linn (leaves) ±0.2	7.51	10	15 ±0.1	>90	45 ±0.5	70
		20 ±0.1	12 ±0.5	50 ±0.5	36 ±0.4	60
		50 ±0.3	7 ±0.3	10 ±0.8	18 ±0.5	55
		80 ±0.3	4 ±0.5	8 ±0.2	8 ±0.2	30
		100 +0.2	2 +0.4	5 +0.3	5 +0.1	11
E. milii (leaves) ±0.9	12.3	10	16 ±0.2	>60	28 ±0.3	40
		20 ±0.5	13 ±0.5	58 ±0.2	25 ±0.2	39
		50 ±0.5	10 ±0.3	50 ±0.5	20 ±0.1	35
		80 ±0.5	5 ±0.8	10 ±0.2	10 ±0.2	35
		100 ±0.4	2 ±0.2	5 ±0.2	6 ±0.3	13
Piperazine citrate ±0.05		10	20 ±0.3	60 ±0.5	1.5 ±0.05	40

[a] Extracts /reference drug were dissolved with distilled water.

[b] All values were significant (P <0.05). In the control (distilled water treated), P. posthuma lived 48 hr, T. solium lived 24 hr.

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