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Evaluation of anthelmintic and antimicrobial activities of *Euphorbia tirucalli* L. latex

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ABSTRACT

Dichloromethane- methanol (1:1 ratio) extract and petroleum ether extract of *Euphorbia tirucalli* L latex was investigated for anthelmintic activity. The activity of extracts at 10 different concentrations (0.1% to 1.0%) was performed on Indian earthworm *Pheretima posthuma* using Albendazole as standard reference. The results of the anthelmintic activity revealed that, all the test groups of the study exhibited reduction in time duration for the time of paralysis and death in both the extracts. Further, the petroleum ether extract was proved to be more potent among the two extracts.The antimicrobial activity was carried out against four pathogenic organism *viz. Bacillus subtilis, Klebsiella pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa, Aspergillus niger, Penicillium chrysogenum, Tricoderma viridiae* and *Candida albicans* using aqueous extract of the latex. The zone of inhibition was determined against the bacteria at the concentration of 5, 10, 15 & 20% and among the bacteria tested, *Bacillus subtilis* was resistant for the extract used. Zone of inhibition against fungi was determined at the concentration of 3, 6, 9 & 12% in the aqueous extract. The phytochemical screening of the extracts revealed the presence of secondary metabolites like steroids, alkaloids, flavonoids, glycosides, phenols and tannins.

KEYWORDS

Euphorbia tirucalli L.;
Anthelmintic activity;
Antimicrobial activity;
Phytochemical screening;
Latex.

INTRODUCTION

For thousands of years medicines and natural products have been closely linked through the use of traditional medicines and natural poisons[10] Plants have always had an important place in the therapeutic armory of mankind. The milk bush (*Euphorbia tirucalli* L.) an uncultivated weed belongs to Euphobiaceae family is a quite common plant in semi-arid and drier regions. It is erect, smooth, fleshy, shrub or small tree of 2 to 5 meter in height. The branches are green, cylindrical, clustered or scattered. It is leafless drought resistant pant and possess white milky latex[8,10]. The plant is used as a phytomedicine. Many investigators evaluated the pharmacological properties of latex such as anticancer[5], antiviral[11] and antiparasites[3,6]. The present study was aimed at evaluation of *in vitro* anthelmintic and antimicrobial activity using the extracts of *E.tirucalli* latex.

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TABLE 1: Qualitative analysis of phyto-chemicals of extracts from latex of *Euphorbia tirucalli* L

Extracts	Alkaloids	Flavonoids	Steroids	Saponins	Cardiac glycoside	Phenols	tannins	Anthraquinones
Dichloromethane -methanol	+	+	+	-	+	+	+	+
Petroleum ether	+	+	+	-	-	+	+	-
Aqueous	+	+	-	-	+	+	+	+

MATERIALS AND METHODS

Plant material

The latex of *E.tirucalli* was collected from the regions of Chitradurga district, Karnataka, India and authenticated from the Department of Botany, Sahyadri Science College, Shimoga.

Preparation of extracts

The latex was shade dried, reduced to coarse powder and subjected to extraction with two different solvents, dichloromethane-methanol (1:1 ratio) and pet ether by keeping on a rotary shaker for 24 hrs. Thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated[4]. The extracts were vacuum dried and stored at 4^0C in airtight bottles for further studies.

The aqueous extraction was done by hot extraction. The coarse powder of the latex was taken in conical flask and was extracted in distilled water for 6 hrs at slow heat. Every 2 hrs, it was filtered through 8 layers of muslin cloth and centrifuged at 5000g for 15 min. The supernatant was collected. This process was repeated twice and after 6 hrs, the supernatant was concentrated to make the final volume one-fourth of the original volume and stored at 4^0C in airtight bottles for further studies.

Drugs used

Albendazole was used as reference standard for the study of anthelmintic activity. Streptomycin was used as a standard drug for antibacterial activity and Fluconazole was used for antifungal activity.

Animals

Earthworms *Pheretima posthuma* of uniform length were procured from earthworm rearing centre, Dummalli, Shimoga, Karnataka, India.

Cultures

Bacillus subtilis (NCIM 2920), *Klebsiella pneumoniae* (NCIM 2883), *Staphylococcus aureus* (NCIM 5022) and *Pseudomonas aeruginosa* (NCIM 2945), *Aspergillus niger* (NCIM 798), *Penicillium chrysogenum* (NCIM 735), *Trichoderma viridiae* (NCIM 1195) and *Candida albicans* (NCIM 3103). These cultures were procured from National Collection of Industrial Micro organism (NCIM), Pune, India.

Phytochemical investigation

Phytochemical tests were carried out to find out the presence of alkaloids, carbohydrates, glycosides, flavonoids and saponins[1]. Results of phytochemical investigation of the crude extracts and pet ether extracts were shown in (TABLE 1).

1. Evaluation of anthelmintic activity

The Anthelmintic activity was evaluated on earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings[12,13,14]. Ten serial suspensions of extracts were prepared in DMF ranging from 0.1% to 1 %(1mg/ml to 10mg/ml). The standard reference drug Albendazole was prepared at 0.1% in DMF. All the groups are maintained in 6% dextrose solution. Thus a total of twelve groups comprising ten tests (0.1% to 1%) and one negative control (Solvent) and one positive control (Albendazole) were subjected to evaluation of anthelmintic study. Each group consists of six approximately equal sized earthworms, which were placed in a large Petri plate containing 50ml of each suspension. The method of Ghosh[2] was followed for anthelmintic screening. Observations were made for the time taken to paralyze, and or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal dextrose solution. Death was concluded when the worms lose their motility followed with fading away of their body colour; and the inactive worms were subjected against the steam from hot water bath[7].

Statistical analysis

Data were expressed as mean ± SE. Statistical

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TABLE 2: Anthelmintic activity of dichloromethane-methanol and petroleum ether extracts of *Euphorbia tirucalli* L against *Pheretima posthuma*

Extracts/ Conc.	Dichloromethane-Methanol		Petroleum ether	
	Time of paralysis (min)	Time of death (min)	Time of paralysis (min)	Time of death (min)
Standard	8.88 ± 0.19	9.07± 0.39	8.88 ± 0.19	9.07± 0.39
0.1%	9.25 ± 0.31	9.54 ± 0.27	8.70 ± 0.21	9.15 ± 0.26
0.2%	9.23 ± 0.31	9.44 ± 0.15	8.52 ± 0.32	8.90 ± 0.51
0.3%	9.02 ± 0.22	9.12 ± 0.21	8.35 ± 0.38	8.61 ± 0.57
0.4%	8.40 ± 0.22	8.77 ± 0.18	7.71 ± 0.55	8.14 ± 0.59
0.5%	8.26 ± 0.28	8.48 ± 0.38	6.80 ± 0.28*	7.81 ± 0.67
0.6%	7.96 ± 0.40*	8.16 ± 0.45	6.53 ± 0.31*	7.35 ± 0.48*
0.7%	7.38 ± 0.57*	7.86 ± 0.33*	6.32 ± 0.39*	6.89 ± 0.42*
0.8%	7.20 ± 0.51*	7.65 ± 0.47*	6.10 ± 0.41*	6.18 ± 0.36*
0.9%	6.56 ± 0.58*	7.33 ± 0.41*	4.58 ± 0.41*	4.74 ± 0.28*
1.0%	6.18 ± 0.38*	6.61 ± 0.40*	2.46 ± 0.36*	2.67 ± 0.39*

(Results expressed as Mean ± SE from six observation; control worms were alive up to 24hrs of observation. Time taken for Paralysis and death: Standard Vs Test *P<0.05)

analysis was done for ANOVA. All tests were considered statistically significant at $p < 0.05$.

2. Evaluation of antimicrobial activity

The antimicrobial property of the aqueous extract was carried out by agar well diffusion method in order to measure the zone of inhibition[2].

(a) Antibacterial activity

The extract was dissolved at different concentrations i.e. 5%, 10%, 15% and 20% in water. Streptomycin (5%) was used as reference standard and solvent control was also maintained throughout the experiment. The screening was initiated by inoculating the test bacteria onto nutrient broth and incubation at the temperature of 37^0C for 24hrs. From the broth, Lawn of each test bacteria was made with the help of sterile cotton swabs on nutrient agar plates. Well of 0.5 cm in diameter was punched on the plate with the help of sterile cork borer. The well was filled with 100µl of different concentration of extract and the experiment was carried out in triplicate. Plates were incubated for 24hrs at 37^0C after application of extracts. The plates were observed for clear zone formation around the well. Zone of inhibition is expressed in millimeter.

Antifungal activity

The extract was dissolved at different concentrations i.e. 3%, 6%, 9% and 12% in water. Fluconazole (3%) was used as reference standard and solvent control was also maintained throughout the experiment. The screening was initiated by inoculating the test fungi onto Potato dextrose broth and incubation at the temperature of 28^0C for 48hrs. From the broth, Lawn of each test fungi were made with the help of sterile cotton swabs on Potato dextrose agar plates. Well of 0.5 cm in diameter was punched on the plate with the help of sterile cork borer. The well was filled with 100µl of different concentration of extract and the experiment was carried out in triplicate. Plates were incubated for 48hrs at 28^0C of after application of extracts. The plates were observed for clear zone formation around the well. Zone of inhibition is expressed in millimeter.

RESULTS

Anthelmintic activity

The data of the mean values of the time of paralysis and time of death of both the extracts i.e. dichloro methane-methanol and petroleum ether are presented in (TABLE 2).

The two different extracts from the latex of *E.tirucalli*, i.e. Dichloromethane-Methanol and petroleum ether showed dose dependent Anthelmintic activity against earthworms tested for 10 different concentrations (0.1-1%). The maximum time duration taken for paralysis and death was found to be at 0.1% (9.25 minutes and 9.54 minutes respectively), whereas the minimum time taken was at the highest concentration 1.0% (6.18 minutes and 6.61 minutes respectively).

Similarly, the effect of petroleum ether extract was also proved to be dose dependent, wherein the maxi-

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TABLE 3: Antibacterial screening of aqueous extract of *Euphorbia tirucalli* L latex against four pathogenic bacteria

Organism	Zone of inhibition in mm					
	Control	Concentrations				
		Standard (5%)	5 %	10%	15%	20%
B.subtilis	-	9	-	-	-	-
K.pneumoniae	-	7	2	3.5	4	5
S.aureus	-	7	2	4	5.5	7
P.aerogenosa	-	10	2.2	3	5.5	6

TABLE 4: Antifungal screening of aqueous extract of *Euphorbia tirucalli* L latex against four pathogenic fungi

Organism	Zone of inhibition in mm					
	Control	Concentrations				
		Standard (3%)	3 %	6 %	9 %	12 %
A.niger	-	13	2	3	8	12
P.chrysogenum	-	9	1	2	7	9
T.viride	-	8	2	3	6	10
C.albicans	-	20	1	4	4	4

mum time duration taken for paralysis and time of death was found to be at 0.1% concentration (8.70 and 9.15 minutes respectively) while, the minimum time was recorded for the same parameters at highest concentration of the extract *i.e.* at 1.0% (2.46 and 2.67 minutes respectively).

Further, the one way ANOVA analysis indicated significant statistical differences ($p < 0.05$) between the standard and tests at concentrations of 0.6% and above for the time of paralysis and 0.7% and above for the time of death in dichloromethane-methanol extract.

For the petroleum ether extract, significant statistical differences were found to be at 0.5% and above for the time of paralysis while at 0.6% and above for the time of death with respect to standard.

The result indicates a negative correlation between time and concentration of the extracts. Comparison of effect of the extracts revealed that, petroleum ether extract was proved to be more potent than the Dichloromethane- methanol as evidenced in the lower mean values for both the parameters of the activity.

Antimicrobial activity

Antibacterial

Among the various concentrations of the aqueous extract which were screened, highest inhibition zone were observed at 20% concentration in all the test organisms. And the zone of inhibition was noted as 5mm, 6mm and 7mm in *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively whereas, least inhibition zone was observed at 5% concentration. The inhibition zones at 5% concentration were found to be 2.0 mm, 2.0 mm and 2.2 mm among the test organisms i.e. *Klebsiella pneumoniae, Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively (TABLE 3). The results also indicated that with increase in the concentration of the extract, the zone of inhibition was also increased. The antibacterial activity of the extract was slightly lesser when compared with the standard, streptomycin.

Antifungal

Among the four different concentrations of the Aqueous extract (3%, 6%, 9% and 12%), highest inhibition zone was observed at a concentration of 12%. The zone of inhibition was measured and noted as 12mm, 9mm, 10mm and 4mm against *Aspergillus niger, Penicillium chrysogenum, Trichoderma viride* and *Candida albicans* respectively and least inhibition zone was observed at 3% concentration. The zone of inhibition was found to be 2mm, 2mm, 1mm and 1mm among the four tested organisms *Aspergillus niger, Trichoderma viride, Penicillium chrysogenum* and *Candida albicans* respectively (TABLE 4). It is evident from the data that increase in the concentration of the extract; there was a simultaneous increase in the inhibition zone. The antifungal activity of the extract was compared with the standard, Fluconazole.

DISCUSSION

The study revealed that the two extracts of i.e. dichloromethane-methanol and petroleum ether possess anthelmintic activity. The comparison of results on the potency of the two extracts revealed that among the two extracts *viz.* dichloromethane-methanol and petroleum ether, the latter proved to be more effective for anthelmintic activity as evidenced in the better mean values in terms of reduction in time duration at similar concentrations. Anthelmintic activity may be due the various constituents present in the extract such as alkaloids and steroids. Specifically tannins present in the two extracts may be attributed to the anthelmintic activity[9].

Antimicrobial activity of the extract refers to its inhibitory activity towards the growth of a specific microorganism. This property may be due to presence of the alkaloids or secondary metabolites of the plant latex, like terpenoids, polyphenols, flavonoids, anthraquinones and tannins, *etc.* which are toxic to the microorganisms. The mechanisms include enzyme inhibition by oxidation[9]. Further, the variation in antimicrobial activity may be due to the differences in the chemical nature of the cell wall and cell membrane of each micro organism. Thus, the inhibition of growth of micro organisms by the extracts of *Euphorbia tirucalli* documents the medicinal importance of the plant which will be helpful in preventing and curing of diseases that are caused due to these microbes.

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REFERENCES

[1] W.C.Evans, G.E.Trease; 'Text book of Pharmacognosy', 13th Ed, ELBS, London, 289 **(1994)**.
[2] T.Ghosh, T.K.Maity, A.Bose, D.K.Dash; Indian J.Nat.Prod., **21**, 16-19 **(2005)**.
[3] S.Imai; Anticancer Res., **14(3A)**, 933- 961 **(1994)**.
[4] Jigna Parekh, Darshana Jadeja, Sumitra Chanda; Turk J.Biol., **29**, 203-210 **(2005)**.
[5] A.Q.Khan; Planta Medica, **55**, 290-291 **(1989)**.
[6] A.D.Kinghorn; J.Nat.Prod., **42(1)**, 112-115 **(1979)**.
[7] I.J.Kuppasta, Vasudeva Nayak; Indian journal of natural product, **19(3)**, 27-29 **(2003)**.
[8] M.Lotter, W.Mc Cleland, E.Schmidt, Trees and shrubs of Mpumalanga and Kruger National Park, Johannesburg, **(2002)**.
[9] Marjorie Murphy Cowan; Clinical Microbiology Reviews, **12(4)**, 564-582 **(1999)**.
[10] L.Mitich; Euphorbia Journal, **2**, **(1984)**.
[11] S.Prince; J.Virol, **77(8)**, 5000-5007 **(2003)**.
[12] G.W.Thorn, R.D.Adams, E.Braunwald, K.J. Isselbacher, R.G.Petersdorf; Harrison principle of internal Medicine, Mc Graw Hill Co., New York, 1088 **(1977)**.
[13] R.D.Vidyarthi; 'A Textbook of Zoology', 14th Ed., S.Chand and Co., New Delhi, 329, **(1977)**.
[14] Z.Vigar; 'Atlas of Medical Parasitology', 2nd ed., P.G.Publishing House, Singapore, 216 **(1984)**.