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SCREENING OF *IN-VITRO* ANTHELMENTIC ACTIVITY OF *KALANCHOE PINNATA* ROOTS

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ABSTRACT
The plant *Kalanchoe pinnata* is widely used in ayurvedic system of medicine as astringent, analgesic, carminative and also useful in diarrhea and vomiting. Naturalized throughout the hot and moist parts of India. In this first roots are subjected to pet.ether, chloroform, methanol and aqueous solvent respectively for extraction. And the in vitro evaluation of anthelmentic activity was done against *Pheretima posthuma* (Annelida), *Ascardia galli* (nematode). Methanolic extract of roots of *K. pinnata* was found to be most effective as anthelmentic as compare to other.
KEYWORDS: *Kalanchoe pinnata, Pheretima posthuma*, *Ascardia galli*.

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INTRODUCTION

Helmenthiasis is prevalent globally, but is more common in the developing countries with poorer personal and environmental hygiene. In the human body gastrointestinal tract is the abode of many helminthes, but some also live in tissue. They harm the host by deriving him of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toins[1]. Many humans harbor helminthes (worms) of one species or another. In some cases infection result many in discomfort and do not cause substantial ill health, and example being thread worm in children other worm infections, such as cytosomiasis (Bilharzias) and hook worm disease, can produce very serious mobidity[2]. Infections with helminthes or parasitic worms, affect more than two billion people world wide[3]. It is among the most important animal diseases inflecting heavy production losses. The disease is highly prevalent particularly third world countries[4]. Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. However, the world wide increasing resistance of gastrointestinal trichostrongylids of domestic small ruminants against conventional and anthelmentis[5, 6]. The resulting economical damage demonstrated the urgent need for alternative method to reduce the worm burden in an animal. Plants have been used from ancient time to cure diseases of man and animals. The systematic therapy is commonly referred as urani, folk, eastern or indigenous medicine in India and Pakistan[7].Helminthes infections are prevalent in people all over the world, but most common in the tropical and subtropical regions. The World Health Assembly, in a number of resolution has emphasized the need to the use of natural products with therapeutically proven efficacy particularly in patients residing in tribal areas who are very much prone to attack of several infections due to lack of knowledge about proper sanitation. Search for anthelmentic factor in plants therefore remains a potential area of investigation[8]. Hence this study is undertaken to evaluate anthelmentic activity of plant *Kalanchoe pinnata*.

Kalanchoe pinnata Pers. (synonyms: *Bryophyllum calycinum* Salisb. Parad. Lond., *B. pinnatum* Kurz.) (Family Crassulaceae) is naturalized throughout the hot and moist parts of India. The leaves and bark is bitter tonic, astringent to the bowels, analgesic, carminative, useful in diarrhoea and vomiting[9]. Antiulcer[10], antiinflammatory[11,12] and antimicrobial activity[13] of leaf extract was reported. Oral treatment with leaf extract significantly delayed onset of disease in BALB/c mice infected with *Leishmania amazonensis* as compared to untreated mice or mice receiving *K. pinnata* by the intravenous or topical routes[14]. Potent cytotoxic compounds bersaldegenin-1,3,5-orthoacetate[15] and bufadienolide-bryophyllin B[16] were isolated. Other chemical constituents from this plant are bryophyllol,

bryophollone, bryophollenone, bryophynol and two homologous phenanthrene derivatives 2(9-decenyl)-phenanthrene (I) and 2-(undecenyl)-phenanthrene (II) from leaves; 18α-oleanane, ψ-taraxasterol, α- and β-amyrins and their acetates also isolated[17]. Isolation and structure elucidation of 24-epiclerosterol [24(*R*)-stigmasta-5, 25-dien-3β-ol], 24(*R*)-5α-stigmasta-7, 25-dien-3β-ol, 5α-stigmast-24-en-3β-ol and 25-methyl-5α-ergost-24 (28)-en-3β-ol from aerial parts was done[18]. This species is also included in the plants species, which are used by the tribals of Kerala for treating cancer symptoms[19]. Juice of the fresh leaves is used very effectively for the treatment of jaundice in folk medicines of Bundelkhand region of India, but no systemic study to assess this activity has been carried out. As the aerial parts of plant have many pharmacological activity but roots of this plant was not focused yet hence the present investigations were carried out to evaluate the root of kalanchoe pinnata for its anthelmentic activity.

MATERIALS AND METHODS

Collection of plant material

The roots of *Kalanchoe pinnata* were collected from Satpuda hills near Akkalkuwa, Dist: Nandurbar, Maharashtra, India, in June 2010, cleaned and dried at room temperature in shade and away from direct sunlight. The plant authenticated by T. Chakraborthy, Deputy Director Botanical Survey of India, Koregaon Road Pune, by comparing morphological features and a sample voucher specimen of plant was deposited for future reference (Voucher specimen number QMAKP1).

Preparation of Extract

The roots of *Kalanchoe pinnata* were collected and dried in the shade and then pulverized in a grinder. The powdered drug was utilized for extraction. Material was passed through 120 meshes to remove fine powders and coarse powder was used for extraction. A method described in Mukherjee was used for extraction of powdered plant. Extraction was done by Pet. Ether, Chloroform, Methanol, and Aqueous.[20]

Preliminary Phytochemical Screening

The extracts were then subjected to preliminary phytochemical screening to detect the presence of various phytoconstituent. The results shows that petroleum ether extract contain steroids, the chloroform extract contain steroids and alkaloids, the methanolic extract contain Steroids, Saponins, Alkaloids, Glycosides, Flavonoids, Tannins, Carbohydrates, Proteins and aqueous extract contain Saponins, Glycosides, Flavonoids, Tannins, Carbohydrates, Amino acids.[21]

Animal Selection

Pheretima posthuma (Annelida), Commonly known as earthworm collected from the water logged areas and *Ascardia galli* (nematode) worms were obtained from freshly slaughtered fowls (Gallus gallus). Both the worm types were identified at S.S.V.P.S. College, Dhule.

Evaluation of Anthelmentic Activity

The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasite of human beings. Because of easy availability, earthworms have been used widely for initial evaluation of anthelmentic compounds *invitro*. *Ascardia galli* worms are easily available in plenty from freshly slaughtered fowls and their use, as a suitable model for screening of anthelmentic drug was advocated earlier. Fifty milliliter of solution containing three different concentrations, each of crude extract (10, 50 and 100 mg/ml) were prepared and six worms (same type) were placed in it. Time of paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 °C). Piperazine citrate (10 mg/ml) was used as reference standard while distilled water as control.[22]

RESULTS AND DISCUSSION

The results reveal that, [**Table 1**] chloroform, methanolic and aqueous extract of *Kalanchoe pinnata* having significant anthelmentic activity. While petroleum ether extract does not show activity against helminth.

Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis. The roots extract of *Kalanchoe pinnata* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 100 mg/ml, in shorter time as compared to reference drug Piperazine citrate. Phytochemical analysis of the crude extracts revealed presence of tannins as one of the chemical constituent. Tannins were shown to produce anthelmentic activity. Tannins are polyphenolic compounds. Some synthetic phenolic anthelmentics eg niclosamide, oxycylozanide and bithionol are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation. It is possible that tannins contained in the exrtracts of *Kalanchoe pinnata* produced similar effects. Another possible anthelmentic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death.

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Table 1: Results of Anthelmentic activity

Test substances/ Extract	Concentration (mg/ml)	Time taken in minutes	
		For Paralysis	For Death
Petroleum ether	20	No paralysis	No death
	40	No paralysis	No death
	60	No paralysis	No death
	80	No paralysis	No death
	100	No paralysis	No death
Chloroform	20	No paralysis	No death
	40	60	87
	60	48	69
	80	41	56
	100	35	48
Methanolic	20	43	54
	40	34	49
	60	27	43
	80	19	38
	100	11	27
Aqueous	20	61	93
	40	53	76
	60	45	57
	80	39	51
	100	27	46
Piperazine citrate	20	29	47
	40	21	39
	60	13	28
	80	09	19
	100	03	09

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