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Research Article

IN–VITRO ANTHELMINTIC ACTIVITY OF PET – ETHER EXTRACT *OF PORTULACA OLERACEA* (LINN.) AGAINST *PHERITIMA POSTHUMA*

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ABSTRACT : Present study was carried out to investigate the anthelmintic potential of petroleum-ether extract of *Portulaca oleracea* against *Pheretima posthuma* as a species of earthworm. Various concentrations (25-100 mg/ml) of petroleum-ether extract were evaluated for anthelmintic activity by recording the time required for paralysis and death of worms. Albendazole was used as standard. Result indicates that petroleum-ether extract significantly (p<0.01) exhibited paralysis at lower doses of 50, 75 and 100 mg/ml and causes death of worms at doses of concentrations 75 and 100 mg/ml when compared with standard. The present study indicates that the petroleum-ether extract of *Portulaca oleracea* has a potential anthelmintic activity can be used as anthelmintic drug.

Keywords: Anthelmintic activity, petroleum-ether extract, *Portulaca oleracea*, Albendazole, *Pheretima posthuma*

INTRODUCTION

Portulaca oleracea (*P. oleracea*) belonging to the family "Portulacaceae" is an herbaceous plant widely distributed throughout the world. It contains many biologically active compounds and is a source of many nutrients like free oxalic acids, alkaloids, omega-3 fatty acids, coumarins, flavonoids, cardiac glycosides, anthraquinone, protein, (Ezekwe MO et, al., 1999) a-linolenic acid and b-carotene (Liu LX et, al., 2000, Barbosa – Filho JM et, al., 2008) mono terpene glycoside (Sakai NK et, al., 1996) N-trans-feruloyltyramine (Mizutani M et, al., 1998). It was also found to contain vitamin C, oleoresins-I and II, saponins, tannins, saccharides, triterpenoids, 0a-tocopherol and glutathione (Chatterjee A et, al., 1956, Simopoulous AP et, al., 1992, Prashanth KL et, al., 2005). The high contents of a variety of phytoconstituents present in this plant were considered to be responsible for the biological activities like antibacterial, antifungal (Oh KB et, al., 2002), anti-fertility (Verma OP et, al., 1982), muscle relaxant (Parry O et, al., 1993) and wound healing properties, (Rasheed AN et, al., 2003) analgesic and antiinflammatory activity (Jagan Rao N et, al., 2012). This plant which is normally used as a vegetable to prepare curry by the native people of Andhra Pradesh is used in combination with tomato. Earlier studies revealed the above pharmacological properties of *Portulaca oleracea*. However, no study was done on its anthelmintic activity of petroleum-ether extract of *Portulaca oleracea*. Therefore, the present study has been designed to investigate the petroleum-ether extract of *Portulaca oleracea* for its anthelmintic activity.

MATERIALS AND METHODS

The leaves of *Portulaca oleracea* were collected from a local vegetable market in Kanchipuram in the month of January 2011. The identification and authentication of the plant done at the department of Botany, Government Degree College, Kanchipuram.

Worm Collection and Authentication:

The Indian earthworm *Pheritima posthuma* was collected from ponds & marshlands in Kanchipuram, Tamilnadu, India. The earth worm was authenticated by Dr Sudarsanam veterinary Microbiologist and Chief Veterinary officer, Meenakshi Medical College and Research Institute, MAHER (Meenakshi University) Kanchipuram, India.

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Preparation of extract:

Portulaca leaves were shade dried and one kg of coarse powder was soaked in 4 litres of petroleum-ether for 3 days at room temperature. The extract was evaporated to dryness by using a rotary vacuum flash evaporator and the yield was10% w/w.

Phytochemical screening:

The petroleum ether extract of *Portulaca oleracea* leaves were subjected to qualitative chemical investigation for the identification of phyto constituents (Khandelwal KR et, al., 2000) like triterpenoids, saponins, alkaloids, carbohydrates, tannins, flavonoids and glycosides using appropriate reagents. The extracts were treated with dilute hydrochloric acid and filtered. The filtrate is used in the following tests.

Test for alkaloids (Mayer's test):

The extract was treated with Mayer's reagent and the appearance of cream color indicates the presence of alkaloid.

Test for tannins:

The extract was treated with 10% lead acetate solution; appearance of white precipitate indicates the presence of tannins.

Test for flavonoids (Shinoda test):

To the extract, add 5 ml 95% ethanol, few drops of conc. HCl and 0.5g magnesium turnings. Pink coloration indicates the presence of flavonoids.

Test for saponins (froth test):

1ml of the extract was diluted to 20 ml with distilled water and shaken well in a test tube. The formation of foam in the upper part of the test tube indicates the presence of saponins.

Test for terpenoids (Salkowski test):

Five ml of extract was mixed in 2 ml of chloroform, and concentrated H2S04 (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

Test for carbohydrates (Molisch's test):

The extract was treated with 3ml of alpha–napthol in alcohol and concentrated sulphuric acid was added along the sides of the test tube carefully. Formation of violet colour ring at the junction of two liquids indicates the presence of carbohydrates

Test for glycosides (modified Borntrager's test):

To 5 ml of extract add 5ml of 5% FeCl3 and 5ml dil. HCl. Heat for 5 min. in boiling water bath. Cool and add benzene or any organic solvent. Shake well. Separate the organic layer and add equal volume of dil.Ammonia. Ammonical layer shows pinkish red color.

Anthelmintic activity

The anthelmintic activity was performed according to the method of Ghosh et, al. (Ghosh T et, al., 2005) on adult Indian earthworm *Pheritima posthuma* as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings (Girme AS et, al., 2006. Tambe VD et, al., 2006) *Pheritima posthuma* worms are easily available and used as suitable model for screening anthelmintic drugs (Dash GK et, al., 2002). In the 50 ml of formulations containing four different concentration of methanol extract (25, 50, 75 and 100 mg/ml in normal saline) and standard (20 mg/ml) were prepared and approximately equal sized six earthworms were released in each group. Observations were made for the time taken to paralyse or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed with fading away of their body color. Albendazole (20 mg/ml) was used as standard while normal saline as control.

RESULTS

Phytochemical Screening:

The percentage yield of petroleum-ether extract of leaves *Portulaca oleracea* was found to be 10.6%w/w.The chemical tests indicate the presence of Phytoconstituents like the flavonoids, tannins, saponins, Terpenoids and Alkaloids in the petroleum-ether extract.

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Table 1: Anthelmintic activity of petroleum-ether extract of Portulaca oleracea

Teat substance	Concentration(mg/ml)	Time taken for paralysis(min)	Time taken for death(min)
Vehicle	-	-	-
Albendazole (std)	20	12.27±0.23	17.27±0.18
Methanolic extract	25	54.18±0.39*	76.09±0.26**
	50	31.53±0.98**	49.74±0.31**
	75	18.15±0.43**	27.13±0.46**
	100	10.55±0.15**	19.15±0.32**

Values are expressed as MEAN±SEM, One way ANOVA followed by Dunnett's test. n=6 in each group. *P<0.05, **P<0.01.

Experimental data showed that, the petroleum-ether extract of Portulaca oleracea has showed anthelmintic activity in dose dependent manner as shown in Table 1. The shortest time required for paralysis and death was observed with concentration of 100 mg/ml.Higher concentration of petroleum-ether extract showed maximum effect as 10.55±0.15 min paralysis time and 19.15±0.32 min death time. Standard Albendazole showed the paralysis at 12.27±0.23 min and death at 17.27±0.18 min after release of worms in it.

DISCUSSION

Helminthic infections of the gastrointestinal tract of human beings and animals have been recognised to have adverse effects on health standards with a consequent lowering of resistance. In search of the compounds with anthelmintic activity, a number of substances were screened using different species of worms, for example, earthworms, Ascaris, Nippostronylus and Heteakis of all these species earthworms have been used widely for the initial evaluation of anthelmintic compounds in vitro because they resemble intestinal "worms" in their reaction to anthelmintic and are easily available. It has been demonstrated that all anthelmintics are toxic to earthworms and a substance toxic to earthworms is worthy for investigation as an anthelmintic (Patil AP et, al., 2010)

Availability drugs like Albendazole act against intraluminal parasites and tissue parasites by either killing or expelling the infesting helminthes without harming the host. Albendazole the congener of mebendazole acts by increasing chloride ion conductance of worm muscle membrane leading to hyperpolarisation causing flaccid paralysis that result in expulsion of the worm by peristalsis of host gastrointestinal tract [Tripathi K D, 2008], But they have limitations for use in pregnancy and in children younger than 2 years of age [Katzung, Bertram G, 2009]. Because of its high prevalence rate since ages traditional and folklore medicines existed.

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols, etc. The successive extracts of plant have been revealed the presence of flavonoids, tannins, saponins, Terpenoids and Alkaloids. From the above results, it is concluded that the extracts of the plant have potent anthelmintic activity when compared to the conventionally used drugs and is equipotent to standard antihelmintic drug. Further results, using in vivo models are required to carry out and establish the effectiveness and pharmacological rationale for the use of the plant as an anthelmintic drug.

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