Anthelmentic Activity of Crude Ethanolic Extract of Euphorbia Hirta in Indian Earthworm Phereetima Posthuma

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Abstract: Euphorbia hirta is a pantropical weed, possibly native to India. It is a hairy herb that grows in open grasslands, roadside and pathways. It is used in traditional herbal medicines. Euphorbia hirta is often used traditionally for female disorders, respiratory ailments like cough, coryza, bronchitis and asthma worm infestation in children, dysentery, jaundice, pimples, gonorrhoea, digestive problems and tumours. It is reported to contain alkanes, triterpenes, phytosterols, tannins, polyphenols, and flavonoids. The results of the present study clearly indicated that the crude ethanolic extract of Euphorbia Hirta did produce anthelmentic activity against Indian earthworm Phereetima posthuma of Euphorbia Hirta have potent anthelmentic activity when compared with the conventionally used drug.

Keywords: Helminths, Potent anthelmentic, Ethanolic extract of euphoria hirta, phereetima posthuma, Albendazole

1. Introduction

Helminthes infection repeatedly entitled helminthiasis is among the most passive infection & foremost degenerative diseases distressing a proportion of world population.

In developing countries they pose a large threat to public health & contribute to the prevalence of malnutrition, anaemia, & pneumonia. The helminth’s parasite mainly subsist in human body in intestinal track, but they are also found in tissue, as their larvae migrate forward them.

Different type of helminthes infection the human and animal out of which intestinal round worm (ascardia sp.) are most common approximately 300 million people suffer severe morbidity associated with this parasite and halp of which are school going children affected by massive infection variety of several clinical symptom arise due to this infection include respiratory symptom, dermatological consequence & epilepsy as a result of neurocysticercosis. Helminthic infection may also subvert immune response to pathogen of other diseases such as T.B, HIV & Malaria.

The euphorbiaceae family comprises over list of plant belong to 229 plant genera, which are Found along roadsides, and Karnataka: Chikmagalur, Coorg, Dharwar, Mysore, N. Kanara, Shimoga Kerala, all districts Tamil Nadu, also in Maharastra in western ghats, in Bangladesh, Andmaan sea.

The main chemical constituent present in that is E. hirta mainly contains flavonoids, terpenoids, phenols, essential oil, and other compounds. The major chemical structures of these compounds are shown in Figure 1. One type of the important constituents of E. hirta is flavonoids including quercetin, quercitrin, quercitol, and derivatives containing rhamnose, quercetin-rhamnose, a chlorophenolic acid, rutin, leucocyanidin, leucocyanidol, myricitrin, cyanidin 3,5-diglucoside, pelargonium 3,5-diglucoside, and, camphol. The flavonol glycoside xanithorhamnin was also isolated from E. hirta. The stems contain the hydrocarbon henriacantone and myricyl alcohol. The latex contains inositol, taraxerol, friedelin, β-sitosterol, ellagic acid, kaempferol, quercitol and quercitrin.

2. Scientific Classification

Kingdom:-Plantae
(unranked):-Angiosperm.
(ranked):- Eudicots.
Order:-Malpighiales.
Family:- Euphorbiaceae.
Genus:-Euphorbia
Species:-E.hirta.
Synonym:-Chamaesiye hirta(L)millsp.
Bionomial name:- Eub.H.L.
English name:- Pill-bearing spurge, Asthma plant, Hairy spurge, Garden spurge, Pillpod sandman. 

Morphological Description

Slender, erect, pubescent herbs about 20-35 cm tall. Leaves decussate, 1-2.5 x 0.7-1.5 cm, broadly oblong to elliptic-lanceolate, base obliquely truncate, margin serrulate, apex acute, hispid on both sides, basally 3-nerved; petiole to 3 mm long. Cyathia aggregated in single or paired axillary clusters. Involucre minute, c. 1 mm long; glands 5, red. Male flowers 4-6, ebracteolate. Female florets laterally pendulous; styles 2-fid from base. Capsule 1.5-2.2 mm across, pubescent. Seeds minute, red, 4-angled, minutely furrowed.
Main Chemical Constituent
Afzelin, quercitin, and myricitin have been isolated from the methanolic extract of *E. hirta*. The chemical investigation of *E. hirta* has led to the isolation of rutin, quercitin, euphorbin-A, euphorbin-B, euphorbin-C, euphorbin-D, 2,4,6-tri-O-galloyl-β-D-glucose, 1,3,4,6-tetra-O-galloyl-β-D-glucose, kaempferol, gallic acid, and protocatechuic acid. *E. hirta* also contains β-amyrin, 24-methylene cycloartenol, β-sitosterol, heptacosane, nonacosane, shikmic acid, tinyatoxin, choline, camphol, and quercitol derivatives containing rhamnose and chotphenolic acid.

Afzelin, quercitin and myricitin have been isolated from the ethanolic extract of *E. hirta* leaves the purpose of this was to be evaluate the anthelmintic property of euphorbiace.

Administration of Extract
The suspension of ethanolic extract of leaves of Euphorbia Hirta of different concentration(25.50mg/ml) were prepared by using 0.5% of carboxy methoxy cellulose(CMC) as a suspending Agent & final Volume Was made up to 10ml for respective concentration. Albenbazole (50mg/ml) are used as standard. Group of approximately equal size worms consisting of three earthworm in each group were released into in each 40ml of desired concentration of drug & extract in Petri dish.

Experimental Design
The anthelmintic activity was performed according to the method:

On adult Indian Pheretima posthuma as it has anatomical & physiological resembles with the intestinal roundworm parasites of human beings. Earthworm was placed in petridish containing three different concentration (25.50mg/ml)of ethanolic extract of leaves of Euphorbia Hirta. Each petridish was placed with three worm & observed for paralysis or death. Mean time for paralysis was noted when no movement of any sort could be observed except when the worms was taken vigorously .The time death of worms (min) was recorded. After ascertaining the worm neither moved when shaken nor when given external stimuli. The test result were compared with Albenbazole (50mg/ml) treated samples. Death was included when the worms lost their motility followed by while secretion & fading away of their body colour.

5. Qualitative Chemical Analysis
The ethanol extract of Euphorbia Hirta leaves subjected to Qualitative chemical analysis to detect the presence of various phytochemical screening.

Test of Carbohydrate

A) Molisch’s Test
Small portion of ethanolic extracts was dissolved in 5 ml of distilled water and filtered. To this solution three drops of alpha-naphthol was added and 1 ml of con. Sulphuric acid was added along the sides of inclined test tube so as to form two layers and observed for formation of violet coloured ring at the interface to detect the presence of carbohydrates.

B) Fehling’s Test:
Few drops of fehlings solution A and B in equal volume were added in dilute extracts and heated for 30 min and observed for the formation fo brick red coloured precipitate.

Test for Glycosides
A) Glycosides-keller-killiani test:
0.5 ml of alcoholic extracts was taken and subjected to the following test, 1 ml of glacial acetic acid containing traces of ferric chloride and 1 ml of con. Sulphuric acid was added to extract and observed for the formation of reddish brown color at the junction of two layers and the upper layer turned bluish green in the presence of glycosides.

4. Preparation of Plant Extracts of Euphorbia Hirta Leaves
Air dried Euphorbia Hirta leaves were powdered using mechanical grinder .50gm of fine powder was weighed into 500ml ethanol (95%) in aq conical flask . This was covered , shaken every 30min. for 6 hrs. and then allowed to stand for about 48 hrs. The solution was s filtered using Watman filter paper. The filtrate was evaporated to dryness using rotary evaporator . It was stored in refrigerator in amber coloured bottle to avoid degradation.

Administration of Albenbazole
Albenbazole (50mg/ml) was prepared by using 0.5% w/v CMC as a suspending agent as administered as per method of extract.
Test for Steroids
2 ml of acetic anhydride was added to 0.5 g of extract and 2 ml of sulphuric acid was added by the sides of the test tube and observed the colour change from violet or blue-green.

Test for Protein-Xanthoprotein:
To 1 ml of extract few drops of nitric acid was added by the sides of the test tube and observed for formation of yellow colour.

Test for Tannin
0.5 g of the extract was taken in a boiling tube and boiled with 20 ml of distilled water and the filterer added few drops of 0.1% ferric chloride was added mixed well and allowed to stand few seconds. Observed for brownish green or a blue-black colour.

6. Constituents Euphorbia Hirsta Leaves

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Qualitative analysis of Phytochemical Components parameter</th>
<th>Ethanolic extraction of Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Reducing Sugar</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Acidic compound</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Cardiac glycoside</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Proteins</td>
<td>+</td>
</tr>
</tbody>
</table>

Symbol: Positive (+), Negative (-).

Preliminary phytochemical screening of crude ethanolic extract of Euphorbia Hirsta leaves, the presence of glycosides, Saponins, Tannins, Steroid carbohydrate, reducing sugar, cardiac glycosides showed anthelmintic activity at a concentration of 25 mg/ml and 50 mg/ml. The anthelmintic effect of extracts is comparable with that of the effect produced by the standard drug albendazole. Parasitic helminths affect animals and man, causing considerable hardship and stunted growth. Hundreds of millions if not billions of human infections by helminthes exist worldwide and increased world travel and immigration from the developing countries. Evaluation of activities of medicinal plants claimed for possessing the anthelmintic property is getting the attention these days. Screening and proper evaluation of the claimed medicinal plants could offer possible alternatives that may be both sustainable and environmently acceptable. The results of this study have shown promising anthelmintic activity suggesting the possible use of Euphorbia Hirsta in intestinal nematode control

7. Conclusion
The results of the present study clearly indicated that the crude methanol extract of Euphorbia Hirsta did produce anthelmintic activity against Indian earthworm Pheretima posthuma. He plant possesses significant anthelmintic activity at 50 mg/ml concentration measured by time taken for paralysis / death of the earth worms. The current investigation leads to conclusion that the leaves of Euphorbia Hirsta have potent anthelmintic activity when compared with the conventionally used drug. Further studies using in vivo models and to isolate active constituents from extract a required to carry out and established the effectiveness and pharmacological rational for the use of as Euphorbia Hirsta an anthelmintic drug.

References
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[4] Department of Chemistry, Periyar University, Salam: 11, India.
[6] Chemistry.enge@gmail.com.
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Table 2: Activity in Pheretima Posthuma

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Extract</th>
<th>Concentration (mg/ml)</th>
<th>Pheretima posthuma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control(0.5%CMC)</td>
<td>0.0</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Standard (albendazole)</td>
<td>50 mg/ml</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Ethanolic Extract of Euphorbia Hirsta</td>
<td>25 mg/ml</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50 mg/ml</td>
<td>-</td>
</tr>
</tbody>
</table>