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Evaluation of Anthelmintic Activity of Methanolic Extracts of Five Medicinal Plants

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ABSTRACT: Helminth parasitic infections has affected nearly one third of the world's human population in developing countries. The children are the most prone to infection. Chemical supplements used are ineffective in course of years as the worms develop resistance to it. Obviously safe and inexpensive medicinal plant extracts attracts interest of anthelmintic drug research. In the present study the methanolic extracts of *Basella alba*, *Caesalpinia pulcherrima*, *Cissus quadrangularis*, *Cassia occidentalis* and *Carica papaya* were investigated for anthelmintic activity. The extracts were tested on *Eisenia foetida* (red wiggler worm, an experimental model) and were compared with Albendazole citrate. The results revealed that *Cassia occidentalis* and *Caesalpinia pulcherrima* test groups proved to be more effective compared to *Cissus quadrangularis* and *Carica papaya* in a dose dependent manner. While *Basella alba* did not show any effect. The phytochemical screening of the extracts was also done. All together, these five medicinal plants warrants further scientific research and clinical investigation as it holds promise as a anthelmintics.

KEY WORDS: Anthelmintic activity; *Eisenia foetida*; Medicinal plants; albendazole.

I. INTRODUCTION

Helminthiasis or Soil-transmitted helminth (STH) parasitism are among the most prevalent human infections in tropical and sub-tropical regions of the world. Lack of adequate water and sanitation in urban slum communities has impact of this parasitism worldwide [1]. Parasitic infection enervates the health by causing anorexia which leads to malnutrition, diarrhea, anemia, eosinophilia and pneumonia [2] [3]. It adversely affects physical and mental growth in childhood [4]. The drastic part of the infection is it physically impairs their hosts and, in severe cases, intestinal blockage leads to death [5]. Parasitic helminthic worms also infect livestock and crops, resulting in major economic impact. In spite of rampant occurrence of parasitic infections, the research on the anthelmintic drug is sparse.

Anthelmintic drugs are either used to control the helminthes or expel helminthes from the body, by stunning or killing them [6]. Albendazole (a benzimidazole), is anthelmintic agent commonly used against the four main geohelminths which causes the human infections are *Ascaris lumbricoides* (roundworm), *Ancylostoma duodenale*, *Trichuris trichiura* (whipworm) and *Necator americanus* (hookworms) [7]. According to the World Health Organization (WHO), only a few effective anthelmintic drugs are available of which some are expensive and while some lose their efficacy in 20 years. Emergence of resistant parasites due to frequent use, high dosage administration and increased application of the drug, has become a major challenge to patient safety. Plant derived medicines on which 80% of world's population rely for primary health care needs is proved to be the best alternative [8]. Medicinal plants have been used by indigenous peoples for

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centuries as sources of extracts used to treat infectious diseases [9]. In the current study, we have attempted to investigate six medicinal plants *Basella alba*, *Caesalpinia pulcherrina*, *Cissus quadrangularis*, *Cassia occidentalis* and *Carica papaya* for anthelmintic activity.

II. MATERIALS AND METHODS

Collection and authentication of plant resource: Fresh and healthy plant material of *Basella alba*, *Caesalpinia pulcherrina*, *Cissus quadrangularis*, *Cassia occidentalis* and *Carica papaya* were chosen and collected from my garden. The plants were thoroughly washed separately with tap water and were air dried in shade. The dried plant material were grounded into coarse powder and stored in air tight polythene containers at room temperature until further analysis. The plants were identified, authenticated and voucher specimen was deposited in the herbarium of Unani research Institute, Hyderabad, India.

Extract preparation for the experiment: About 200g of powdered plant material was extracted two to three times with methanol at room temperature for 72 h with mass to volume ratio of 1:10 (g/ml). The solvent extracts were evaporated to dryness under vacuum on rotary evaporator between 37°C to 40°C. The extracts were stored at -20°C until further analysis. The percentage yields of the extractives were calculated [10].

Qualitative phytochemical screening: A small portion of the dry extract was used for phytochemical screening. It was done by following the standard qualitative methods with slight modifications as described by Harborne (1998) [10].

Test organism: The adult Indian earthworm, *Eisenia foetida* (red wiggler worm) used in this experiment has anatomical and physiological resemblance with the intestinal human parasite, Roundworm. The earthworms of 3-5 cm in length and 0.1- 0.2 cm in width were collected from commercial compost pits. The collected worms were washed with normal saline before the experiment. The sample extracts were prepared freshly by suspending in Tween 80 (0.1%) in normal saline.

Anthelmintic Activity: The anthelmintic activity of methanolic extract of six plants was evaluated following method of Dash et al with slight modification [11]. Eight groups consisting of six earthworms in each group were used for the present study.

Group-1 : (Distilled water) ; Group-2 : Control (normal saline with 0.1% Tween 80) ; ; Group- 3; 4; 5; 6; 7: Methanolic extract of *Basella alba* , *Caesalpinia pulcherrina*, *Cissus quadrangularis*, *Cassia occidentalis* and *Carica papaya* of different concentration (5mg/ml, 10mg/ml, 20mg/ml) respectively. Group- 8: Standard (Albendazole- 10mg/ml). The observation was made up to 4 h of the test period. The time taken to paralysis (the worms did not revive even in normal saline) and death (when the worms lost their motility followed by fading away of their body colors) of individual worms were recorded and tabulated [12].

Statistical analysis: The experiments were replicated three times and the mean and standard deviation ($X \pm SD$) of the data were calculated statistically.

III. RESULTS AND DISCUSSION

Phytoconstituents screening of the methanolic extracts of the five plants are tabulated (Table 1). All the plant extract contained flavonoids, phenolic compounds, steroids, carbohydrates, reducing sugars and glycosides. Flavonoids are known as natural biological modifiers. As biological modifiers flavonoid modifies the degradative effects of allergens, viruses and carcinogens [13]. The most ubiquitous group of plant metabolites are Phenolic compounds [14]. These molecules exhibit their protective action as blocking agents of the carcinogenic precursors [15]. It revealed that alkaloids and tannins was present in all the four plants except *Carica papaya* while vice versa for the content of terpenoids. Alkaloids are the secondary metabolites with therapeutic potential. Knowledge about this diverse group of secondary metabolite helps in identifying and designing lead drug molecules. It has been reported that alkaloids inhibits DNA topoisomerases to exhibit

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antimicrobial action [16]. Experimental evidence has revealed that tannin-rich plants have anthelmintic properties [17]. Proteins, anthocyanidin and anthraquinones were proved to be completely absent in the methanolic extract of all the five plants. Saponins are present in the four plants except *C.pulcherrima* methanolic extract. While lignins was absent in the both *C. occidentalis* and unripe fruit of *C.papaya* methanolic extract.

Anthelmintic activity of methanolic extract of five medicinal plants against *Eisenia foetida* was evaluated (Table 2) in which *C. occidentalis* exhibited best activity in dose dependent manner giving shortest time of paralysis (4.5 min) and death (27.5 min) at 20 mg/ml concentration compared to other test plant and the drug albendazole. Since the presence of tannins in the plant extract may be the reason for paralysis and death of the *Eisenia foetida*. *Carica papaya* induced paralysis and death in 12.5 min and 35 min respectively. While this extract did not show any positive test for tannins. A group of researchers have proved that the latex of *Carica papaya* showed significant anthelmintic activity in a dose dependent manner as compared to standard reference (piperazine citrate) and control (distilled water) [18]. *C. pulcherrima* extract induced paralysis in 8.5 minutes (min) and death in 103 minutes while albendazole induced paralysis in 23 min and in 38 min death. Albendazole exhibits anthelmintic activity by blocking uptake of glucose and depleting glycogen stores in the parasite [19]. Earlier it was reported that the petroleum ether and chloroform extracts of pods of *C.pulcherrima* exhibited significant anthelmintic activity than methanolic extract[20]. *Cissus quadrangularis* showed the least effect compared to all the other three plants. There was recent report on alcoholic root extract of *C.quadrangularis* exhibiting significantly determined anthelmintic activity at 100 mg/ml, caused paralysis in 17 min and death in 30.67 min while, 200 mg/ml, caused paralysis in 8.33 and and death in 18.50 minutes against earthworm *Pheretima posthuma* [21]. This shows that the aerial methanolic extract of *Cissus quadrangularis* is better than it's root extract. *Basella alba* did not show any effect at this concentration. If a plant extract does not shows any anthelmintic activity, one cannot designate it to be completely inactive. The extraction method using methanol does not guarantee a complete extraction of bioactive molecules with potential anthelmintic activity. A specific formulation or the synergistic interaction of phytocomponents or combination of extracts could show additive or inhibitory effect [19]. In the present study, Methanolic extract of the test plants have proved to be very effective than the standard drug. Knowledge of indigenous plants and its bioactive principles molecular role exposes a protocol for drug discovery and compatible treatment.

Table 1. Qualitative Phytochemical analysis of methanolic plant extracts

Phytochemical tests	Methanolic plant extracts				
	Basella alba Aerial part	Caesalpinia pulcherrina Aerial part	Cissus quadrangularis Aerial part	Cassia occidentalis Aerial part	Carica papaya Unripe fruit
Colour	Greenish brown	Dark green	Green	Dark brown	Light brown
Alkaloids	+	+	+	+	-
Flavonoids	+	+	+	+	+
Phenolic compounds	+	+	+	+	+
Terpenoids	-	-	-	-	+
Steroids	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Proteins	-	-	-	-	-
Reducing sugars	+	+	+	+	+
Anthocyanidin	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Saponins	+	-	+	+	+
Tannins	+	+	+	+	-

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Lignins	+	+	+	-	-
Glycosides	+	+	+	+	+

Table 2. Anthelmintic activity of methanolic extract of medicinal plants against *Eisenia foetida*

S.No./ Group No:	Groups with equal amount of each extract	Time taken for paralysis (min.) at different Concentrations (mg/ml) of extract			Time taken for death(min.) at different Concentrations (mg/ml) of extract		
		5mg/ml	10 mg/ml	20 mg/ml	5mg/ml	10mg/ml	20mg/ml
1.	Distilled water as control	>250	>250	>250	>250	>250	>250
2.	Normal saline with 0.1% Tween 80	>250	>250	>250	>250	>250	>250
3.	<i>Basella alba</i> (A)	>200	>200	>200	>200	>200	>200
4.	<i>Caesalpinia pulcherrima</i> (B)	23.0±0.89	13.3±1.21	8.5±1.62	130.6±1.96	105.6±3.82	103.3±3.55
5.	<i>Cissus quadrangularis</i> (C)	36.2±2.13	18.5±6.03	14.5±1.76	220.5±9.22	170.8±15.9	106.6±4.88
6.	<i>Cassia occidentalis</i> (E)	16.3±2.50	8.8±1.72	4.5±1.85	166.6±9.30	70.3±9.17	27.5±5.00
7.	<i>Carica papaya</i> (F)	31.6±2.06	12.8±3.13	12.5±1.04	201.6±5.04	68.6±10.9	35.0±6.26
8.	Albendazole (Drug)	23.0±1.42			38.0±1.87		

Each value represents mean value ± standard deviation of six replicates (n=6).

IV. CONCLUSION

This investigation has revealed that the methanolic extract of *C. pulcherrima*, *C. quadrangularis*, *C. occidentalis* and *Carica papaya* showed significant anthelmintic activity against *Eisenia foetida* when compared to the common chemical drug albendazole. Among these medicinal plants *C. occidentalis* exhibited potential anthelmintic effect. Further chemical characterization of active fraction of the extract against parasitic helminthes is under study.

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