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Research Article

EVALUATION OF ANTHELMINTIC AND ANTIOXIDANT ACTIVITIES OF *EPIPRINUS MALLOTIFORMIS* LEAF EXTRACTS

CHANDRASHEKAR M.B.[1]*, RAJA NAIKA[1], VINAYAKA K.S.[1], JOY HOSKERI H.[2]

[1]Department of Post Graduate Studies and Research in Applied Botany, Kuvempu University, Shankarghatta – Shimoga, Karnataka, India.
[2] Department of Biotechnology, The Oxford College of Science, HSR Layout, Bangalore – Karnataka, India.
Email: chandrashekarbotany25@gmail.com

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ABSTRACT

Objective: Development of anthelmintic resistance and high cost of conventional anthelmintic drugs led to the evaluation of medicinal plants as an alternative source of anthelmintics. The aim of the present study was to investigate the anthelmintic activity and antioxidant activity of the leaves of *Epiprinus mallotiformis* extract using adult earthworm, *Pheritima posthuma*.
Methods: The leaves extracts of *Epiprinus mallotiformis* at different concentrations of 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL were tested, which involve determination of paralysis time and death time.
Results: It was found that the extract exhibited significant dose dependent anthelmintic activity. Piperazine citrate 10 mg/ml as standard reference. Whereas antioxidant activity of the extracts were performed by four method, DPPH free radical scavenging activity, H_2O_2 scavenging activity, Reducing power assay and Metal chelating activity using ascorbic acid as a standard. The antioxidant activity was found more in the methanol extracts show comparable activity with ascorbic acid. In the metal chelating activity the petroleum ether show maximum activity when compare with chloroform and methanol.
Conclusion: The leaves extracts of *Epiprinus mallotiformis* possess antioxidant and anthelminthic property. Further investigations on *in vivo* antioxidant activity need to be carried out to unmask its mode of action.

Keywords: Epiprinus mallotiformis; anthelmintic; antioxidant; leaf extracts.

INTRODUCTION

People living in poverty in developing countries often suffer from helminthic infections, which more often physically impair their hosts then kill them. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travelers who have visited those areas and some of them can develop in temperate climates [1]. As an important component of complementary and alternative medicine, traditional Ayurvedic medicinal plants may be useful model for the discovery and development of new chemical substances for helminth control which are generally considered to be very important sources of bioactive substances [2]. Helminthiasis, or infection with parasitic worms, affects over two billion people worldwide. Human beings can spread these pathogens to previously uninvolved population through travel, migration, and military operations.

Worms pathogenic for human beings are Metazoa, classified into roundworms (nematodes) and two types of flatworms, flukes (trematodes) and tapeworms (cestodes). These biologically diverse eukaryotes vary with respect to life cycle, bodily structure, development, physiology, localization within the host, and susceptibility to chemotherapy. Immature forms invade human beings via the skin or gastrointestinal tract and evolve into well-differentiated adult worms that have characteristic tissue distributions. With few exceptions, such as *Strongiloides* and *Echinococcus*, these organisms cannot complete their life cycles, that is they replicate themselves, within the human host. Therefore, the extent of exposure to these parasites dictates the severity of infection, and reduction in the number of adult organisms by chemotherapy is sustained unless reinfection occurs. The prevalence of parasitic helminthes typically displays a negative binomial distribution within an infected population such that relatively few persons carry heavy parasite burdens. Without treatment, those individuals are most likely to become ill and to perpetuate infection within their community [3].

Antihelmintics are drugs that either kill (vermicide) or expel (vermifuse) infesting helminths. *Helminthiasis* is prevalent globally (One third of world's population harbors them), but is more common in developing countries with poorer personal and environmental hygiene. Multiple infestations in the same individual are not infrequent. In the human body, gastrointestinal tract is the abode of many helminths, but some also live in tissues or their larvae migrate into tissues. They harm the host by depriving him of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toxins. *Helminthiasis* is rarely fatal, but is a major cause of ill health [4].

Free radicals are found to be a product of normal metabolism. Although oxygen is essential for aerobic forms of life, oxygen metabolites are highly toxic. As a consequence, reactive oxygen species (ROS) are known to be implicated in many cell disorders and in the development of many diseases including cardiovascular diseases, atherosclerosis, chronic inflammation etc [5, 6]. Although organisms have endogenous antioxidant defenses produced during normal cell aerobic respiration against ROS, other antioxidants are taken both from natural and synthetic origin [7]. Antioxidants that can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction, therefore, appear to be very important. Synthetic antioxidants are widely used but their use is being restricted nowadays because of their toxic and carcinogenic effects. Thus, interest in finding natural antioxidants, without any undesirable effect, has increased greatly [8].

Epiprinus mallotiformis Muell. belongs to the family Euphorbiaceae, it is distributed throughout Western Ghats, South Canara, Coorg, Niligiris, Kigga, evergreen and semi evergreen forest of Karnataka [9, 10] The plant is traditionally used in traditionally used to treat the diuretic, digestive problems, dysentery, external wounds, antimicrobial, laxative, remedy for vesical calculi, ulcers, gonorrhea

etc. The present study is carried out the anthelmintic activity of leaf extracts of *E. mallotiformis*.

MATERIALS AND METHODS

Plant material

The leaves material of *E. mallotiformis* were collected from the Agumbe (13° 30′ N 75° 05′ E, 2154 Ft), Shimoga district of Karnataka. The region comes under malnad region, receives the maximum rain during the South West Monsoon. The samples were authenticated and herbarium was kept in the Department of Applied Botany Kuvempu University Shankaraghatta, Shimoga district of Karnataka. Leaf material was cleaned and air dried, then powdered for future work.

Preparation of extract

The powdered material was subjected to soxhlet extraction with various solvents ranging from non-polar to polar. The solvents used were Petroleum ether, chloroform and methanol. Each time before extraction with next solvents the marc was air-dried. All the extracts were concentrated by rotary flash evaporator. Three different solvent extracts was selected for anthelmintic and antioxident activity on the basis of phytochemical screening and literature survey.

Animals

Indian adult earthworms (*Pheritima posthuma*) were used to study anthelmintic activity. The earthworms were procured from the Department of Horticulture Shimoga, Karnataka collected from moist soil and washed with normal saline to remove all fecal matter earthworms were identified in the Department of Applied Zoology Kuvempu University Shankaraghatta, Shimoga district of Karnataka.

Anthelmintic activity

The anthelmintic assay was carried as per the method of [12] with minor modifications. The assay was performed on adult Indian earthworm, *Pheretima posthuma due* to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings [13-16]. Because of easy availability, earthworms have been used widely for the initial *in vitro* evaluation of antihelmintic compounds [17]. Three sets (petroleum ether, chloroform and methanol extract treatment) in seven groups were taken each containing six earthworms of approximately equal size (6.0 ± 1.0 cm). Piperazine citrate (10 mg/mL) was taken as standard drug. Leaves extracts of *E. mallotiformis* at different concentrations were prepared by dissolving in minimum quantity of DMSO and making upto the final volume with normal saline to obtain 20 mg/mL, 40 mg/mL, 60 mg/mL, 80 mg/mL and 100 mg/mL concentrations. One of the groups is taken as control group, which was treated with normal saline. Time for paralysis was noted when no movement of any sort could be observed except the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°C. All the test solution and standard drug solution were prepared freshly before starting the experiments [18].

Chemicals and Reagents for antioxidant activity

The drug piperazine citrate purchased from commercial sources and all other chemicals were of analytical grade. Nicotinamide adenine dinucleotide (NADH), butylated hydroxyanisole (BHA), l-ascorbic acid, nitroblue tetrazolium (NBT), phenazine methosulphate (PMS), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 3-(2-Pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), polyoxyethylene sorbitan monolaurate (Tween – 20) and trichloroacetic acid (TCA).

DPPH scavenging activity

Free radical-scavenging capacities of different extracts were determined according to the previously reported procedure, using the stable 2,2-diphenyl-1-picryhydrazyl radical (DPPH) [19].

Prepare the stock solution of extracts (1 mg/mL) and DPPH (0.004%) using 95% of methanol. Freshly prepared DPPH solution were taken in test tubes and extracts are to be added followed by serial dilutions (1 µg to 1000 µg) to every test tube so that the final volume will be 3 mL and after 10 min, the absorbance will be read at 517 nm using a spectrophotometer UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). Ascorbic acid has to be used as a reference standard and dissolve in distilled water to make the stock solution with the same concentration (1 mg/mL). Control sample prepared containing the same volume without any extract and reference ascorbic acid. 95 % methanol will serves as blank.

Metal chelating activity

The chelating of ferrous ions by the test sample and standard (EDTA) were estimated by the method of [20]. Briefly, add 3ml of test sample/standard (100-500 µg/ml) to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction will be initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixture has to be shaken vigorously and leave at room temperature for 10 min. Absorbance of the solution measured at 562 nm. The control contains $FeCl_2$ and ferrozine, complex formation molecules. The percentage of inhibition of ferrozine-Fe2+ complex formation calculated.

$$\text{Inhibition (\%)} = [(A_{control} - A_{test}) / A_{control}] \times 100$$

Where, $A_{control}$ is the absorbance of the control and A_{test} is the absorbance of the test sample /standard.

Total reductive capability

The reductive ability is determined according to the (21) method. Briefly, different concentrations of the test compound and the standard compound (Quercetin) in 1ml of methanol will be mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1 % potassium ferricyanide [$K_3Fe(CN)_6$]. Reaction mixture will be incubated for 20 min at 50oC. Then add 2.5 ml of 10 % trichloroacetic acid to the mixture, then centrifuge (650 rpm at room temperature) for 10 min. The mix upper layer of solution (2.5 ml) with 2.5 ml of distilled water and 0.5 ml of 0.1 % $FeCl_3$. Absorbance of the reaction mixture was read spectrophotometrically at 700 nm, increased absorbance of the reaction mixture indicates greater reducing power.

Hydrogen peroxide scavenging activity:

The ability of test sample to scavenge H_2O_2 will be determined according to [22] method. Prepare H_2O_2 in phosphate buffer (pH 7.4). Determine H_2O_2 concentration spectrophotometrically by measuring absorption with extinction coefficient for H_2O_2 of 81 M-1cm-1. Take test sample (200-600 µg/ml) in distilled water and add H_2O_2 solution (0.6 ml, 40 mM). Measure the absorbance of H_2O_2 initially and after 10 min against a blank solution containing the phosphate buffer without H2O2 at 230 nm.

RESULTS AND DISCUSSION

The methanolic leaves extract of the *Epiprinus mallotiformis* Muell showed significant anthelminthic activity compared to the other extracts. From the above study, it was observed that the ethanolic extract showed dose dependent anthelmintic activity as compared to a standard drug piperazine citrate (Table 1). The mean paralyzing time of *Pheritima posthuma* with the concentrations 20, 40, 60, 80 and100 mg/mL of petroleum ether extract were found to be 46.79, 45.60, 44.81, 43.25 and 40.34 mean respectively. Whereas in chloroform extracts 45.3, 44.04, 40.25, 39.28, 36.78 minutes. In methanol extract shows 35.17, 32.98, 31.54, 29.00, 25.98 minutes respectively. The standard piperazine citrate (10 mg/ml) was found to be 21.98 minutes. The mean Death time of *Pheritima posthuma* with the concentrations 20, 40, 60, 80 and100 mg/mL of petroleum ether extract were found to be 93.51, 92.00, 91.83, 85.93 and 83.51 minutes. Whereas, in chloroform 92.11, 91.86, 87.91, 86.57 and 84.91 minutes. In methanol extract shows 70.59, 69.63, 68.39, 66.84 and 65.11 minutes respectively. Death time of standard piperazine (10 mg/ml) citrate was found to be 59.92 minutes.

Table1: Anthelmintic activity of leaves methanolic extract against *Pheritima posthuma.*

	Petroleum ether		Chloroform		Methanol	
Concentration (mg/ml)	**TTP in minutes (Mean and SD)**	**TTD in minutes (Mean and SD)**	**TTP in minutes (Mean and SD)**	**TTD in minutes (Mean and SD)**	**TTP in minutes (Mean and SD)**	**TTD in minutes (Mean and SD)**
Control	-	-	-	-	-	-
Piperazine citrate 10 mg/ml	21.98±0.84	59.92± 0.80	21.98±0.84	59.92±0.80	21.98±0.84	59.92± 0.80
Methanolic extract 20 mg/ml	46.79±0.98	93.51± 1.67	45.3±1.09	92.11±0.75	35.17±0.72	70.59±1.19
Methanolic extract 40 mg/ml	45.60±1.33	92.00±1.59	44.04±0.68	91.86±0.65	32.98±0.59	69.63±1.06
Methanolic extract 60 mg/ml	44.81±1.10	91.83±0.82	40.25±0.63	87.91±0.99	31.54±1.26	68.39±0.91
Methanolic extract 80 mg/ml	43.25±1.45	85.93±2.06	39.28±1.07	86.57±0.71	29.00±0.66	66.84±0.98
Methanolic extract 100 mg/ml	40.34±1.01	83.51±2.04	36.78±0.90	84.91±0.73	25.98±0.69	65.11±1.04

Values of anthelminthic activity are represented as mean ± S.E.M., TTP = Time taken for Paralysis, TTD = Time taken for Death.

DPPH Scavenging activity

Antioxidant properties *E. mallotiformis* extracts were evaluated to find a new natural source of antioxidant. This assay is known to give reliable information concerning the antioxidant ability of the tested compounds [23]. The plant extracts were screed for DPPH radical scavenging activity according to the method described and the results of the screening are shown in Figure 1, as comparable with ascorbic acid, known antioxidant. The methanol extracts show comparable activity with ascorbic acid , but other extracts were not shows good activity when compare with the standard.

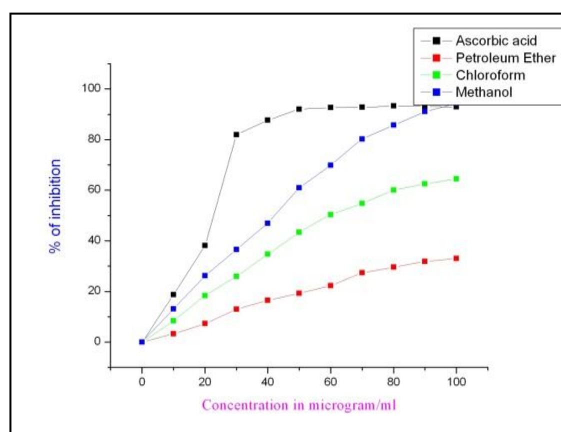

Figure 1: DPPH Scavenging activity on leaf extracts of *E. mallotiformis*

Hydrogen peroxide scavenging activity

Hydrogen peroxide gives rise to hydroxyl radicals. Removing hydroxyl radicals (●OH) is very essential for the protection of living system as they react with most biomolecules and other cellular components to cause tissue damage leading to cell death [24, 25]. In this present study, ability of the different plant extracts at different concentrations (200-600 µg/mL) to scavenge H_2O_2 was evaluated. Figure 2 shows the percentage H_2O_2 scavenging activity result by different concentrations of the plant extract and standard compound (ascorbic acid). The methanol extract showed comparable H_2O_2 scavenging ability when compared to standard. The percentage H_2O_2 scavenging effect by the same concentration (400 µg/mL) of the plant extract and ascorbic acid was found as 38.6 and 48, respectively. But other extracts were not shown good result when compared with the methanol extract.

Reducing power assay

Reducing power assay of sample extract compared to Ascorbic acid are shown in the Figure 3. For the measurements of the reductive ability, we investigated the Fe^{3+}–Fe^{2+} transformation in the presence of different plant extracts [21]. The antioxidant activity of potent antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [26]. The reducing properties are generally associated with the presence of reductones [27], which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [28]. The reductones present in the plant extracts react with certain precursors of peroxide, thus preventing peroxide formation. The result shows that methanol extract have significant reductive capacity when compare to other extracts.

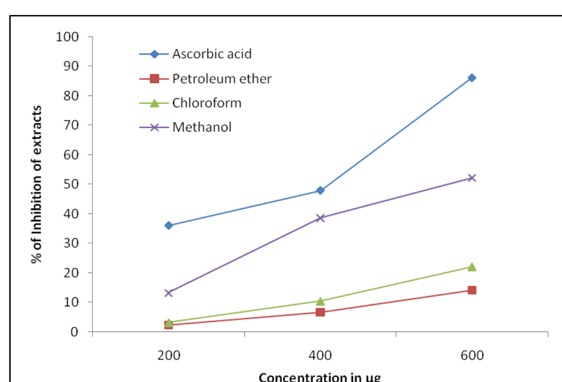

Figure 2: Hydrogen peroxide scavenging activity on leaf extracts of *E. mallotiformis*

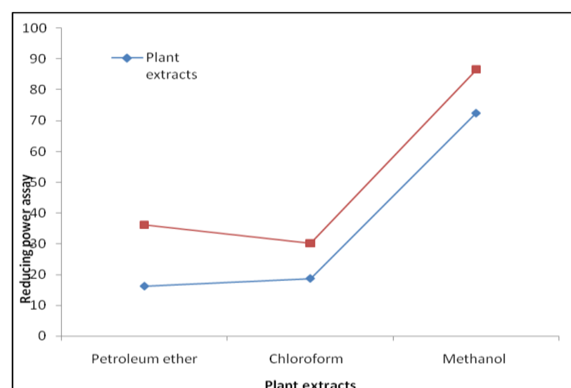

Figure 3: Reducing power assay on leaf extracts of *E. mallotiformis*

Metal chelating activity

Transition metals such as ion can stimulate lipid peroxidation of generating hydroxyl radicals through fenton reaction and accelerate lipid peroxidation into peroxyl and alkoxyl radicals therefore drive

the chain reaction of lipid peroxidation. Chelating agents may inhibit radical generations by stabilizing transition metals, consequently reducing free radical damage. In addition, some phenolic compounds exhibit antioxidant activity through the chelation of metal ions. Phenolic compounds may permit for bonding to metal ions due to their chemical structures. The chelating activity of plants was evaluated against Fe^{2+} for estimate the potential antioxidant activities of the methanol extracts. Petroleum ether extract of plants exhibited high metal chelating activity when compare to methanol and chloroform extracts shown in Figure 4. The chelating activity of pet ether was found to be 36.06 mM EDTAE/g whereas methanol and chloroform extracts have 18.03 and 9.83 mol EDTA /g kb respectively.

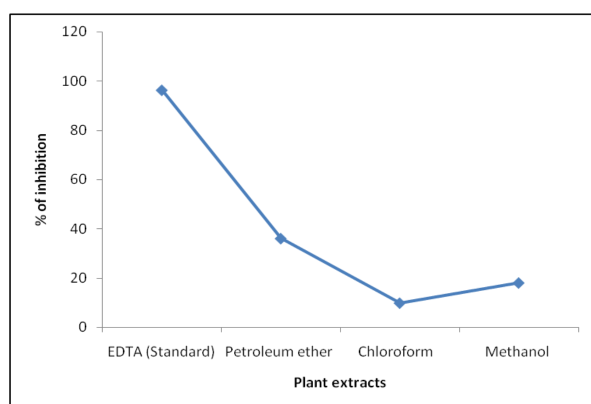

Figure 4: Ferrous metal ion chelating activity on leaf extracts of *E .mallotiformis*

CONCLUSION

From the above results, it is concluded that *E. mallotiformis*, showed significant anthelmintic activity and antioxidant activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional uses of this plant, rich in phytochemicals and highly valuable source of natural antioxidants and free radical scavengers. These extracts can increase the shelf life of food products, as well as, they can be used against damaging effects of free radicals and can inhibit degenerative disorders and carcinogenesis and delay aging. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for anthelmintic and antioxidant activity.

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