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Research Article

PHYTOCHEMICAL INVESTIGATION AND ANTHELMINTIC ACTIVITY OF *CELOSIA CRISTATA* LEAF EXTRACTS

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ABSTRACT
Development of anthelmintics from medicinal plants might be served as an alternative source for the conventional anthelmintic drugs. The present study was attempted to assess the possible anthelmintic activity for chloroform, methanol and aqueous extract of *Celosia cristata* leaves. Two different concentrations (100 mg/ml and 200 mg/ml) of these extracts were used to determine the paralysis and mortality of earthworms, *Pheretima posthuma* and the results were compared with standard anthelmintic drug, albendazole (100 mg/ml and 200 mg/ml). Worms placed in both aqueous and methanol extracts of *C. cristata* showed significant paralysis and leads mortality in dose dependent manner. Chloroform extract showed no significant activity against the worms. The results revealed that the aqueous extract had higher significant anthelmintic activity than methanol extract. The preliminary phytochemical analysis on the extracts showed the presence of alkaloids, flavanoids, triterpenoids, phenolic compounds and tannins, which might be responsible for the anthelmintic activity.
Keywords: *Celosia cristata*, Extracts, *Pheretima posthuma*, Anthelmintic, Paralysis

INTRODUCTION

Prior to the earliest history, helminthes which are also well-known as parasitic worms had been infecting humans[1] and animals. Those parasites also have caused farm and domestic animals businesses to run on loss by impacting the livestock and the animals. Parasitic worms usually cause gastrointestinal infections to mammals. In this era, the common helminthes which affects human are tapeworms, hookworms, roundworms and flukes[2]. Approximately two billion people around the world are infected with parasites according to a survey done by the World Health Organization (WHO). Ideally, an anthelmintic should stand with a broad spectrum of action, cost effective, cause less toxic to the host, and should destroy most of the parasites in a single dose[2]. Nevertheless, these criteria generally are not met by synthetic anthelmintics. This is further supplemented with the resistance issue faced by the synthetic drugs and raises the search for newer anthelmintics[3]. Most of the plants in nature withhold variety of medical values. Medicinal plants like *Ocimum sanctum*[4], *Mimusops elengi*[5] and much more have been studied by researches for their anthelmintic activities based on their folkloric claims.

Malaysia owns a high affluence of flora which withholds a variety of medicinal secrets. The plant, *Celosia cristata* is one of the valuable assets, which belongs to the Amaranthaceae family and commonly known as cockscomb. Locally, it is called as “balung ayam” and “Chi kuan” in Chinese. These non-woody plants often grow up to 1 foot in height, but mostly are smaller in size. It is grown commonly in Africa, South America, India and some parts of Asia. In India, the plant’s leaves are edible[6] and flowers serves as an astringent which are used to treat bloody stool, hemorrhoid bleeding and diarrhea; the seed decoction is used to treat dysentery[7]. The leaves are also utilized in Chinese medicine for the treatment of dysentery, menstrual bleeding, inflammation and against worms[8]. The *C. cristata* seeds were reported to be hepatoprotective[9]. The plant was also found to have anti aging and antioxidant[10] properties as well.

Since there are no scientific reports of anthelmintic studies on *C. cristata* leaves, the present study was attempted to evaluate the anthelmintic activity of various extracts of *C. cristata* leaves to confirm its folkloric claim as a parasiticide[8].

MATERIALS AND METHODS

Collection and authentication of plant

C. cristata plants were collected from Sungai Buloh, Selangor, Malaysia on November 2011. The plant was authenticated by Dr. M. Sugumaran a/l Manickam, Institute of Biological Sciences, Faculty of Science Building, University of Malaya, Malaysia.

Preparation of extracts

The collected *C. cristata* leaves were washed thoroughly and shade dried at room temperature. The dried leaves were size reduced into coarse powder (1000g). The coarse powder were divided into three portions and macerated with distilled water, methanol and chloroform for 6 days[11]. The chloroform extract (CECC), methanol extract (MECC) and aqueous extract (AECC) of *C. cristata* were filtered separately and concentrated using rotary vacuum evaporator under vacuum and were kept in refrigerator till further use. The colour, consistency and percentage yield of the three extracts are shown in Table 1.

Preliminary Phytochemical Analysis

The preliminary phytochemical studies were conducted on *C. cristata* leaves extracts to find out the presence of various active compounds such as alkaloids, carbohydrates and glycosides, fixed oils and fats, flavonoids, mucilages, phenolic compounds, proteins, saponins, sterols, triterpenoids and tannins[12]. The results of preliminary phytochemical investigation are shown in Table 2.

Screening of Anthelmintic activity

Collection of worms

Adult earthworms (*Pheretima posthuma*) were collected from moist soil and washed with distilled water. These worms are commonly used to test anthelmintic activity as they possess resemblances both anatomically and physiologically with the intestinal roundworm parasites found in human beings[13-15].

Anthelmintic activity

The anthelmintic activity of all the extracts of *C. cristata* leaves were studied according to the method developed by

Tamby *et al* [16]. Earthworms of 3-5cm in length and 0.1-0.2cm in width were used. The worms were divided into nine groups (n = 6). Two different concentrations (100 and 200 mg/ml in distilled water) of each extract were prepared. Albendazole (100 and 200 mg/ml) was used as reference standard and distilled water was used as a control vehicle. Both the test solution and standard drug solution were freshly prepared and transferred into respective petridish. The selected earthworms were released into the corresponding petridish. Time for paralysis was noted when no movement of any sort could be observed except when the worms were vigorously shaken. The death time of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50°C. A maximum time period of 120 min was fixed for the paralyzing as well as death time of *Pheretima posthuma*. All experiments were done in triplicate.

Statistical Analysis

The mean ± SEM were analyzed statistically by ANOVA followed by Student's 't' test, the statistical analysis was conducted with SPSS software (v.19, SPSS, USA) at significant levels of 0.05 and 0.01[17-18].

RESULTS AND DISCUSSION

The color, consistancy and yield of extracts are shown in Table 1. The AECC showed a higher percentage yield (28.23%) than MECC (11.58%) followed by CECC (4.62%). The preliminary phytochemical constituent tests illustrated the presence of alkaloids, flavonoids, mucilages, triterpenoids, phenolic compounds and tannins (Table 2). The anthelmintic activity of chloroform, methanol and aqueous extract of *C. cristata* were studied against the adult earthworms, *Pheretima posthuma.* Time for paralysis and death of worms were noted (Table 3) to assess the anthelmintic activity of the extracts of *C. cristata.* The results of anthelmintic activity of the extracts were well compared with standard drug, albendazole (100mg/ml and 200mg/ml). The results demonstrated that AECC and MECC extracts exhibited high significant paralysis and also caused death to worms in dose dependent manner at the concentration of 100mg/ml and 200mg/ml. The worms treated with CECC were not responding significantly at both concentration and also no mortality was observed until 120min.

The results illustrated that the significant anthelmintic property of AECC and MECC might be due to the presence of alkaloids, tannins, phenolic compounds[19-21], flavanoids and triterpenoids[22]. Earlier studies reported that alkaloids present in extracts could be the reason for paralysis of *Pheritima posthuma* worms[19] and some synthetic phenolic compounds like niclosamide and bithinol could pharmocologically interferes with the energy generation of parasites by uncoupling oxidative phosphorylation[21]. Another study stated that tannins might have the anthelmintic properties by binding with free proteins in the gastrointestinal tract of the host animal or the parasite's cuticle and cause death[20]. Olenanae type of triterpenoid saponins, gymnemic acids reported in earlier studies[23] also supported the present study. From these findings, a high significant anthelmintic activity of AECC and MECC could be due to triterpenoids and tannins. Since these bioactive constituents were not identified in CECC, no significant anthelmintic activity was exhibited.

CONCLUSION

The results obtained proved the folkloric claim of *Celosia cristata* leaves for the treatment of helminthiasis. The study could become a stepping stone to rationalize the new findings in developing the possible mechanism for its anthelmintic activity. Further research on phytochemical components in the selected plant will be needed to elucidate the structure of respective phytoconstituent and its mechanism at molecular level which will be very useful for the discovery of newer antihelmintics.

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Table 1: Colour, consistency and percentage yield of *C. cristata* leaves extracts

Extracts	Colour	Consistency	Yield (%)
CECC	Greenish Brown	Powder	4.62%
MECC	Dark Green	Powder	11.58%
AECC	Dark Brown	Solid	28.23%

Table 2: Preliminary Phytochemical Analysis of *Celosia cristata* leaves extracts

Chemical Constituents	CECC	MECC	AECC
Alkaloids	+	+	+
Carbohydrates & glycosides	-	-	-
Fixed oils & fats	-	-	-
Flavonoids	+	+	+
Mucilages	+	+	-
Phenolic compounds & Tannins	-	+	+
Proteins	-	-	-
Saponins	-	-	+
Sterols	-	-	-
Triterpenoids	-	+	+

+ = present; - = absent

Table 3 Anthelmintic Activity of various Extracts of *Celosia cristata*

Treatment	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Control	-	-	-
Albendazole	100mg/ml	5.67 ± 0.33**	7.67 ± 0.33**
	200mg/ml	4.67 ± 0.33**	5.67 ± 0.33**
AECC	100mg/ml	6.7±0.588**	10.67 ±1.33**
	200mg/ml	6.33± 0.33**	6.00 ± 0.00**
MECC	100mg/ml	10.67±0.33*	12.67± 0.88*
	200mg/ml	9.00±0.58*	10.67± 0.88*
CECC	100mg/ml	59.67± 0.88	-
	200mg/ml	59.67± 1.45	-

Values are mean ± SEM; (n = 6).
*P<0.05, **P<0.01 are considered for significance

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