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Qualitative Tests for Preliminary Phytochemical Screening and Antihelmintic Activity of Root Extract of *Crossandra infundibuliformis*

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Abstract

Crossandra infundibuliformis is native to South India and Srilanka. It is a traditional plant having beneficial activities with fewer side effects. The promising effects of the plant includes Anticancer, Antibacterial, Antifungal, Antihelmintic, insecticidal, wound healing properties etc. The active constituents of the plant are alkaloids, flavonoids, phytosterols, and phenolic compounds. In the present study, the root extract was evaluated for antihelmintic activity and the plant showed promising effect at 200mg/kg. Initial phytochemical screening was also done in ethanol, chloroform and acetone extracts. The major constituents in all the three extracts are alkaloids, flavonoids and tannins.

Keywords: *Crossandra infundibuliformis, root extracts, antihelmintic activity*

1. Introduction

In Infectious diseases are an important health hazard all over the world, both in developing and developed countries (Sasikumar *et al.*, 2003). Even though pharmacological industries have produced a number of new antibiotics in the last three decades resistance to these drugs by microorganisms has increased (Nascimento *et al.*,2000). There is an urgent need to alternative medicine for ailments. The increasing interest on traditional ethono medicine may lead to discovery of novel therapeutic agents. Medicinal plants are finding their way into pharmaceuticals, neutraceuticals, cosmetics and food supplements. The World Health Organization (WHO) estimated Preparation of extracts that 80% of the population of developing countries still relies on traditional medicines, mostly plant drugs, for their primary health care needs (Mohanasundari *et al.*, 2007). The medicinal value of these plants lies in some chemical that produce a definite physiological action on the human body.The most important of these bioactive constituents of plants are alkaloids, flavonoids and phenolic compounds (Dhandapani *et al*.,2008). *Asteracantha longifolia (L.) Nees, Psassiflora edulis Sims, Berberis tinctoria Lesch, Sphaeranthus indicus Linn.* and *Solanum trilobatum Linn.* are used in traditional medicine of India. Several studies investigate the fundamental scientific bases for the use of some Indian medicinal plants by defining and quantifying the percentage of crude phytochemical constituents present in these plants.

The use of medicinal plants as a source of relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind. Neanderthals living 60,000 years ago in present day Iraq used plants such as hollyback, these plants are still widely used in ethno medicine around the world (Stockwell, 1988). The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 2,50,000-5,00,000 plant species only a small percentage has been investigated phytochemically and the

fraction submitted to biological or pharmacological screening is even smaller. Thus, any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics (Kroschwitz and Howe-Grant, 1992). Even now, contrary to common belief, drugs from higher plants continue to occupy an important niche in modern medicine. On a global basis, at least 130 drugs, all single chemical entities extracted from higher plants, or modified further synthetically, are currently in use, though some of them are now being made synthetically for economic reasons (Newman et al., 2000).

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava et al., 1996). A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of the raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries (Uniyal et al., 2006). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of them not been adequately evaluated (Balandrin et al., 1985). *Crossandra infundibuliformis* is one of the perennial crops which occupies in the same rhizosphere soil for more than years and remove most of the available nutrients from the rhizosphere soil and leads to pour content of nutrients particularly phosphorus.

Helminth infections are the most common infections in man, and exaggerated worldwide population. It may cause anemia, eosinophilia, pneumonia and prevalence of malnutrition (Bundy DA. *et al*., 1994). Helminthiasis is the most common infection caused by worms that is contaminant to human body parts. Normally, the worms not only live in the gastrointestinal tract but may also reside into liver and other organs. When infected people excrete faeces with helminth eggs, the soil in the areas with poor sanitation will be contaminated (Idika IK et *al*.,2012). There are two clinically important types of worm infections, one is the worms live in the host's alimentary canal and the other is worms live in other tissues of the host's body. Tapeworms or cestodes (*Taenia saginata, Taenia solium, Hymenolepis nana, Diphyllobothrium latum*) and intestinal roundworms or nematodes (*Ascaris lumbricoides, Enterobius vermicularis Trichuris trichiura, Strongyloides stercoralis, Necator americanus, Ankylostoma duodenale*) are live in the host's alimentary canal while trematodes or flukes (*Schistosoma haematobium, Schistosoma mansoni, Schistosoma japonicum*), tissue roundworms (*Trichinella spiralis, Dracunculus medinensis*) and hydatid tapeworm (*Echinococcus species*) are live in the host's tissues[3]. Several nematodes that usually live in the gastrointestinal tract of animals may communicate a disease to humans and penetrate tissues. A skin infestation, termed creeping eruption, is caused by the larvae of dog and cat hookworms. Toxocariasis is caused by larvae of cat and dog roundworms of the Toxocara genus (Dale MM *et al*., 2008).

Epidemiology Helminthiasis is the most common infection mainly caused by the helminths. It is observed in various tropical and subtropical areas, and it is also classified as neglected tropical diseases. They spread the majority of common parasitic infection of human in developing countries. Ascaris limbricoides, Trichuris tritura, Necator americanus, Ancylostoma duodenale, schistosomes and filarial worms cooperatively infect more than one billion people, rivaling AIDS and malaria (James WD *et al*., 2006). As the recent evaluations, over a billion people have been infected due to at least one helminth species in Asia, Africa, America and Su-saharan, which leads to severe morbidity, accompanied by persistent shortage, decreased efficiency, and poor socioeconomic development. Helminthiasis has immunomodulatory effects on the host cells, with implications for many affecting pathogens. In fact, in endemic areas, AIDS, malaria and tuberculosis are recognized to be caused by helminthiasis. In most cases, they can induce severe hypersensitivity reaction that leads to chronic allergic reactions called anaphylaxis (James WD *et al*., 2006).

Helminths have a complex life cycle that often links several species. Helminth infections are mainly caused due to improper sanitation. They enter by mouth in unpurified drinking water or in poorly cooked

meat from infected animals. It is also enter through the skin by a skin cut, an insect bite or even after swimming or walking on polluted soil. Humans are the primary hosts for the helminth infections and most of the worms reproduce sexually in the human host, producing eggs or larvae that pass out of the body and infect the secondary host. In some cases, the eggs or larvae may persevere in the human host and become encysted, enclosed with granulation tissue, giving rise to cysticercosis. This is characterized by encysted larvae in the muscles, viscera and more critically in the eye or the brain ((Dale MM *et al*., 2008; James WD *et al*., 2006)

These features are depending on the helminth species, intensity of infection, and host age. *Taenia solium* can cause not only neurocysticercosis but also mass lesions in brain. Chronic infection with Schistosoma causes granulomas, fibrosis, and inflammation of the spleen and liver. *Echinococcus granulosis* ingested eggs can cause life-threatening anaphylaxis if antigens are released from the cysts. Hookworm and schistosomiasis can infect pregnant women, cause neonatal prematurity and increase maternal morbidity and mortality (Christian P *et al*., 2004). Intestinal worms and schistosomes infection are observed in children at school age or younger as compared with any other age group patients. As a result, the young patients suffer from growth retardation, diminished physical fitness, and impairment in memory and cognition (Crompton DW *et al*., 2002)

Helminthiasis is among the most important animal diseases inflicting heavy production losses. Helminths are the most common infectious agents of humans in developing countries and produce a global burden of disease and contribute to the prevalence of malnutrition, anaemia, eosinophilia, and pneumonia. The disease is highly prevalent particularly in third world countries (Dhar, D.N., *et.al* 1982*)* due to poor management practices. However, increasing problems of development of resistance in helminths (Geert, S *et al*., 1995; Coles, G.C *et al.*, 1997) against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity. The plants are known to provide a rich source of botanical anthelmintics (Satyavati, G.V *et al.*,1976; Lewis, W.H., *et al.*, 1977) As we know very well, now a days the medicinal preparation available in the market from which most of them either not effective up to the mark or has to develop resistance resulting in reoccurrence again. Plant derived drug serve as a prototype to develop more effective and less toxic medicines.

Crossandra infundibuliformis (Acanthaceae family) is a plant which is important in horticulture. This plant is found abundantly in tropical areas such as South India and Srilanka. It reaches 2m in height and can withstand high temperature which makes it to survive in very high humidity. Due to its medicinal value, the various parts of this plant are used for many treatments (Shanmugam S *et.al.,)* Despite of studies that have revealed the efficiency of this plant as potential source of aphrodisiac activity, wound healing, anticorrosive activity, etc, there is a lack of information about the Antihelmintic activity of root extracts. Therefore, this study was proposed to investigate antihelmintic activity, and screening of phytochemical constituents in roots.

2. Materials And Methods

Procurement and authentication of plant

The roots of *Crossandra infundibuliformis* were collected from Vishakapatnam, Andhra Pradesh. The plant material was identified and authenticated by Dr.P.V.Prasanna, Plant Taxologist, Scientist F and HOD Professor, Ministry of environment, Botanical survey of India, Hyderabad, Telangana.

Preparation of Plant Extract

The leaves and stems of *Crossandra infundibuliformis* were collected and washed thoroughly with distilled water to make sure the roots are free of dust and are shade dried. The dried roots are then powdered finely using mechanical grinder. And then, required quantity of the powder is subjected to maceration with ethanol. The obtained extract is dried completely using desiccators. (Hunt J.V.*et al.,* 1988, Aruna P, *et al.,* 2007, and Ahmed S, *et al.*,2016)

Qualitative evaluation of Phytoconstituents of different extracts (Segelman A.B *et al*., 1969 and Siddiqui A.A., *et al*., 1997)

Test for Carbohydrates:
Fehling's test
0.5ml of Fehling's A was added to 0.5ml of Fehling's B solution and to this mixture; 2ml of EECC was added. The mixture was heated in boiling water for 5-10minutes and a yellow followed by a brick red precipitate shows the presence of carbohydrates.

Benedict's test
0.5ml of extract is taken in a test tube and to this; 0.5ml of Benedict's reagent was added. The mixture was boiled for 5 minutes and a brick red precipitate shows the presence of carbohydrates.

Molisch's test
2-3ml of extract was taken in a test tube and to this, few drops of -naphthol solution was added. The test tube was shaken and conc.H_2SO_4 was added from the walls. Violet ring at the junction of two liquids indicates the presence of carbohydrates.

Test for Saponins:
3ml of extract was taken in a test tube and was shaken vigorously and is kept aside for 3 minutes. Formation of honeycomb-like froth indicates the presence of saponins.

Test for Flavonoids:

Shinoda test
0.5ml of extract is taken in a test tube and to it; 0.5ml of dilute hydrochloric acid was added followed by the addition of few pieces of magnesium turnings. A pink or reddish pink color shows the presence of flavonoids.

Lead acetate test
To 1ml of extract, 1ml of lead acetate solution was added and a yellow color precipitate indicates the presence of flavonoids.

Sodium hydroxide test
To 200mg of extract, 2ml of dil. NaOH was added and formation of yellow color which gets decolorized on the addition of dil. HCl shows the presence of flavonoids.

Tests for steroids:
Salkowski reaction
To 2ml of extract, 2ml chloroform and 2ml conc. H_2SO_4 was added. Shake well. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence.

Liebermann-Burchard reaction
2ml of the extract was mixed with chloroform. 1-2ml of acetic anhydride was added and 2 drops conc. H_2SO_4 was added from the sides of the test tube. First red, then blue and finally green color appeared.

Test for Tannins and Phenolic compounds:
To 2ml of EECC, following reagents were added:
5% $FeCl_3$ solution ⟶ deep blue-black color
Lead acetate solution ⟶ White precipitate
Bromine water ⟶ Decoloration of bromine water
Dil. Iodine solution ⟶ Transient red color
Dil. HNO_3 ⟶ Reddish to yellow color

Test for Glycosides:
Legal's test

To aqueous or alcoholic extract, 1ml pyridine and 1ml sodium nitroprusside were added. Pink to red color appeared.

Test for Triterpenoids:
About 0.5 g of extract in a separate test tube was taken with 2 ml of chloroform; 5 ml of concentrated sulphuric acid was carefully added to form a layer and observed for the presence of reddish-brown color interface to show positive results for the presence of terpenoid.

Test for Proteins:
Biuret's test
1mlof hot extract was taken in a test tube and to it, add 0.5ml of w/v of sodium hydroxide and 0.1ml of 3% copper sulfate solution. A red or violet color shows the presence of proteins.
Millon's test
A small quantity of extract was treated with few drops of Million's reagent and observed for the formation of the white precipitate which indicates the presence of protein.

Test for Amino acids:
Ninhydrin test (General test)

3ml of extract was heated and to it 2-3 drops of 5% Ninhydrin solution was added. The mixture was kept in a boiling water bath for 10 minutes. The purple or bluish color indicates the presence of amino acids.

Test for Fats and Oils
The extract is taken on a filter paper and permanent stain on filter paper indicated the presence of fats and oils.

Test for Gums
Extract was hydrolyzed using dil. HCl. This solution was subjected to Fehling's test and red color indicates the presence of gums.

Antihelmentic Activity:

Helminthiasis is the most common infection caused by macro parasitic worms that is contaminant to human body parts. An antihelmintic drug can act by causing paralysis of the worm, or by damaging its cuticle, which lead to partial digestion or rejection by immune mechanisms. Most of the existing synthetic antihelmintic drugs produce serious adverse effects. In order to eliminate the harmful side effects of these synthetic antihelmintic drugs, it is important for us to promote the studies of traditionally used antihelmintic plant-based drugs. The ethanolic root extracts of *Crossandra infundibuliformis* was prepared by maceration method. The phytoconstituents like carbohydrates, glycosides, alkaloids, tannins, saponins and flavonoids were observed in the extracts. The antihelmintic activity was investigated on Indian earth worm (*Pheritima posthuma*) (Ajaiyeoba E. O. *et al*., 2001). Antihelmintic activity was performed in three different concentrations such as 20 mg/ml, 50 mg/ml and 100 mg/ml. Albendazole (10 mg/ml) was used as reference standard drug whereas distilled water as control. Determination of paralysis time and death time of the worms were recorded.

3. Results and Discussion

Preparation of Plants Extract

The roots of the plant were collected washed properly to remove the mud and dried to remove the moisture. The obtained dried roots are then powdered finely using mechanical grinder. And then, required quantity of the powder is subjected to maceration with ethanol. The obtained extract is dried completely using desiccators

Preliminary phytochemical screening

The percentage yield of the ethanolic root extract of *Crossandra infundibuliformis* was about 97.04%. The phytoconstituents present in the plant are found to be Phenols, tannins, cardiac glycosides, flavonoids, amino acids, carbohydrates, Proteins, saponins and alkaloids. The results are summarized in below.

Table (1): Preclinical phytochemical screening of extract of *Crossandra infundibuliformis.*

Phytochemical constituent	Ethanol	Acetone	Chloroform
Carbohydrates	+	-	-
Amino acids	+	-	-
Proteins	+	-	+
Alkaloids	+	-	+
Glycosides	+	-	-
Saponins	+	+	+
Phytosterols	-	+	+
Steroids	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Phenols	+	+	-

ANTIHELMENTIC ACTIVITY:

Extract exhibited more significant antihelmintic activity at the concentration of 100 mg/ml. The results reported in the present work shows evidence that the roots of *Crossandra infundibuliformis* possess antihelmintic activity.

STANDARD | EXTRACT 100mg/ml | CONTROL

Antihelmintic activity of the ethanolic root extract of *Crossandra infundibuliformis*

S.No	Concentration (mg/ml)	Time (min)	
		Death	Paralysis
1	40	20	82
2	60	15	75
3	80	14	40
4	100	10	35
5	CONTROL	NIL	NIL
6	ALBENDAZOLE (10 mg/kg)	4	50

4. Conclusion

Phytochemicals have attracted the attention of scientists due to the development of new and sophisticated techniques. These techniques play a significant role in giving the solution to systematic problems on the one hand and the search for additional resources of raw materials for compounds which can be shorted by the chemical class, bio synthetic origin on functional groups into primary and secondary metabolites. After thorough literature survey the plant was selected for its unique chemical constituents that are expected to show different activities. The aim of the present study was to investigate the presence of Phytochemical constituents of roots of ethanol, chloroformic and acetone extracts of *Crossandra infundibuliformis.* Our results clearly indicates that the presence of flavonoids, tannins, saponins and steroids. In the present study, it was found that ethanolic root extract of *Crossandra infundibuliformis* showed promising antihelmintic activity.The wormicidal activity of ethanol extract suggests that it is effective against parasitic infections of humans. Further, in future it is necessary to identify and isolate the possible active phytoconstitutents responsible for the anthelmintic activity and study its pharmacological actions

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