Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(5):260-264

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Invitro Anticancer and Insecticidal activity of *Crossandra infundibuliformis*

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ABSTRACT

Crossandra infundibuliformis is a popular tropical flower known as "Firecracker", It is a species of flowering plant in the family Acanthaceae, native to southern India and Sri Lanka. It is traditionally used for the treatment against various ailments in tropical and subtropical parts of India without any scientific knowledge. In the present study the anticancer and insecticidal activity of the ethanolic extract of the leaves of Crossandra infundibuliformis were investigated. Anticancer activity evaluated against human breast cancer cell line (MCF-7) by MTT assay method and the insecticidal activity was evaluated against Wheat weevil (Sitophilus oryzae). Both the activities were dose dependent. IC_{50} value of anticancer activity against MCF-7 cell line was found to be 404.66 µg/ml. Maximum insecticidal activity (100%) was attained at the concentration of 0.16% and 0.2% ethanolic extract of Crossandra infundibuliformis. Our results confirm that the ethanolic extract of Crossandra infundibuliformis exhibited significant effect against MCF-7 cell lines and Wheat weevil. Anticancer and Insecticidal activity lead some support to the use of Crossandra infundibuliformis for various ailments in the traditional medicine of India.

Keywords: Anticancer activity, MCF-7 cell lines, Insecticidal activity, Wheat weevil, *Crossandra infundibuliformis*

INTRODUCTION

The history of herbal medicines is as old as human civilization. The plants were used medicinally in China, India, Egypt and Greece long before the beginning of the Christian era. Drugs such as acacia, castor oil and fennel are mentioned along with apparent references to such compounds as iron oxide, sodium chloride, sodium carbonate and sulphur. Charaka made fifty groups of ten herbs each of them sufficient for an ordinary physician's need. Sushruta arranged 760 herbs in 7 distinct sets based on some of their common properties [1]. Medicinal plants are the source of great economic value in the Indian subcontinent. Herbal medicine is still the main source of medicine and about 75-80% of the whole population, mainly in developing countries depends on herbal medicines for their primary health care because of better cultural acceptability and better compatibility with the human body.

Crossandra infundibuliformis (*Acanthaceae*) is an important plant in horticulture [2]. It is abundantly present in tropical areas such as South India and Sri Lanka. The flowers of *C. infundibuliformis* are also referred as 'Tropical flame' or 'Firecracker' has been used as hairdo by the Puliyars tribal women. The leaf extracts of *Crossandra infundibuliformis* show aphrodisiac, anti-inflammatory and analgesic properties. The leaf extracts also reported for wound healing, antimicrobial, antioxidant, antisolar and larvicidal activities. Due to its medicinal value, this plant is used to treat various ailments. Phytochemical screening of various solvent extracts of *C. infundibuliformis* flower revealed the presence of carbohydrates, flavonoids, alkaloids, saponins, tannins, steroids and terpenoids

Cancer is a disease which occurs when changes in a group of normal cells within the body lead to uncontrolled growth, causing a lump called a tumor; this is true of all cancers except leukemia (cancer of the blood). If left untreated, tumors can grow and spread into the surrounding normal tissue, or to other parts of the body via the bloodstream and lymphatic systems and can affect the digestive, nervous and circulatory systems [3]. The Indian subcontinent is home to 16.5% of the world's population and it is estimated that there are over 2 million people with cancer. Presently in India, out of a million newly diagnosed cancer patients each year, more than 50% die within 12 months of diagnosis and another one million cancer survivors show progressive disease within five years of diagnosis. In 2005, cancer killed approximately 826,000 people in India; 519,000 under the age of 70. This is predicted to rise disproportionately compared with cardiovascular and communicable diseases by 2030, to nearly 1.5 million deaths annually. Based on the cancer registry data it is estimated that there will be about 8,00,000 new cancer cases in India every year.

Insecticides play an important role in the way food is grown and prepared these days. Insects, rodents and bacteria are capable of destroying the crop and contaminating the rations people consume. As a solution to control this problem, insecticides were manufactured. These products can be natural or chemical based. There are various types of insecticides that you can use depending on the nature of the problem. Without any control, the grains can be destroyed up to 100% of the stored commodity. There are three species of Sitophilus which are *S. oryzae* (wheat weevil), *S. zeamais* (maize weevil) and the *S. granarius* (granary weevil). All of them are the most important pests of stored grain in the world. *S oryzae* can infest many kinds of agricultural crop stores such as rice, wheat, corn, oat, barley, bean, nut, wild bird seed, rye, buckwheat, macaroni, starch, cereal products and all types of stored grains. *S. oryzae* is found in warm and tropical parts of the world. The aim of the present study was to investigate Anticancer activity and the insecticidal activity against human breast cancer cell line (MCF-7) and against Wheat weevil respectively.

EXPERIMENTAL SECTION

Plant collection

The leaves of *Crossandra infundibuliformis* were collected from the Southern Districts of Goa in the month of October-November. The plant *Crossandra infundibuliformis* was identified and confirmed by the botanist Dr. M. K. Janarthanam, Head, Department of Botany, Goa University, Goa, India.

Extract preparation

The leaves of the *Crossandra infundibuliformis* were shade-dried and pulverized to a powder in a mechanical grinder. The powder of the plant (1 kg) was extracted by maceration with solvent ethanol. The ethanol extract of the leaves of *Crossandra infundibuliformis* was used for the further studies.

Preliminary phytochemical studies

The ethanolic extract of the leaves of *Crossandra infundibuliformis* was screened for preliminary phytochemical constituents.

Anti-cancer activity

MCF-7 cell lines were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in DMEM and MEM respectively supplemented with 10% inactivated Fetal Bovine Serum (FBS), Penicillin (100 IU/ml), Streptomycin (100µg/ml) and Amphotericin B (5µg/ml) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India). Anticancer studies were performed as per Francis [4] *et al*, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM and MEM respectively supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serial two fold dilutions were prepared from this for carrying out anticancer studies**.**

The monolayer cell culture was trypsinized as and the cell count was adjusted to 1.0 x 105 cells/ml using DMEM and MEM respectively containing 10% FBS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100µl of different test concentrations of test drugs were added on to the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 3

days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations were noted every 24 h intervals. After 72 h, the drug solutions in the wells were discarded and 50µl of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values was determined.

$$\% \text{ Growth Inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{OD of Control group}} \text{ X } 100$$

Table.1.Preliminary Phytochemical Studies of *Crossandra Infundibuliformis*

Sr.no.	EXPERIMENT	OBSERVATION	INFERENCE
1	**TEST FOR STEROIDS:** Test solution (1 ml) + 10 ml $CHCl_3$ + 10 ml conc. H_2SO_4 by sides of test tube.	Upper layer turns red and H_2SO_4 layer showed yellow with green fluorescence.	Presence of Steroids.
2	**TEST FOR ALKALOIDS:** Test solution + 2N HCl. Aqueous layer formed decanted and to which are added 1-2 drops of Hager's reagent.	Yellow precipitate forms.	Presence of Alkaloids
3	**TEST FOR FLAVONOIDS:** Test solution (1 ml) + 1 drop of dil. NaOH + 1 drop of dil. HCl.	Yellow color produced becomes colorless on addition of dil. HCl.	Presence of Flavonoids
4	**TEST FOR SAPONINS:** Test solution + 20 ml H_20 + Agitation in graduated cylinder for 15 minutes.	1cm layer of foam forms.	Presence of Saponins
5	**TEST FOR TANNINS**: Test solution (5 ml) + drop of 1% lead acetate.	Yellow precipitate forms.	Presence of Tannins
6	**TEST FOR TERPENOIDS (SALKOWSKI TEST):** Plant extract (0.5g) + 2 ml $CHCl_3$ + 3 ml conc. H_2SO_4 carefully added to form layer.	A reddish brown coloration of the interface.	Presence of Terpenoids
7	**TEST FOR REDUCING SUGAR:** Test solution + 2 ml of Fehling solution A + Fehling solution B + 3 ml of H_20 + Boil.	Red or orange color precipitate forms.	Presence of Reducing Sugars.
8	**TEST FOR CARBOHYDRATES:** Test solution + 1 ml of H_20, Shake to get a clear solution + 2 drops of alcoholic α-napthol + conc. H_2SO_4 along the side of the test tube.	Purple ring at the junction forms.	Presence of Carbohydrates.
9	**TEST FOR PROTEINS:** Test solution + 1% NaOH + Few drops of Cu (II)SO_4.	Purple color.	Presence of proteins.
10	**TEST FOR GLYCOSIDES** The plant ethanolic extract is dissolved in Pyridine and a few drops of 2% Sodium Nitroprusside together with a few drops of 20% NaOH.	Deep red color.	Presence of glycosides.

Insecticidal activity

The Insecticidal activity was performed according to the Chandra Shekhar Pathak [5] *et al* method. Film residue method was used to test the mortality of the adults of *Sitophilus oryzae*.

For testing mortality, nine doses were used, including control and standard. Five insects were used at 0.01, 0.02, 0.04, 0.08, 0.12, 0.16 and 0.2% concentrations. The ethanol extract was weighed and dissolved in acetone for dosing. The doses were prepared by mixing the requisite quantities of the extract with acetone. After mixing properly the liquid was dropped in a petri dish. After drying at room temperature, five insects of Wheat weevil were released in each Petri dish. Mortality was assessed after 24, 36, 48, 60 and 72 hours of the treatment. Commercially available DDT powder was used as a standard substance in the experiment. The calculation of mortality rate was corrected for control mortality, according to Abbott'sformula:

$$\% \text{ of Mortality} = \frac{\text{No. of dead insects}}{\text{No. of insects introduced}} \text{ x } 100$$

RESULTS AND DISCUSSION

India is one of the most promising regions for discovering novel biologically-active substances from its flora. More efforts are needed to explore potent anticancer and insecticidal plants from the mother earth and save humans around the world.

Preliminary phytochemical studies

The various phyto-chemical tests performed are presented in Table.1 and the results were presented in Table.2.

Table.2. Preliminary phytochemical studies of the leaves of *Crossandra Infundibuliformis*

S.No	Compound	Ethanol extract
1	Steroids	+
2	Alkaloids	+
3	Flavonoids	+
4	Saponins	+
5	Tannins	+
6	Terpenoids	+
7	Reducing sugar	+
8	Carbohydrates	+
9	Proteins	-
10	Glycosides	-

(+) present and (–) absent

The ethanolic extract showed the presence of Steroid, Alkaloid, Flavonoid, Saponins, Tannins, Terpenoids, Reducing Sugar, Carbohydrates.

Anticancer activity

In-vitro anti-cancer activity of ethanolic extract of *Crossandra infundibuliformis* against human breast cancer cell line (MCF-7) using MTT assay method was presented in Table.3.

Table.3 *In-vitro* Anticancer properties of the ethanolic extract of the leaves of *Crossandra infundibuliformis* against MCF-7 cell line

S. No.	Test conc. (µg/ml)	% cytotoxicity	%cell viability	CTC_{50} (µg/ml)
1.	60	11.76470588	88.24	404.66
2.	50	8.345902464	91.65	
3.	40	5.83207642	94.17	
4.	30	4.172951232	95.83	
5.	20	2.513826043	97.49	
6.	10	1.005530417	98.99	

The present study shows a dramatic *In-vitro* anticancer activity of ethanolic extract of the leaves of *Crossandra infundibuliformis* on human breast cancer cell line (MCF-7) at increasing concentrations. CTC_{50} was found to be 404.66µg/ml. Phyto-constituents such as triterpene, flavonoids and tannins are biologically active against different strains of bacteria and many human cancer cell lines [6]. The presence of these phytoconstituents may be responsible for the anticancer activity of *Crossandra infundibuliformis*

Insecticidal activity

The plant extract showed the potent insecticidal activity against the storage pest *Sitophilus oryzae*. The ethanol extracts showed the dose dependent response. The maximum mortality of 100% was achieved at the concentrations of 0.16% and 0.2% (Table.4). Even the least concentration of 0.01% showed the moderate mortality of 20%. The standard also showed the maximum mortality of 100%.

The insecticidal activity of saponins is due to the interaction with cholesterol, disturbance in ecdysteroid synthesis, protease inhibition or cytotoxicity of insects [7]. Therefore, the leaf extract of *Crossandra infundibuliformis* is a promising option for developing pesticides to manage *Sitophilus oryzae* being natural insecticide it is active at highly acceptable levels, biodegradable and do not leave toxic residues while the commonly used phosphorous and chlorinated insecticides contaminate the environment. The Insecticidal activity of *Crossandra infundibuliformis* may be due to the presence of flavonoids and saponins.

Table.4: *In-vitro* Insecticidal activity of ethanolic extract of the leaves of *CrossandraInfundibuliformis* against *Sitophilus oryzae*

S.No.	Sample Con (%)	Number of insects added	NUMBER OF INSECTS DEAD					% of mortality at 72 h
			24 h	36 h	48 h	60 h	72 h	
1	CONTROL	5	0	0	0	0	0	0
2	0.01	5	0	0	0	0	1	20
3	0.02	5	0	0	0	1	2	40
4	0.04	5	0	0	0	2	2	40
5	0.08	5	1	1	2	3	4	80
6	0.12	5	2	2	2	4	4	80
7	0.16	5	3	3	4	5	5	100
8	0.2	5	3	4	4	5	5	100
9	STANDARD	5	5	5	5	5	5	100

CONCLUSION

This study suggests that the ethanolic extracts of the leaves of *Crossandra infundibuliformis* possess significant *In-vitro* anticancer and insecticidal effect with increasing concentrations. It is anticipated that this plant would be a useful pharmaceutical material to treat breast cancer. It also could be a potential grain protectant against *Sitophilus oryzae*. The present investigations give the evidence that it may be a fruitful medicine of tomorrow. Future research should focus on the molecular mechanism of *Crossandra infundibuliformis*. There is a need for further investigation of this plant in order to identify and isolate its active principle(s) to treat Cancer and Insect infestations.

Acknowledgement
The authors are grateful to Dr. Kishore G. Bhat, Department of Microbiology Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belgaum for his valuable helps to do the anticancer activity. We would like to thank Research Centre and the Management of Dnyanprassarak Mandal’s College for their encouragement and financial support in carrying out the work.

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