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EVALUATION OF PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF *CROSSANDRA INFUNDIBULIFORMIS (L) NEES* FLOWER EXTRACT

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ABSTRACT

Phytochemical 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 pharmaceutical industries on the other hand the plant synthesizes a wide variety of chemical compounds which can be shorted by the chemical class, bio synthetic origin on functional groups into primary and secondary metabolites. The aim of the present study was to evaluate *Crossandra infundibuliformis* (Acanthaceae family) for its antibacterial activity and phytochemical constituents. The flowers of *C. infundibuliformis* were screened for antibacterial activity and antifungal activity. Aqueous, acetone, methanol and chloroform extract. Methanol extract exhibited inhibition zone against all the pathogens comparable to the standard (ciprofloxacin and Grisefulvin). Phytochemical analysis showed the presence of flavonoids, saponins, terpenoids, cardiac glycoside, reducing sugars and tannins. Antimicrobial activity was confirmed by the presence of zone of inhibition. Due to the promising results obtained by this study; the range of avenues can be developed for drug development giving high hopes to scientific community to serve humanity with utmost benefiting drugs with little or no side effects. Hence, leaves extract of *C.Infundibuliformis* could be proved as a potential drug component if designed in a hybrid manner along with allopathic drugs or a standalone drug formulation to subsidize/ nullify the side effects of allopathic drugs.

KEYWORDS: *Crossandra infundibuliformis*, Flower extracts, Antibacterial activity, Phytochemicals.

INTRODUCTION

Since the 20th century, scientific research has developed a large number of drugs in an effort to battle various infectious diseases, starting with the discovery of antibiotics. The phenomena of pathogenic microbes co-evolving with drugs that either kill or hinder them is clearly caused by "changing" nature. Resistance is thus a major problem that the scientific community must deal with in order to create novel drug formulations (combinations of two or more) to combat the potentially potent microorganisms.[16] In order to control the spread of resistant bacteria throughout the world, the scientific community is currently being forced to investigate safe, effective, yet non-toxic alternative medications due to a discernible increase in resistance development against current therapies. In an attempt to find a solution, researchers are focusing on medicinal plants. These plants have a high concentration of phytochemicals and a variety of chemical structures, which could make them effective antimicrobial formulations if thorough in-vitro research is done and accompanied by encouraging clinical trials. Numerous examples exist in the literature regarding the application of herbal medicine to cure infectious diseases; this practice is thought to be inherent indigenous knowledge connected to a certain community or plant origin.[16] Crossandra The decorative flowering plant Infundubiliformis is used to spruce up the kitchen. Research has shown that it has antibacterial properties, which are backed by the people's apparent use of it as a source of traditional wisdom. As a medicinal plant, it has been shown to combat a range of nosocomial infections brought on by human pathogens by extracts prepared from various plant components using various solvents.[17] The aim of this study is to determine the antibacterial characteristics of C. infundibuliformis, namely the leaf extracts that are applied to a range of test organisms and made using different solvent systems.

The plant Crossandra Infundibuliformis belongs to the Acanthaceae family. There is evidence that this herb has several medicinal use. "Firecracker" is the name given to the well-known tropical flower Crossandra Infundibuliformis.[1] The plant is native to Southern India, Malaysia, and Sri Lanka.[2] Because of the plant's many medicinal qualities, its various parts are utilized to treat a variety of diseases. Antimicrobial compounds found in many plant components have a wide range of medical uses. They mitigate many of the negative effects commonly associated with synthetic antimicrobials, while also effectively treating infectious

diseases.[1,2] When male rats are exposed to ethanol-induced testicular toxicity, the leaf extract of C. infundibuliformis exhibits aphrodisiac properties.[3] Medicinal plants possess active chemicals that have the potential to treat a wide range of human ailments. According to the current study, phytochemicals help in drug molecule formulation and drug discovery. There are medicinal benefits to phytochemicals such terpenoids, flavonoids, and sugars.[4]

Antimicrobials derived from plants are a hugely unexplored medical resource.

Antimicrobial compounds of plant origin have enormous therapeutic potential. They are effective in the treatment of variety of infectious diseases.[5] The aqueous and methanol extracts of *C.Infundibuliformis* leaves possess antimicrobial activity tested against some common bacterial and fungal pathogens.[6] The evidence suggests that the leaf extracts of *C.Infundibuliformis* possesses antibacterial, antifungal & anticandidal activities.[7] It is an erect, evergreen sub-shrub growing to 1 m with glossy, wavy-margined leaves and fan-shaped flowers, which may appear at any time throughout the lifetime.[8] The flowers are irregularly shaped with 3 to 5 asymmetrical petals. They grow from four-sided stalked spikes, and have a tube-like 2 cm stalk. Flower colors range from the common orange to salmon-orange or apricot, coral to red, yellow and even turquoise.[8] This plant requires a minimum temperature of 10°C and in temperate regions it is cultivated as a houseplant. It is usually grown in containers but could be attractive if grown in beds. The flowers have no perfume but stay fresh for several days on a bush. A well-tended specimen will bloom continuously for years. It propagates either by seeds or cuttings. The tiny flowers are often found strung together into strands, sometimes along with white jasmine flowers and therefore in a great demand for making garlands which are offered to temple deities or used to embellish women's hair.

Kingdom: Plantae

Order: Lamiales

Family: Acanthaceae

Genus: Crossandra

Species: C.infundibuliformis

Common name: Aboli, Ambol

MATERIALS AND METHODS

Collection of Plant material

The fresh flowers of *Crossandra infundibuliformis* were purchased from local market. The plant was identified and authenticated by the Tamilnadu Agricultural university, Coimbatore. The flowers were collected and healthy flowers were shade dried and then powdered using electric blender to get a coarse powder. All solvents used were grade ethanol, chloroform, acetone were obtained from college store.

Extraction

The powdered material was extracted with solvents like chloroform, acetone, methanol and water by cold maceration process. The extract were prepared by taking 30g of dried flower powder in separate containers and to this 200ml of each solvent was added and kept in a shaker for 24hrs. The extracts were collected by filtered through 5 layers of muslin cloth. The extraction was repeated twice. Then the collected filtrates were pooled, concentrated and dried at a mild temperature.

Phytochemical screening

The phytochemical screening for the extracts was carried out by standard protocolsAlkaloids (Mayer's test), Glycosides (Legal's test), Saponins (Froth formation test), Carbohydrates (Molisch's test), Proteins (Xanthoproteic test), Aminoacids (Ninhydrin test), Flavanoids (Lead acetate test), Steroids (salkowski test), Tannins (Ferric chloride test), Volatile oils (hydro distillation method), Were analysed.

Test for Carbohydrates: (MOLISCHS TEST)

To the test solution add 1ml of water and shake well to get a clear solution.add2drops of alcoholic napthol and conc. sulphuric acid on the sidewalls of test tube. violet ring formed at the junction of 2layers indicates the presence of carbohydrates.

Test for Steroids (Salkowski Test)

10mg of extractvwas dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a low layer. A reddish brown colour at the interference indicate the presence of steroids.

Test for Cardiac Glycoides(Legal's Test)

The extracts was dissolved in pyridine and a few drops of 2% Sodium nitroprusside with 20% NaOH was added. deepred colour indicate the presence of cardiac glycosides.

Test for Flavonoids

To 1ml of the extract, a few drops of dilute NaOH were added. A intense yellow colour was produced, which became colourless on addition of few drops of dilute acid indicating the presence of flavonoids.

Test for Alkaloids(Mayers's Test)

A few drops of Mayer's reagent were added to 2 ml of the extract. formation of pink yellow precipitate indicate the presence of alkaloids.

Test for Tannins

To 1ml of extract, a few drops of 1% lead acetate was added. Yellow precipitate formed indicate the presence of tannins.

Test for Saponins

1ml of extract was diluted with 5ml of distelled water and it was agitated for 10mins. Formation of foam showed the presence of saponins.

TLC ANALYSIS

Silica gel plate was prepared (layer thickness 0.20mm). The lower end was marked with a slim line with a HB pencil to identify spotted end. 1mg of the extract was dissolved in 1ml of methanol as solvent. 0.5ml was drawn in a capillary and spotted in the labelled spots. The plate were allowed to run in the mobile phase (ethyl acetate: n- butanol: formic acid: water) (10:6:2:2 v/v/v/v) vanillin sulphuric acid reagent was used as spray reagent for colour, development reaction of tannins The spot were located on the plate under uv lamp. The Rf values were calculated.

$$\text{Rf value} = \frac{\text{Distance travelled by Solute}}{\text{Distance travelled by Solvent}}$$

Antibacterial and Antifungal activities of *Crossandra Infundibuliformis*

Preparation and sterilization of culture media and preparation of agar plate

Medium is defined as any substrate or material that will enable the micro organism to grow and multiply. A common nutrient medium used for cultivating the bacteria consist of beef extract- 0.35, peptone- 0.5%, sodium chloride-0.1% in water and it is called liquid medium or nutrient broth. All medium should provide carbon, nitrogen sources in addition to mineral and other growth factors for the growth and multiplication microbes, the basal nutrient

medium can be supplemented with different substance such as sugar, proteins, alcohol or inorganic salts to satisfy the requirement of particular organisms.

MEDIA COMPOSITION

- Beef extract-0.3g
- Peptone-0.5g
- Nacl-0.1g
- Water-100ml

PROCEDURE

Accurately weighed amount of ingredients are added to required quantity of water in a conical flask. Gently stir and keep it on the boiling water bath. Add 2% of agar to convert the liquid medium in to solid nutrient agar medium. Make sure that all ingredients were dissolved. Take out the flask from the water bath and check the pH for 7-2 by using pH meter. If necessary bring out the pH to 7-2 by adding buffers. Carry out sterilization by autoclaving Label the petri plates and organisms kept on the inoculation cabin. Hold the bottom of the conical flask on your right hand. Remove the cotton plug from your flask by using your left hand in proper way. Hold your petri plate in your left hand with a specified position. Flame the mouth of the conical flask. Pour the sterilized agar liquid medium into the bottom of the petri plate just to form one circle. Again flame the mouth of the flask and replace the cotton. Immediately place the plate on the table from the edge. Leave the plates for solidification.

Determination of Anti Bacterial Activity

The extracts sample were dissolved in a solvent at final concentration of 10mg/1ml was pipette into the different wells in a sterilized environment at different volumes(0.1,0.2,0.3,0.4,0.5,0.6) in plates using a micro pipette. Control wells were inoculated by ciprofloxacin for bacteria (E.coli, Staphylococcus aures). The plates were incubated for 24h for bacteria and the Zone of inhibition were measured. Control wells were inoculated by griseofulvin for fungi (A.niger, C.albicans). The plates were incubated for 72hrs for fungi and the Zone of inhibition were measured.

RESULTS AND DISCUSSION

Table 1: Preliminary Phytochemical Screening of Flower Extracts of *Crossandra infundibuliformis.*

TESTS	CHLOROFORM	ACETONE	METHANOL	AQUEOUS
Carbohydrate	-	+	+	-
Gums	-	-	-	-
Mucilages	-	-	-	-
Proteins	-	-	-	-
Amino acids	-	-	-	-
Fats&oils	+	-	+	-
Steroids	-	-	-	-
C.Glycoside	-	+	+	-
Flavonoids	-	-	+	+
Alkaloids	-	+	-	+
Tannins	-	+	+	+
Vitamins	-	-	-	-

'+' indicates presence '-' indicates absence

Table 2: Antibacterial activity of flower extracts of *Crossandra ifundibuliformis.*

Bacterial strain	Zone Of Inhibition(mm)					
	Chloroform	Acetone	Methanol	Aqueous	DMSO	Ciprofloxacin
E.coli	15	15	10	10	Nil	31
S.aureus	13	13	11	10	Nil	32

Table 3: Antifungal activity of flower extracts of *Crossandra Infundibuliformis.*

Fungal strain	Zone of Inhibition (mm)					
	Chloroform	Acetone	Methanol	Aqueous	DMSO	Griseofulvin
A.niger	10	10	10	09	Nil	26
C.albican	09	10	12	10	Nil	28

CONCLUSION

The results of preliminary phytochemical screening revealed the presence of various phytoconstituents (Table:1). The carbohydrates and cardiac glycosides were present in acetone and methanol extracts. The alkaloids were present in acetone and aqueous extracts The presence of tannins and flavonoids were observed in methanol and aqueous extract. The present study shows the evaluation of in vitro antimicrobial activity of *Crossandra infundibuliformis* against Gram-positive, Gram-negative and fungi, using agar well diffusion method. The results of antimicrobial activity were given in (Table:2 and Table:3), which clearly show that all the extracts have shown antimicrobial activity equivalent to that of the standard against entire tested organisms. Antimicrobial activity was confirmed by the presence of zone of inhibition. Due to the promising results obtained by this study; the range

of avenues can be developed for drug development giving high hopes to scientific community to serve humanity with utmost benefiting drugs with little or no side effects. Hence, leaves extract of *C.Infundibuliformis* could be proved as a potential drug component if designed in a hybrid manner along with allopathic drugs or a standalone drug formulation to subsidize/ nullify the side effects of allopathic drugs.

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