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Phytochemical screening and Antimicrobial activity of Crossandra Infundibuliformis

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ABSTRACT

Since the advent of 21st century, a phenomenon of co-evolution of pathogens as well as the drugs to combat concerned diseased state is of keen interest to scientific community due to their evolving nature. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed. Acanthaceae, a family which is well known for its medicinal values due to the presence of valuable and range of phytochemical compounds. Nowadays, the scientific community is exploring the effective alternatives of allopathic medicine by gaining more knowledge on pharmacologically important properties of medicinal plants as a safer yet efficacious option. Crossandra Infundibuliformis is a very popular plant which belongs to the acanthaceae family. In this review, the qualitative and quantitative data is presented for selective plant Crossandra Infundibuliformis. The sample was dried in a shadow for about 3 to 4 days and then crushed. The crushed samples were mixed with different solvents and were subjected to Soxhlet apparatus for phytochemicals extraction. The qualitative tests were performed aiming at determining the presence of various phytochemical compounds by tests such as Tannin test, flavanoid test, Steroid test, Starch test and Alkaloids test and many others following the standard protocols. The antimicrobial activity of plant species was evaluated by Agar well diffusion method for the solvent extract using Nutrient Agar medium for the assay.

Keywords: Medicinal plant, phytochemicals, antimicrobial activity, 3phytomedicine

Introduction

Since 20th century, the discovery of antibiotics is followed by extensive drug development in the field of Science in order to combat several infectious diseases. The phenomenon of co-evolution of pathogenic microorganisms and drug that either inhibit/kill them is quite evident as due to 'changing' nature. As a result, resistance is a key issue faced by scientific community in order to develop new formulations or drugs. (combinations of two or more) to address the potentially powerful microbes. [16].

Currently, due to noticeable increase in resistance development against present drugs; it has compelled scientific community to explore safe, effective yet non-toxic alternative drugs to manage the spread of resistant microbes across the globe. For seeking the solution to this problem, the scientific community's attention is diverted to medicinal plants, rich in phytochemicals content along with diverse chemical structures, may be proved as potent antimicrobial formulations if detailed studies are carried out in-vitro followed by supportive clinical trials. There are several reports in the literature describing the use of herbal medicine for the treatment of infectious disease, considered as innate indigenous knowledge associated with particular community or origin of plants [16].*Crossandra Infundubiliformis*, an ornamental flowering plant; used to beautify the kitchen, literatures are found revealing antimicrobial effects supported by the evident use by the folks in the form of traditional knowledge. As a medicinal plant, the different solvents extracts from various parts of the plant are proven to combat various nosocomial infections of human pathogens. [17] The aim of this study is to determine the antimicrobial effects of *C.Infundibuliformis*; in particular the leaf extracts using different solvent systems on several test organisms.

Crossandra Infundibuliformis belongs to the Acanthaceae family. This plant has been proved to have various medicinal properties. *Crossandra Infundibuliformis* is a popular tropical flower known as "Firecracker" [1]. The plant is native to Southern India, Malaysia, and Srilanka [2]. Due to its medicinal value, the various parts of this plant are used to treat many diseases. Antimicrobial compounds found in various parts of plant have enormous therapeutic potential. They effectively treat infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials[1, 2].The leaf extract of *C. Infundibuliformis* shows aphrodisiac activity on ethanol induced testicular toxicity in male rats [3]. Medicinal plants are the potential source of active compounds to cure many diseases associated with human beings. The present study reports that phytochemicals are beneficial to drug molecule formulation and drug discovery. The phytochemicals like terpenoids, flavonoids, carbohydrates have therapeutic values [4]. Plant-based antimicrobials represent a vast untapped source of medicines. Antimicrobial compounds of plant origin have enormous therapeutic potential. They

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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



Methodology

Extract preparation

Collect the plants leaves. Dry it in a shadow for 3 to 4 days. Crush this leaves in mixture jar to a fine powder. Use 10gm of leaf powder and perform extraction using soxhlet extraction methods. Use different solvents like ethylene acetate, methanol and chloroform in 100ml volume.[9,10].Filter this solution using whattman filter paper. Store the filtrate in an air tight glass bottle under refrigeration condition.

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Crossandra infundibuliformis –fig1. Dry leaves, fig 2. Extracts of -A. Methanol, B. Ethanol, C. n-Hexane, D. Chloroform, E. Ethyl acetate

Phytochemicalanalysis:

The extracts were analyzed for the presence ofdifferent phytochemicals like tannins, alkaloids, steroids etc.

Tannin's test:

Take 1ml of sample solution in a test tube. Add few drops of 0.1% ferric chloride and observe for a color change from brownish green or blue-black color.

Carbohydrate test:

Take 2ml of sample solution in a test tube. Add 2 ml each Fehling's solution A and B. Subject it to boiling water bath for about 10-15 minutes. Observe the red precipitateformation.[11]

Quinone test:

Take 1ml of sample solution and 1ml of concentrated sulfuric acid in all test tubes. Red color confirms the presence of quinone.

Terpenoid test:

In this test, take 2 ml chloroform and 3 ml of concentrated H_2SO_4 in 5ml of sample solution to form a monolayer of reddish-brown color. Reddish brown monolayer denotes the positive result.

Starch test:

Take 1 to 2ml of sample solution. Add iodine solution to it. Incubate it for few minutes. The starch containing part renders iodine color change from brown to blue/black. Color change to blue/black indicates the presence starch.

Phenolic compound:

The Compound with phenol group will form a blue violet, purple, green or red brown color upon the addition of 1% aqueous ferric chloride[12].

Steroid test:

The sample was taken in a test tube and dissolved in chloroform. Then add equal volume of concentrated H_2SO_4 by sides. The upper layer in the test tube turning red and H_2SO_4 layered showed with yellow with green fluorescence and it denotes the presence of steroid.

Anti-microbialactivity:

Antimicrobial activity refers to the process of analyzing the effect of variety of compounds or extract on microbial growth via inhibiting or lethal effect. Various antimicrobial agents are used for this purpose and antimicrobial activity may be checked against variety of microbes as anti-bacterial, anti-fungal or antiviral. They all differ in the mode of actionsby which they either suppress the growth or kill them. Antimicrobial activity was measured by agar well diffusion method. [13]

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Preparation of nutrient agar medium

Dissolve the dehydrated agar medium in the appropriate volume of distilled water.Take 15gm dehydrated nutrient agar in 500ml distilled waterand dissolve it properly.[14]Heat with frequent agitation and boil it for 1 minute to completely dissolve the dried agar powder. Pour the medium into the petri-plates and let it solidify. Now, spread the bacterial suspension on agar medium and allow it to dry for 5 minutes. Make a well on agar surface. Add a sample in well with micro pipette and incubate it at 37c for 24 hours. The resulting inhibition zones are measured in millimetres.[15]

Result

In this study, the primary investigation revealed presence of several phytochemicals that are native to this plant leaves. It showed the presence of secondary metabolites and other compounds such as tannins, saponins, alkaloids, flavonoids, steroids, glycosides, terpenoids and carbohydrates.

Sr. no	Phytochemicals	Presence in C. infundibuliformis
1	Tannins	+
2	Saponins	+
3	Alkaloids	+
4	Terpenoids	+
5	Flavanoids	+
6	Steroids	+
7	Glycosides	+
8	Carbohydrates	+

Table 1: Result of Phytochemical Analysis

Among the present phytochemicals,the presence ofdifferent phytochemicalsknown to be solvent specific as quinone, starch and terpenoids were present in methanol and chloroform extract. Steroid and phenolic compounds are present in ethyl acetate extraction. Ethyl acetate, a solvent system containing plant extracts showed the maximum zone of inhibition; also denoted the presence ofsteroids and phenolic compounds. Hence, it can be deduced that compounds are dissolvable in specific solvent over others.

Extract	E.Coli	B. Subtilis	S.Aureus
Methanol	8±0.06	7±0.11	8±0.04
Ethanol	8±0.10	8±0.05	9±0.00
N-Hexane	10±0.14	9±0.00	9±0.04
Chloroform	8±0.10	7±0.02	6±0.06
Ethyl Acetate	11±0.15	9±0.12	10±0.05

Table 2 : *Crossandrainfundibuliformis* (Zone of Inhibition in mm)

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Antimicrobial activity:

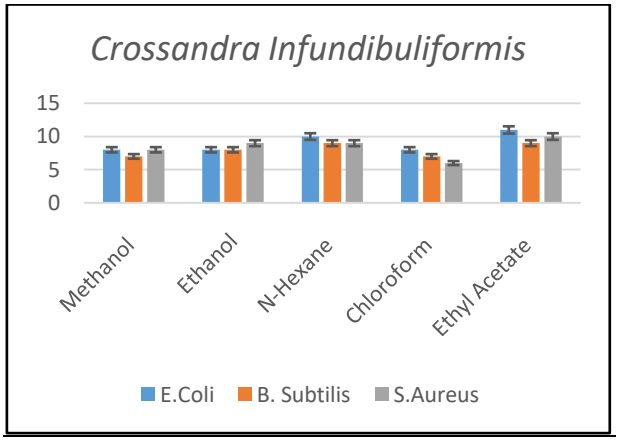


Figure 1 Bacillus Subtilis



Figure 2E. Coli

In the experiment performed, the various types of solvent extracts were prepared, tested and analysed for the inhibitory mode depending on zone of inhibition on a media petri-plate. Overall, the most effective solvent for leaf extract was found to be Ethyl acetate which recorded the highest zone of inhibition against the test organisms *E.coli*, *B.subtilis* and *S.aureus* giving 11mm, 9mm and 10mm inhibition zone respectively.



Graph 1: Antimicrobial Activity- zone of inhibition(mm)

Discussion

The emergence of therapeutic formulation from medicinal plants is now being accepted gradually across the globe due to its vital healing power. Nowadays, researchers are inclined towards the traditional medicines for curing infectious disease. As modern antibiotics have various toxic effects, these plants extract could serve as an alternative antimicrobial agent. These medicinal plants hold prime importance in combating several infections due to the innate presence of secondary metabolitesrendering medicinal effects. This study has revealed that methanol extract of leaves contains high phytochemical content.

In general, the phytochemical analysis detects the presence of various active and non-active compounds rendering the medicinal effects. The constituents such as tannins, saponins, alkaloids, terpenoids, carbohyrdrates, flavonoids, steroids and glycosides were found significantly during the current investigation.

The leaves extract of *C.Infundibuliformis* was extracted for determining the antimicrobial effect on mainly three microbial strains namely *E.coli*, *B.subtilis* and *S.aureus*. The inhibitory effect was analyzed using leaves extract of *C.Infundibuliformis*based on determining the zone of inhibition(mm) on a petri plate. For extraction of phytochemicals;

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various solvents including methanol, ethanol, N-hexane, chloroform and Ethyl acetate were utilized which were further subjected as a testing sample aiming at analysing inhibition activity against test organisms. The most profound antimicrobial effect was observed with Ethyl acetate extract againstall the microorganisms mentioned above. The zone of inhibition recorded for *E.coli*, *B.subtilis* and *S.aureus* were 11(mm), 9(mm) and 10(mm) respectively for Ethyl acetate solvent extract(table 2). On a comparative note with Ethyl acetate solvent, similar yet somewhat less effect was observed when the test organisms were subjected to N-hexane extracts.

Interestingly, the alcohol based solvent extracts showed almost same results in terms of zone of inhibition when the values were obtained. Methanol and Ethanol based solvent extracts based inhibition zones obtained were 8(mm),7(mm), 8(mm) and 8(mm), 8(mm), 9(mm) for *E.coli*, *B.subtilis* and *S.aureus* respectively. Among the described solvents extract, the least antimicrobial effect was evident with chloroform extract suggesting the absence or presence of phytochemicals in low amount pertaining to the concept of solubility in a solvent.

Overall, the highest inhibitory activity was found with Ethyl acetate solvent which can be utilized for extraction of suitable inhibitory phytochemicals for rendering maximum inhibition effect. Lastly, the notable antimicrobial effect was observed with Ethyl acetate solvent system which can be correlated with the number of carbon atoms in terms of proportionality as less carbon atoms based extract including ethanol and methanol showed lesser activity as compared to ethyl acetate suggesting the proportionality of number of carbon atoms with its antimicrobial effect. However, it is an observation, the study regarding its proportionality has to be yet proved by the experiments in-vitro.

Conclusion

In 21st century, the pathogenic microorganismsare gaining resistance at a rapid pace suggesting a need of an hour to develop the drug formulations with broad spectrum activity and least side effects that's where the concept of phytomedicine comes into existence. Phytomedicine offer great advantage such as broad spectrum antimicrobial effects due to presence of diverse phytochemicals in plants extract. This study proved presence of diverse phytochemicals in the leaf extract(Ethyl acetate) which are considerably responsible for highest antimicrobial effect on potentially pathogenic microbes such as *E.coli*, *B.subtilis* and *S.aureus*. 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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