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PHYTOCHEMICAL ANALYSIS AND ANTI-MICROBIAL ACTIVITY OF *COSTUS IGNEUS* (INSULIN PLANT) LEAF EXTRACT

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ABSTRACT

Extract from leaves of *Costus igneus* (insulin plant) are investigated for phytochemical constituents and antimicrobial activity. The leaves of *Costus Igneus* for this experiment were collected from RBVRR woman's college, Hyderabad. Leaf extract with ethyl alcohol revealed the presence of Tanins, Anthraquinones, Flavonoids, Alkaloids, Terpenoids, Saponins, Cardiac glycosides, Glycosides, Reducing sugars, phlobatins, steroids, phenolic, amino acids, and proteins. It is a medicinal herb and shows good anti-microbial activity against microbes. We tested its anti-microbial activity against *E.coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella typhi*, *Aspergillus niger*, *Candida albicans*, *Candida parapsilosis*. In view of this, it is aimed to study whether the plant can be use of fighting against diabetes.

Keywords: *Costus igneus*, insulin plant, phytochemical constituents, antimicrobial activity, Diabetes

INTRODUCTION

Costus igneus Nak is commonly known as fiery costus, Step ladder or Spiral flag or Insulin plant belongs to the family Costaceae. It is native of South to Central America and now introduced as a herbal cure for diabetes in India, it is also called as

“Insulin plant” (Jose B, 2010). It is widely grown in gardens as an ornamental plant in south India (Benny M, 2004). These are spirally arranged leaves and rhizomes free from aromatic essential oils. Based on the spirally arranged leaves it is first ranked to

the family Nakai. Before the elevation to family status, Engler and Prantl identified Costoideae as a subfamily under Zingiberaceae. It has a strong belief that consuming one leaf of *costus igneus* can lower blood glucose level and diabetes (Devi VD, Urooj A, 2008). Identification of the secondary metabolites from plant in therapeutic application of diabetes is of growing interest as they contain many active phytochemical constituents against the hyperglycaemic condition. These phytochemical constituents are of organic in nature. Bioprocess can convert simple compounds to complex compounds and it uses in several medicines and therapeutics. *Costus igneus* contains phytochemicals (Devi VD 2010), such as flavonoids, terpenoids, alkaloids and is used to control diabetes (Devi VD 2008). In a recent study the extract from the plant is subjected to column chromatography and the purified fractions were bioprocessed in HPTLC to identify active compounds. The compounds were assessed for antidiabetic activity in L6 myoblasts (swarnalatha.Y,2015).

Innumerable biologically active compounds that are found in plants (Alade,1993,Clark A.M, Samy 1993) possess antibacterial properties (Brantner1994, Samy 1999). Biologically produced compounds are more effective, safer than synthetically produced antimicrobial agents (Baladrin M.F.1985)

In present years, phytochemical compounds have been reported as a source of medicinal agents (Krishnaraju A.V, 2005). Thus, it is anticipated that phytochemicals with adequate antibacterial potential will be used for the treatment of bacterial infections (Baladrin M.F, 1985). Since, man has used various parts of plants for the treatment of various diseases (Tanaka, 2002).

The present study is focused to evaluate phytochemical composition and antimicrobial activity of insulin plant *Costus igneus* leaves extract.



Picture from RBVRR woman's college of *Costus igneus*

Microorganisms:

To determine the viability of organisms, those are cultured on nutrient agar (Nand kshah). The test organisms are *staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Trichophyton rubrum*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Candida parapsilosis*, *Candida albicans*, *Aspergillus niger*. They are maintained as Glycerol stocks at 4°C to check their viability from time to time.

Evaluation of antimicrobial activity

The total experiment was undergone on aseptic conditions. Nutrient agar medium (25ml) was taken in a sterile petridish and broth cultures of the test isolate(0.1 ml) containing 1.0×10^5 CFU/ml of organisms were used(Sah N K et al,2005). The culture extracts were dissolved in ethyl alcohol and used. Different concentrations were used to test the extracts i.e., 10, 20, 40 and 50 mg/ml.

Ampicillin (10 μ g/ml) was used as standard antibacterial agent and Griseofulvin was used as standard antifungal agent (Sah P 2008).

MATERIALS AND METHODS

Plant material was collected from the RBVRR college campus, Hyderabad. Chemicals such as wagner's reagent, chloroform, 2% H₂SO₄, concentrated sulphuric acid, 10% lead acetate, Benedicts reagent, 0.1% ferric chloride, Fehlings solution, dilute NaOH, 2% HCL, 10% ammonia, 10% HCL, distilled water, Ethyl alcohol are provided by the management of the college.

PREPARATION OF SOLUTIONS

- a) **Fehling's solution:** A mixture of equal volume of copper sulphate, sodium potassium tartarate and sodium hydroxide is prepared in a beaker.

- b) **Wagner's Reagent:** Mixing 2gm of Iodine, 6gm of potassium iodide in 100ml of water

Collection of sample: Healthy leaves of the *Costus igneus* plant were taken and washed under running water to remove the dust and other external pollutants. The plant leaves were air dried for few days (normally 15 to 21 days). Leaves which are collected for this experiment were taken from RBVRR woman's college, Hyderabad.

Grinding the sample:

The dried leaves are grind to fine powder in a mixer and the powder is collected in clean polythene bags.

Preparation of plant extract with Ethyl alcohol: 10gm of leaf powder was taken and added 50ml of ethyl alcohol stirred it constantly for 30minutes and the solution was kept in room temperature for 24 hours (minimum) and then filtered. The filtered solution is again filtered with whatman filter paper No.3 and then it was stored at 4 $^{\circ}$ c (in a freezer) until use.

TESTS AND RESULTS

a) **Phytochemical screening:**

Chemical test is carried out on the ethyl alcohol extract of all spice using standard procedure to identify the constituents.

- b) **Procedure for alkaloids:** 2ml of extract is taken and added 2ml of

wagner's reagent a brownish precipitate indicate the presence of alkaloids.

- c) **Cardiac glycosides:** 2ml of extract is dissolved with 2ml of chloroform and concentrated sulphuric acid is carefully added to form a layer. Deep reddish brown colour at the inter face of steroid ring indicates the presence of cardiac glycosides.
- d) **Flavonoids:** 2ml of extract is treated with 2ml of 10% lead acetate. Yellowish green colour indicates the presence of flavonoids.
- e) **Saponins:** 2ml of extract is dissolved with 2ml of benedicts reagent. Blue black ppt indicates the presence of saponins.
- f) **Tanins:** 2ml of extract with 0.1% of ferric chloride. Brownish green indicates the presence of tannins.
- g) **Terpenoids:** (salkowski test) 2ml of extract is dissolved with 2ml of chloroform and concentrated sulphuric acid is carefully added to form a layer. A reddish brown colour indicates the presence of terpenoids.

- h) **Anthraquinones:** 1ml of extract is boiled with 10% HCL for few minutes in a water bath. It is filtered and allowed to cool. Equal volume of CHCL₃ is added to the filtrate few drops of 10% Ammonia is added to the mixture and heat. Formation of rose pink colour indicates the presence of reducing sugars.
- i) **Reducing sugars:** The extract was shaken with distilled water and filtered. The filtrate was boiled with Fehlings solution A and B for few minutes an orange red ppt indicates the presence of reducing sugars.
- j) **Glycosides:** The extract was hydrolysed with HCL solution and neutralized with NAOH solution. A few drops of Fehlings solution A and B are added, red ppt indicates the presence of glycosides.
- k) **Phlobatanins:** The extract is dissolved in distilled water and filtered. The filtrate is boiled with 2% HCL solution. Red precipitate shows the presence of phlobatanins.

Table showing results of Phyto chemical Analysis Costus igneus (Insulin Plant) Dt 8.6.2015

Sl. No	Phytochemicals	Distilled Water	Methanol	Acetone	Ethanol
1	Tanins	Positive	Positive	Positive	Positive
2	Anthraquinones	Positive	Positive	Positive	Positive
3	Flavanoides	Positive	Positive	Positive	Positive
4	Alkaloides	Positive	Positive	Positive	Positive
5	Terpenoids	Positive	Positive	Positive	Positive
6	Saponins	Positive	Positive	Positive	Positive

7	Cardiac glycosides	Positive	Positive	Positive	Positive
8	Glycosides	Positive	Positive	Positive	Positive
9	Reducing Sugars	Positive	Positive	Positive	Positive
10	Phlobatanins	Positive	Positive	Positive	Positive
11	Steroids	Positive	Positive	Positive	Positive
12	Phenolic	Positive	Positive	Positive	Positive
13	Aminoacids	Positive	Positive	Positive	Positive
14	Proteins	Positive	Positive	Positive	Positive

Human usage and disease management

- ❖ Alkaloids are anaesthetic agents and found in medicinal plants
- ❖ Cardiac glycosides are strong and direct action on the heart and used for diuretic and lowering the blood pressure.
- ❖ Flavonoids are anti oxidant elements strengthen capillary valves of heart and improve blood circulation.
- ❖ Saponins are useful as expectorants, anti-bacterial and anti-viral.
- ❖ Tanins draw the tissue closure together and fights infection.
- ❖ Terpenoids are useful as aromatic agents and used for flavouring the food.
- ❖ Anthraquinins are having laxative properties and useful to large intestine.
- ❖ Flavonoids are identified with antioxidant properties retards breast cancer growth and progression.

Anti-microbial activity

Collection of insulin plant leaves:

For the present study, *Costus igneus* leaves are collected from RBVRR College, Hyderabad and dried under shade and made

into fine powder. The powder (100 grams) was extracted with methanol and rota vaporized at 40-50°C for 3-4 hours. This extract was further used to evaluate antimicrobial activity.

Equipment Preparation

To conduct the experiment, 28g of nutrient agar is dissolved in 1000ml distilled water. Along with the agar medium, petri dishes, forceps, spreader, cotton balls, and 25ml conical flasks, whatmann no 1 filter paper are kept in autoclave. The sterilized agar was then transferred to petri-dishes and allowed to solidify. The anti-microbial activities were formed using whatmann no 1 paper.

The fine round paper discs were obtained and were sterilized. The whole process executed under aseptic conditions under laminar air flow.

RESULTS AND DISCUSSION

Results of phytochemical analysis: By this analysis we can conclude that *Costus igneus* consists of Tannins, Anthraquinones, flavonoids, alkaloids, Terpenoids, Saponins, Cardiac glycosides, Glycosides, Reducing sugars, phlobatanins, steroids, amino acids, phenolic, and proteins.

Results of antimicrobial activity:

The antimicrobial activity of leaf extract was examined against Gram positive and Gram negative bacterial and fungal strains by measuring the zone of inhibition.

The activity was performed by Agar disc diffusion method at concentration level of 2.5,5.0,7.0,10µg/ml respectively.

Organism/conc of extract	2.5µg/ml	5µg/ml	7.5µg/ml	10µg/ml
<i>E.coli</i>	1.0cm	1.2cm	1.2cm	1.1cm
<i>Staphylococcus aureus</i>	1.1cm	1.1cm	1.2cm	1.3cm
<i>Bacillus subtilis</i>	1.2cm	1.1cm	1.1cm	1.4cm
<i>Salmonella typhi</i>	1.0cm	1.0cm	1.2cm	1.2cm
<i>Aspergillus niger</i>	0.9cm	0.9cm	0.9cm	1.0cm
<i>Candida parapsilosis</i>	1.2cm	1.2cm	1.3cm	1.2cm
<i>Trichophyton rubrum</i>	1.1cm	1.2cm	1.2cm	1.2cm
<i>Candida albicans</i>	1.0cm	1.1cm	1.1cm	1.0cm

The *Costus igneus* leaf extract showed high activity against *Bacillus subtilis*, *Candida parapsilosis* at low concentration (2.5µg/ml). The zone of inhibition measured in cm.

CONCLUSION

The preliminary phytochemical screening of *Costus igneus* gives good results in the presence of presence of flavonoids, alkaloids, anthraquinones, taninns, phenolic compounds, terpenoids, saponins, carbohydrates and proteins.

The anti-microbial activity screening also shows good results at *Bacillus subtilis*, *Candida parapsilosis* at lower concentration.

This phytochemical and antimicrobial bioprocesses concludes that *Costus igneus* which popularly named as insulin plant have good medicinal and therapeutic compounds.

Ampicillin (anti-bacterial), Itraconazole or Griseofulvin (antifungal) as the standard drug at a concentration of 200µg/ml. LB Agar was used as the culture media and potassium dextrose was used as antifungal activity. Table shows antimicrobial activity of *Costus igneus* leaves extract.

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