Antibacterial Activity and Screening of Antibacterial Compounds of *Costus pictus* D.Don Using GC-MS

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**Abstract:** The medicinal plants are the reservoir of potential microbial compounds that are useful as an alternative to synthetic microbicides and are used to develop drugs. In the present study an ethanomedical plant *Costus pictus* was analysed for preliminary phytochemical screening, GCMS and antimicrobial activity. The phytochemical analysis revealed the presence of active compounds such as phenols, flavonoids, tannins, terpenoids, alkaloid, steroids, glycosides and saponin in the ethanolic leaf extract of *costus pictus*. Different concentration of ethanolic leaf extracts were tested using disc diffusion technique for the activity against *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* , *Escherichia coli* and *Bacillus subtilis*. The ethanolic leaf extract showed concentration –dependent activity at various concentrations against all tested bacteria expect *E.coli* with the zone of inhibition. GC-MS analysis also confirmed the presence of bioactive compounds. The powerful antibacterial effect is attributed due the presence of active compounds present in the ethanolic leaf extract of *Costus pictus*.

**Keywords:** Phytochemical, antimicrobial, disc diffusion, Concentration –dependent, GC-MS analysis

1. Introduction

Medicinal plants are the valuable source of natural active compounds for maintaining human health and treatment of many human diseases. The bioactive compounds are alkaloids, phenols, flavonoids, vitamins and minerals which are found to have antioxidant, antitumour, antibacterial, anticarcinogenic and diuretic properties. [1][2] Therefore there is a need to study the ethnomedicinal use and importance of herbal medicinal plants in the discovery of novel drugs. Most of the synthetic drugs used to cure human ailments have their origin from plant products.[3]

*Costus pictus* D.Don, commonly known as fiery costus, spiral ginger, step ladder or insulin plant is a native of south and central America.[4] In India the plant is grown as ornamental plant especially in Kerala.[5] The plant is used in India as an herbal cure for diabetes hence commonly called as “ Insulin plant”. [6] It is a perennial erect herb growing up to three meters tall and leaves arranged spirally around the stem. The plant belongs to the family costaceae. The plant can be cultivated either by stem cutting or vegetative propagation.

In India the plant is used to control diabetes, people consume one leaf daily to keep their blood sugar level low.[7] It is also reported to have anti-inflammatory and hypoglycemic action. [8] The parts of the plant are used in the treatment of renal disorders and possess diuretic activity. [9] Several studies have been carried out in the leaf extract of *Costus pictus* to evaluate the antiabetic properties. [10] The leaf and the rhizome are known for the antidiuretic, antihelminthic, antibacterial and antitumour activities. [11] The present study was undertaken to study the antibacterial activity of the leaf extract of *Costus pictus*. Phytochemical screening and GCMS analysis were also performed.

2. Materials and methods

**Plant Material**

Fresh healthy plants of *Costus pictus* was collected from Kerela and brought to the laboratory after identification. Fresh leaves were washed under running tap water, shade dried at room temperature and then homogenized to fine powder and stored in air tight bottles.

**Test organism used**

The test microorganism like *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* were collected from MTCC. All the bacterial strains were suspended in nutrient broth and incubated at 37°C for 24 hrs.

**Preparation of Plant extract**

Preparation of the extracts was done by the following method. One gram of dried powder of plant materials was extracted with 20 ml of ethanol (75%), acetone, chloroform, aqueous and petroleum ether (Merck, extra pure) for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 filter paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rotavator at 40°C to a constant weight and re dissolved in the same solvent namely aqueous, ethanol, chloroform, petroleum ether and acetone for extraction. The solution was stored at 18°C for further studies.

**Phytochemical Screening**

Phytochemical screening was carried out for analysing the secondary metabolites which are responsible for curing various human ailments. The phytochemical screening of the leaf extract was accessed by the standard method. [12] [13] [14] to detect the presence or absence of certain bioactive compounds. Five different solvent extracts were used to...
identify the major natural chemical compounds such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides and steroids.

**GC–MS Analysis**

For the Identification of bioactive components, the extract was subjected to GC-MS analysis. GC-MS analysis was carried out on a GC-MS -5975C agilent system comprising an auto sampler and a gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument, employing the following conditions: column Elite-1 fused silica capillary column (30×0.25 mm ID × 1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1.51 ml/min and an injection volume of 1μl was employed (split ratio of 10:1) injector temperature 2000°C. The oven temperature was programmed from 700°C (isothermal for 2 min), with an increase of 100°C/min, to 3000°C/min, ending with a 9 min isothermal at 3000°C. Mass spectra were taken at 70eV; with a scan range 40-1000 m/z. Solvent cut time was 5 min; MS start time being 5 min; MS end time being 35 min; Ion source temperature set to 2000°C and interface temperature being 2400°C.

**Identification of Components**

Interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the component of the test materials were identified.

**Anti-bacterial activity**

The ethanolic leaf extract of *Costus pictus* was used for antibacterial study.[16] [17] Different concentrations (10mg, 20mg and 30mg /ml) of the concentrated leaf extract was tested for its antimicrobial activity against pathogenic bacterial strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The bacterial cultures were grown in Mueller Hinton Agar and Mueller Hinton Broth (Himedia).[18]

**Antibacterial activity assays**

Antibacterial activity was measured using the standard method of diffusion disc plates on agar.[19] For antimicrobial assay, all bacterial strains were grown in Mueller HintonBroth Medium (Himedia) for 24 hours at 37°C and plated on Mueller Hinton Agar (Himedia) for agar diffusion experiments. Then 0.1ml of each culture of bacteria was spread on agar plate surfaces. Sterile disc (Hi Media, 6mm in diameter) were placed on the agar medium to load 20μl of different concentration (10 -30mg /ml) of ethanolic leaf extracts of *Costus pictus* was tested. Inhibition diameters were measured after incubation for 24 hours at 37°C. Blanks of solvent only (processed in the same way), were also tested for antibacterial activity.

**Minimum inhibitory concentration**

Minimum inhibitory concentration (MIC) of leaf extract against both the bacteria (*Bacillus cereus* and *Pseudomonas aeruginosa*) was assessed by serial dilution method. Ten different concentrations of the ethanolic extract of *Costus pictus* (0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1.0%) were incorporated into nutrient broth in different test tubes. In each test tube, 5 ml of extract was added to 4.9 ml of nutrient broth and 0.1 ml of bacterial culture. A control tube containing the growth medium and the bacteria was set up. The mixtures were incubated at 37°C for 24 h and analyzed for turbidity. The minimum concentration of leaf extract that will inhibit the growth of the microorganism was determined as MIC.

**3. Results**

**Phytochemical screening**

In the present study phytochemical screening was performed with aqueous, ethanol, chloroform, acetone and petroleum ether. The ethanolic leaf extract of *Costus pictus* was found to be rich in terpenoids, Quinones, glycosides, alkaloids, flavonoids, steroids, Phenols, tannins and saponins followed by other extracts. The results were presented in the Table 1.

**GC-MS Analysis**

Gas Chromatography –Mass Spectrometry is a potent tool for identifying the bioactive compounds present in the natural product. The GC-MS chromatogram of *Costus pictus* showed twenty three peaks indicating the presence of twenty chemical constituents. The twenty chemical constituents were characterised and identified on comparison of the mass spectra of the constituents with the NIST library. The active compounds with their retention time, molecular formula, molecular weight and peak area (%) are presented in the Table 2. GC-MS analysis revealed the presence of active metabolites comprising of fatty acids, alcohols, methylesters, terpenoids, alkaloids and heterocyclic compounds. Out of twenty compounds identified major components were Vitamin E(20.47%),Gamma Tocopherol (12.08%)...

**Antibacterial assay**

The ethanolic leaf extract of *Costus pictus* were tested for the antimicrobial activity against *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* and the results are presented in Table 3. The ethanolic leaf extract shows activities against all test organisms except *Escherichia coli*.

**4. Discussions**

In the present study, the phytochemical screening of leaf extract of *Costus pictus* showed that, among the five different solvent extracts, the ethanolic leaf extract was found to be rich in Tannins, Saponins, Flavonoids, Quinones, Glycosides, Terpenoids, Phenols, Steroids, Alkaloids followed by other extracts.

Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites that serve as defence mechanism against many microorganisms [20]

Flavonoids are potent water soluble antioxidants which prevent oxidative cell damage and possess anticancer...
activity and anti-inflammatory activity. [21] Tannins are found to have anti-bacterial, antiulcer and antiviral properties. [22] In the present study the antimicrobial activity is mainly attributed due to the presence of phenolic compound, tannin and saponins.[23] [24] The ethanolic crude extract showed inhibitory activity against gram positive and gram negative bacteria. [25] The ethanolic leaf extract shows activity against Bacillus Subtilis, Bacillus Cereus, Pseudomans aeruginosa, Staphylococcus aureus but no activity against E Coli. The bioactive compounds present in the extract elicit the antibacterial activity against microorganism.

The antimicrobial potential of Costus pictus was tested by using agar well diffusion method. The ethanol extract of Costus pictus (30 mg/ml) showed maximum zone of inhibition (13mm) against Pseudomonas aeruginosa whereas Staphylococcus aureus showed less zone inhibition (9mm). E. coli does not show any activity against any concentration.

Further GC – MS analysis of the plant extract has established 23 bioactive compounds which possess several pharmacological properties. Phytochemical screening and GC-MC studies confirm presence of phenolic compound mainly responsible for the antimicrobial property of the plant. The present study unveil the medicinal important of Costus pictus. The antibacterial properties of the extract may be due to the presence of above mentioned phytochemicals

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6. Conclusion

The medicinal plant screened was found to be rich in secondary metabolites which are used in traditional medicine to combat and cure various diseases.

Phytochemical analysis showed that antibacterial activity of Costus pictus was due to the presence of phytochemical such as Tannins, Saponins and Phenols. Thus the plant can be utilized as an useful source to formulate new antimicrobial drugs of natural origin. Further studies are needed to isolate and characterize the structure of bio active compounds for drug formulation.

Table 1: Phytochemical screening of Costus pictus

| Phytochemicals | Aqueous | Ethanol | chloroform | Acetone | Petroleum ether |
|----------------|---------|---------|------------|---------|-----------------|
| Tannins        | +       | +       | -          | +       | +               |
| Saponins       | -       | +       | -          | -       | -               |
| Flavonoids     | +       | +       | -          | +       | -               |
| Quinones       | +       | +       | +          | +       | +               |
| Glycosides     | -       | +       | -          | -       | -               |
| Cardiac glycosides | -     | +       | -          | +       | +               |
| Terpenoids     | +       | ++      | -          | +       | +               |
| Phenol         | +       | ++      | +          | +       | -               |
| Alkaloids      | +       | +       | -          | -       | +               |
| Steroids       | +       | ++      | +          | +       | +               |
| Betacyanin     | +       | +       | -          | -       | -               |
| Anthocyanin    | -       | -       | -          | -       | -               |

Figure 1: Shows GC-MS Chromatogram of Costus pictus

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Table 2: Phytoconstituents identified in Costus pictus by GC-MS analysis

| RT   | Area Percentage | Name                          | Molecular Formula | Molecular structure |
|------|-----------------|-------------------------------|-------------------|--------------------|
| 13.919 | 1.74          | Caryophyllene oxide           | C15H18O            | 220.35046          |
| 16.638 | 0.67          | Bicyclo[3.1.1]heptane, 2,6,6-trimethyl- | C18H30O2           | 256.43             |
| 17.537 | 1.67          | Hexadecanoic acid             | C17H34O2           | 278.55             |
| 17.849 | 2.86          | Dibutyl Phthalate             | C20H24O2           | 294.4721           |
| 19.164 | 4.71          | 9,12-Octadecadienoic acid (Z,Z)-methyl ester | C20H34O2           | 294.4721           |
| 21.006 | 0.94          | Nonadecane                    | C19H38O            | 268.518            |
| 22.648 | 1.33          | Eicosane                      | C20H42O            | 282.5478           |
| 22.893 | 0.39          | Bis(2-ethylhexyl) phthalate   | C24H36O2           | 390.56             |
| 24.170 | 3.27          | Heptadecane                   | C17H34O            | 240.48             |
| 24.505 | 3.21          | 9,12-Octadecadienoic acid (Z,Z) | C19H36O3           | 280.4555           |
| 24.980 | 3.40          | Squalene                      | C30H50             | 410.73             |
| 25.589 | 3.21          | Eicosane                      | C20H42O            | 282.5478           |
| 25.753 | 1.79          | Piperine                      | C21H19NO3          | 285.34             |
| 25.805 | 1.27          | Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-henicicosapentaenyl)-(all-E) | C30H46O3           | 426.7174           |
| 26.637 | 3.73          | Beta Tocopherol               | C28H50O2           | 416.68             |
| 26.748 | 12.08         | Gamma Tocopherol              | C28H50O2           | 416.68             |
| 27.313 | 20.47         | Vitamin E                     | C28H50O2           | 430.7061           |
| 30.722 | 2.31          | Phytol (1,2,5-Octadecadiol-3,4-methoxyphenoxy) | C19H19NO3          | 207.18606          |

Table 3: Antibacterial activity of ethanol leaf extract of Costus pictus

| Micro-organisms Tested | Concentrations of extract | 10mg/ml | 20mg/ml | 30mg/ml |
|------------------------|---------------------------|---------|---------|---------|
| Bacillus subtilis•     |                           | 10mm    | 12mm    |         |
| Bacillus cereus•       |                           | -       | 12mm    |         |
| Pseudomonas aeruginosa•|                           | -       | -       | 13mm    |
| Staphylococcus aureus• |                           | -       | -       | 9mm     |
| Escherichia coli•      |                           | -       | -       | -       |

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