

# 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 ethnomedicinal 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 ethanol 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 except *E. coli* with the zone of inhibition. GC-MS analysis also confirmed the presence of bioactive compounds. The powerful antibacterial effect is attributed due to 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, tannins, 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 antidiabetic properties. [10] The leaf and the rhizome are known for the antidiuretic, antihelmintic, 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* were collected from Kerala 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 rotator 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 2400C; ion-source temperature 2000C. The oven temperature was programmed from 700C (isothermal for 2 min), with an increase of 100C/min, to 3000C/min, ending with a 9 min isothermal at 3000C. 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 2000C and interface temperature being 2400C.

### 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 ethanol leaf extract of *Costus pictus* was used for antibacterial study.[16] [17] Different concentrations (10mg, 20mg and 30mg /ml) of the concentrated ethanol 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 Hinton Broth 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 *Baiclus*, *Subtilis*, *Bacillus Cereus*, *Pseudomans aeruginosa*, *Staphylococcus auerus* 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* where as *Staphylococcus aureus* showed less zone inhibition (9mm). *E. coil* 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 bioactive compounds present in the ethanol extract of *Costus pictus*. The antibacterial properties of the extract may be due to the presence of above mentioned phytochemicals

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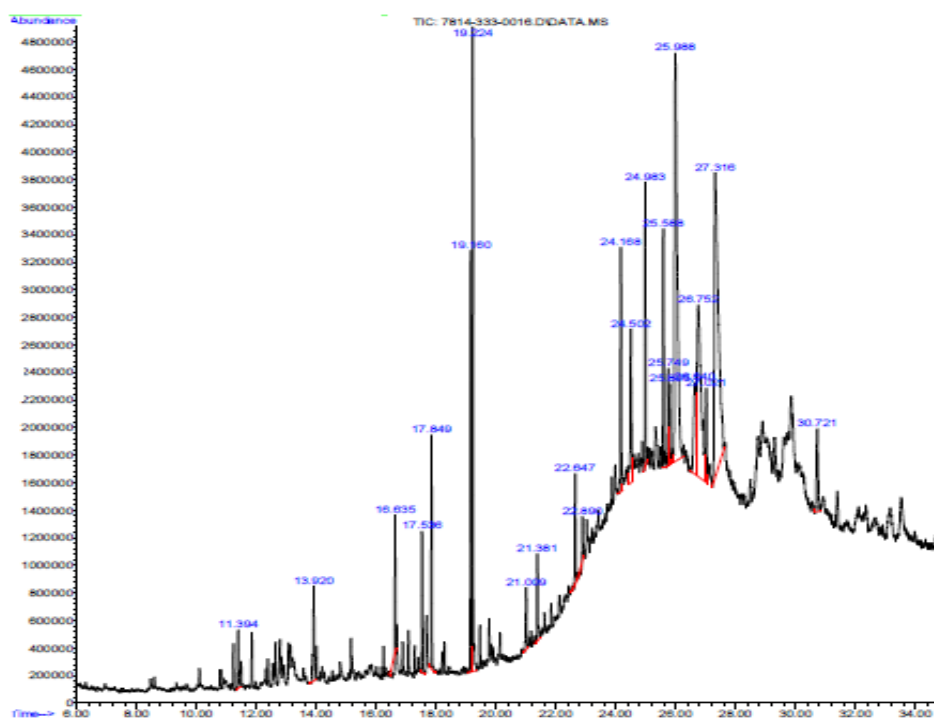
### 6. Conclusion

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	Leaf extract				
	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*

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

RT	Area Percentage	Name	Molecular Formula	Molecular structure
13.919	1.74	caryophyllene oxide	C <sub>15</sub> H <sub>24</sub> O	220.35046
16.638	0.67	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	C <sub>10</sub> H <sub>18</sub>	138.2499
17.537	1.67	Hexadecanoic Acid	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	256.43
17.849	2.86	Dibutyl Phthalate	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278.35
19.164	4.71	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294.4721
21.006	0.94	Nonadecane	C <sub>19</sub> H <sub>40</sub>	268.518
22.648	1.33	Eicosane	C <sub>20</sub> H <sub>42</sub>	282.54748
22.893	0.39	Bis(2-ethylhexyl) phthalate	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390.56
24.170	3.27	Heptadecane	C <sub>17</sub> H <sub>36</sub>	240.48
24.505	3.21	9,12-Octadecadienoic acid (Z,Z)	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280.4455
24.980	3.40	Squalene	C <sub>30</sub> H <sub>50</sub>	410.73
25.589	3.21	Eicosane	C <sub>20</sub> H <sub>42</sub>	282.54748
25.753	1.79	Piperine	C <sub>17</sub> H <sub>19</sub> NO <sub>3</sub>	285.34
25.805	1.27	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)	C <sub>30</sub> H <sub>50</sub> O	426.7174
26.637	3.73	Beta Tocopherol	C <sub>28</sub> H <sub>48</sub> O <sub>2</sub>	416.68
26.748	12.08	Gamma Tocopherol	C <sub>28</sub> H <sub>48</sub> O <sub>2</sub>	416.68
27.313	20.47	Vitamin E	C <sub>28</sub> H <sub>48</sub> O <sub>2</sub>	430.7061
30.722	2.31	1,2,5-Oxadiazol-3-amine,4-(3-methoxyphenoxy)	C <sub>9</sub> H <sub>9</sub> N <sub>3</sub> O <sub>3</sub>	207.18606

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

<i>Inhibition Zone in diameter (mm)*</i>			
Micro-organisms Tested	Concentrations of extract		
Leaf extract	10mg/ml	20mg/ml	30mg/ml
<i>Bacillus subtilis</i> •	-	10mm	12mm
<i>Bacillus cereus</i> •	-	-	12mm
<i>Pseudomonas aeruginosa</i> •	-	-	13mm
<i>Staphylococcus aureus</i> •	-	-	9mm
<i>Escherichia coli</i> •	-	-	-

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