EVALUATION OF ANTIMICROBIAL POTENTIAL OF COSTUS PICTUS LEAF EXTRACTS

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Abstract

Costus pictus is a family of Costaceae, a recently introduced plant from Mexico has shown its potential as herbal drug for diabetes. Costus pictus is a rhizomatous medicinal herb. Leaf of this herbal plant helps to build up insulin by strengthening β-cells of pancreas in the human body thus popularly known as “insulin plant” in India. Many important and beneficial aspects of C. pictus are reported, which include anti-diabetic, anti-microbial, anti-cancer, anti-oxidant, anti-fertility, anti-helminthic, diuretic, anti-inflammatory properties. The antimicrobial activity was evaluated using disc diffusion method and effect of concentration of ethanolic extract on growth of bacteria culture was carried out by tube dilution technique. Different concentration of ethanolic, chloroform and aqueous leaf extracts were tested. The ethanolic extract was found to have significant activity against both gram-positive-Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Lactobacillus and Streptococcus mutansand gram-negative bacteria- Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumonia and proteus mirabilis. The selected medicinal plant is the reservoir of potential anti-microbial compounds that are useful as an alternative to synthetic microbicides and are used to develop combination drugs for many pharmaceutical necessities. The powerful anti-bacterial effect was attributed due the presence of active compounds present in the ethanolic leaf extract of Costus pictus. The present study was conducted to investigate the anti-bacterial activity of Costus pictus leaf extract in three different solvents Chloroform, ethanol and aqueous.

Key words: Antimicrobial potential, Costus pictus.

Introduction

Medicinal plants being an herbal remedy in both developing and developed countries in the management of health care, globally 80% of the population relies on traditional medicine, since ancient times as major source of medicine in treating many diseases, Ayurveda, Unani, homeopathy, naturopathy. Natural products derived from plants on a large scale and attracted by many researchers due to less or no side effects during pharmaceutical approaches. The insulin plant namely Costus pictus and Costus speciosus are commonly known as fiery Costus or Spiral flag. It is widely grown in South India as an ornamental plant in gardens and also in many places. Costus pictus leaves were known to be effectively used for treating diabetes by the tribal people of Kolli hills of Namakkal district, Tamilnadu. In India the plant is used to control diabetes, people consume one leaf daily to keep their blood sugar level low (Devi et al., 2008). It is also reported for their anti-inflammatory and hypoglycemic potential (Ramya and Daniel, 2012 ). The plant parts are used to treat renal disorders and it also possesses diuretic activity (Melendez- Camargo, 2006). Several studies have been evidenced to evaluate the anti-diabetic properties in the leaf extract of Costus pictus (Jothivel, 2007). The leaf and rhizome of plant are known to be anti-diuretic, anti-helminthic, anti-bacterial and anti-tumor activities (Thomas and Devi, 2013). Herbal drugs are considered to be less toxic and free from side-effects compared to synthetic drugs (Pari and Umamaheswari, 2000). Plants have always been an exemplary source of drugs and many of the currently available drugs were derived directly or indirectly from them (Grover, 2002). The family Costaceae consists of 4 genera and over 200 species. Costus is native of tropical areas of Asia, Africa, Australia and the America (Gothandam et al., 2010). Costus under family Zingiberaceae, Costaceae is classified as Costideae by Engler & Prantl. The ethanolic extract of

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C. pictus leaves known to exhibit significant enzymatic action on pepsin, α-amylase, effect on mitochondrial enzymes, carbohydrate hydrolyzing enzymes and induce insulin production.

Materials and Methods

Collection of Plant Material

Fresh healthy plants of Costus pictus was identification and collected from Kanyakumari District, Tamil Nadu and brought to the laboratory for further sample processing. 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.

Preparation of leaf extracts

Sample extraction was prepared by dissolving the one gram of dried powder of leaf in 20 ml of ethanol (75%), chloroform and aqueous (Merck, extra pure) then mixed for 1 min using an Ultra Turax mixer (13,000 rpm) and percolate overnight at room temperature. The sample extracts was then filtered through Whatman No. 1 filter paper in a Buchner funnel. The filtered solution was concentrated under vacuum in a rotary evaporator at 40°C to a constant weight and re-dissolved in the respective solvents such as ethanol, chloroform and aqueous. The samples were stored at 18°C for further studies.

Test Organisms

The test microorganisms like Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Lactobacillus sps, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumonia, Proteus mirabilis and Streptococcus mutans were collected from (MTCC) Chandigarh. The bacterial strains were maintained on Nutrient Agar (NA) for further assays.

Broth culture preparation

Pure culture from the each strains were inoculated into Nutrient Agar plate and sub cultured at 37°C for 24h. culture Inoculums were prepared by aseptically inoculate the fresh culture into 2 ml of sterile 0.145 mol/L saline tube and the cell density was observed with 0.5 McFarland turbidity standard to yield a fine bacterial suspension of $1.5 \times 10^8$ cfu/ml. Standardized inoculm for Anti-microbial assay.

![Mean Value of Activity](image1)

**Fig. 1:** Mean average of C. pictus extracts.

![Antibacterial activity of Leaf extracts of Costus pictus](image2)

**Fig. 2:** Antibacterial activity of Leaf extracts of Costus pictus.
Evaluation of Antimicrobial Potential of Costus Pictus Leaf Extracts

Antimicrobial Test

The assay media was prepared by dissolving 38 g of Muller Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured petriplates (25 ml/plate) the plates were swabbed with Pathogenic Bacteria culture viz. Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Lactobacillus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumonia, Proteus mirabilis and Streptococcus mutans. Finally, the positive control (streptomycin 25mg) and sample loaded disc was then placed on the surface of Mullar-Hinton medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, zone of clearance were measured around the disc with transparent ruler in millimeters. The size of the zone of inhibition (including disc) was measured in millimeters. The absence of zone inhibition was interpreted as the absence or low activity (Kohner et al., 1994, Mathabe et al., 2006). The activities are expressed as resistant, if the zone of inhibition was less than 7 mm, intermediate (8-10 mm) and sensitive if more than 11 mm (Assam, 2010).

Results

The anti-microbial assays of ethanolic and chloroform extracts of Costus pictus showed potential zone of inhibition against Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Lactobacillus sps, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumonia, Proteus mirabilis and Streptococcus mutans. Finally, the positive control (streptomycin 25mg) and sample loaded disc was then placed on the surface of Mullar-Hinton medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, zone of clearance were measured around the disc with transparent ruler in millimeters. The size of the zone of inhibition (including disc) was measured in millimeters. The absence of zone inhibition was interpreted as the absence or low activity (Kohner et al., 1994, Mathabe et al., 2006). The activities are expressed as resistant, if the zone of inhibition was less than 7 mm, intermediate (8-10 mm) and sensitive if more than 11 mm (Assam, 2010).

Mean average of C. pictus Extracts

The mean average of each solvent of anti-bacterial activity was calculated and plotted in a chart diagram with the comparison of standard drug (streptomycin 25mg). The mean value of ethanolic extract was 18.1±0.1 and 12.9±0.1 for chloroform extract.

Discussion

In this present study has shown that the ethanolic and chloroform Leaf extract of C. pictus has a potent antibacterial property against majority of organisms were tested. These plants could serve as useful sources for new anti-microbial agents for various internal and external pathogenic infections. The present study of antimicrobial evaluation of Costus pictus led a primary platform for further phytochemical and pharmacological studies. The result indicates significant prophylactic activity against both gram-positive Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Lactobacillus and Streptococcus mutans and gram-negative bacteria- Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumonia and Proteus mirabilis.

Conclusion

Infectious disease can become a threat to public health in this world. For a long period of time, plants have been a valuable source of natural products for maintaining human health, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purpose has gradually increased. Recent studies stated that the medicinal plants would be the source of drug discoveries (Khalil, 1996; Specht, 2006). Medicinal plants possess many bioactive and pharmaceutically active compounds and can be utilized as anti-infectious agents and for many deadliest diseases. Medicinal plants are readily available in rural areas to be used in traditional way. It is considerably useful and relatively cheaper than modern medicine. According to my experiments,

Table 1: Antibacterial activity of Leaf extracts of Costus pictus.

<table>
<thead>
<tr>
<th>Strain name</th>
<th>Positive control (Streptomycin 25mg)</th>
<th>Ethanolic extracts of C. pictus</th>
<th>Chloroform extracts of C. pictus</th>
<th>Aqueous extracts of C. pictus</th>
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<tbody>
<tr>
<td></td>
<td>Zone of inhibition in mm</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>S. aureus</td>
<td>19</td>
<td>17</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>23</td>
<td>22</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>S. mutans</td>
<td>24</td>
<td>16</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Lactobacillus sps</td>
<td>26</td>
<td>23</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>24</td>
<td>22</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>E. coli</td>
<td>27</td>
<td>15</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>23</td>
<td>19</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>29</td>
<td>16</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>29</td>
<td>13</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>23</td>
<td>18</td>
<td>12</td>
<td>-</td>
</tr>
</tbody>
</table>
Costus has high source of bio active compounds against diabetic and pathogenic microbial infections (Anonymus, 1988). Apart from anti-diabetic potential, the selected plant also exhibits anti-antioxidant and anti-cancer properties (Asolkar et al., 1992; Vijayalaxmi and Sarada, 2008).

Reference