

ORIGINAL RESEARCH ARTICLE

Qualitative Study of *Costus speciosus* (Koen ex. Retz.) Sm. and its potentiality against Human Pathogenic Microbes

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Received 26 Feb 2014; Revised 28 Apr 2014; Accepted 12 May 2014

ABSTRACT

Objectives: To analyze the antimicrobial activity and qualitative phytochemical evaluation of *Costus speciosus* (Koen ex. Retz.) Sm.

Methods: *In-vitro* antimicrobial activity of *Costus speciosus* was evaluated by disc diffusion method, and qualitative phytochemical constituents were analyzed by Harborne method.

Results: Antimicrobial activity of Gram negative bacterial strain *Salmonella typhi* showed maximum inhibition at the concentration of 100 mg (24mm) in methanolic extract. Qualitative evaluation of *Costus speciosus* showed that maximum amount of phytochemical compounds in leaves by methanolic extract than the petroleum ether extract. In leaves more amount of saponins were present.

Conclusions: The results of the present study revealed that the leaves of *Costus speciosus* has high potent against Gram negative bacterial strain *Salmonella typhi* and high degree concentration of Saponins.

Key words: *Costus speciosus*, antimicrobial, qualitative evaluation, Saponin, *Salmonella typhi*.

1. INTRODUCTION

India is a varietal emporium of medicinal plants and is one of the richest country in the world in regard to genetic resources of medicinal plants. It exhibits a wide range of topography and climate, which has bearing on its vegetation and floristic composition. Moreover, the agro-climatic conditions are conducive for introducing and domesticating new exotic plant varieties [19]. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [15]. Several plants containing volatile oils, polyphenols and alkaloids as active constituent are utilized as popular folk medicines, while others gained popularity in the form of finished products collectively named phytomedicines. During the second half of the 20th century, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the classical antibiotics to investigate the antimicrobial activity of several medicinal plants. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [14].

Costus speciosus (Koen ex. Retz.) Sm. belongs to the family Zingiberaceae comprises 175 species in all over the world. It is commonly called Creep ginger. It can grow up to 5ft. tall in frost-free areas, but typically grow to about 6 ft. tall in cooler regions. In India *Costus speciosus* alone widely distributed at Western Ghats of Tamilnadu and some other moist places of India. The plant is mainly used for healing in burning sensation, constipation, leprosy, worm infection, skin diseases, fever, asthma, and bronchitis. The leaf infusion is used by the patients while bathing with high Febrifuge [30]. In the present study, qualitative analyses of phytochemical constituents and antimicrobial potentiality of *Costus speciosus* against human pathogens viz., Gram-negative species: *Escherichia coli*, *Salmonella typhi* and gram-positive species: *Staphylococcus aureus*, *Bacillus subtilis* and the two fungal species: *Aspergillus oryzae* and *Candida albicans*.

2. MATERIAL AND METHODS**2.1. COLLECTION OF PLANT MATERIAL**

The selected medicinal plant *Costus speciosus* was collected in Top slip of Western Ghats, Coimbatore District, Tamilnadu.

2.2. PREPARATION OF EXTR ACTS

The leaves were cleaned and shade dried. The dried leaves were grind into fine powder. The powder was subjected to extraction with petroleum ether, acetone, chloroform and methanol using soxhlet apparatus for 24 hours and the extract was condensed to remove the solvent. The residues from the extract were used for preliminary phytochemical and antimicrobial studies.

2.3. PHYTOCHEMICAL ANALYSIS

Preliminary phytochemical screenings were carried out using petroleum ether, acetone, chloroform and methanol extracts for the present study [13]. The following qualitative tests were performed using same extracts.

2.3.1. ALKALOIDS

2.3.2. MAYER'S TEST (POTASSIUM MERCURIC IODIDE)

1.36g Mercuric chloride was dissolved in 60ml of distilled water and 5g of potassium iodine in 100 ml of distilled water. The two solutions were mixed and diluted to 100ml with distilled acidic aqueous solution of samples, few drops of Mayer's reagent was added. Formation of white or pale precipitate showed the presence of alkaloids.

2.3.3. FLAVONOIDS

In a test tube containing 0.5ml of alcoholic extract of sample, 5-10drops of dilute hydrochloric acid and small piece of Zn or Mg were added and the solution was boiled for few minutes. The appearance of reddish pink or dirty brown colour showed the presence of Flavonoids.

2.3.4. GLYCOSIDES

A small amount of alcoholic extract of sample was dissolved in 1.0ml of water and then aqueous sodium hydroxide solution was added. Formation of yellow colour indicated the presence of Glycosides.

2.3.5. PHENOLS

2.3.6. FERRIC CHLORIDE TEST

To 1.0ml of alcoholic solution of sample, 2.0 ml of distilled water followed by few drops of 10% aqueous Ferric chloride solution were added. Formation of blue or green colour indicates the presence of Phenols.

2.3.7. TANNINS

2.3.8. LEAD ACETATE TEST

In a test tube containing about 5.0 ml aqueous extract of sample, add a drop of 1% solution of

lead acetate. A yellow red precipitate formation indicated the presence of Tannins.

2.3.9. SAPONINS

In a test tube containing about 5.0 ml of an aqueous extract of sample add a drop of solution bicarbonate. The mixture was shaken vigorously and kept for 3 minutes. A honey comb like material formation showed the presence of Saponins.

2.3.10. STERIODS

To 2.0ml of chloroform extract of sample, 1.0 ml of concentrated sulphuric acid was added carefully along the sides of the test tube. A red colour was produced in the chloroform layer showed the presence of Steroids.

2.3.11. RESINS

To 2.0ml of chloroform or ethonolic extract, 5-10 ml of acetic anhydride was added, and dissolved by gently by heating, cooling and then 6.5 ml of sulphuric acid was added. Bright purple colour was produced, indicates the presence of Resins.

3. ANTIMICROBIAL STUDY

3.1. Bacterial and fungal species

The seven bacterial species were used in this study are, the gram-negative species: *Escherichia coli*, *Salmonella typhi* and gram-positive species: *Staphylococcus aureus*, *Bacillus subtilis* and the two fungal species: *Aspergillus oryzae* and *Candida albicans*. They were identified according to standard phenotype tests.

3.2. Media used

Muller Hinton agar medium was used for both culturing and sub culturing of bacteria and Sabauraud agar medium is used for Fungi. Besides nutrient agar medium broth was prepared for culturing bacteria and fungi. All the media were autoclaved before culturing.

3.3. Determination of antimicrobial activity

Antimicrobial activity of each organic extract of plant samples (50 and 100 mg/mL) were evaluated by paper disc diffusion method. Stock cultures of test bacteria and fungi were grown in nutrient broth medium at 37°C for 24 hours. A lawn culture was prepared on Muller-Hinton agar using sterile cotton swabs. All the fungal cultures were inoculated in Sabauraud Dextrose Agar plates. Sterile filter paper discs (6 mm for bacteria and fungi) were placed on these cultures and impregnated with reconstituted extract in minimum amount of solvent at the concentrations of 1mg/ml. These were then placed on the culture

plates previously seeded with the 0.5 McFarland and 10⁶cfu/ml cultures of bacteria and fungi, respectively. Paper discs impregnated with 20 µl of a solution of 10 mg/ml of Streptomycin as standard antimicrobials were used for comparison. Antimicrobial activity was determined by the measurement of the inhibition zone around each paper disc. For each extract three replicate trials were conducted against each organism. Organism of fungal and bacterial strains had significant inhibition in all extracts in the period of 24 hrs. The antimicrobial activity showing zone of inhibition in millimeter and as percentage (calculated by taking Streptomycin for Bacteria and Fungi as positive control with 100 percent inhibition) for Bacteria and Fungi were calculated

4. RESULTS

Table 1 Preliminary Phytochemical test for the presence of active constituents in *Costus speciosus* (Koen ex. Retz.)Sm

| Extracts | Flavanoids | Alkaloids | Glycosides | Steroids | Phenols | Tannins | Saponins | Resins |
|-----------------|------------|-----------|------------|----------|---------|---------|----------|--------|
| Petroleum ether | - | - | + | - | - | - | - | - |
| Acetone | + | - | - | - | - | - | + | - |
| Chloroform | - | + | - | - | - | - | - | - |
| Methanol | + | + | + | + | + | + | + | + |

+: Present; - : Absent

4.2. Antimicrobial activity of *Costus speciosus*

The antimicrobial activity of *Costus speciosus* leaves has been evaluated *in vitro* against four bacterial strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli* and two fungal strains like *Aspergillus oryzae* and *Candida albicans* depicts the results (Table 2). Petroleum ether, Acetone, Chloroform and Methanol extracts were screened against bacteria and fungi using disc diffusion method. Gram negative bacterial strain *Salmonella typhi* showed maximum inhibition at the concentration of 100 mg (24mm) in methanol extract. The gram positive bacterial strain *Staphylococcus aureus* showed higher inhibition in chloroform extract of *C. speciosus* leaves and in acetone extract 100 mg concentration (28 mm). The gram positive bacteria *Bacillus subtilis* showed inhibition in 50 mg (14mm) of *Costus speciosus* leaf extract. The methanol and

4.1. Preliminary Phytochemical test for the presence of active constituents in

Costus speciosus (Koen ex. Retz.)Sm

The results of preliminary phytochemical screening were given in the (Table 1). The compounds like Alkaloids, Flavonoids, Glycosides, Steroids, Phenols, Tannins, Saponins and Resins are the preliminary phytochemicals present in this plant leaves. Maximum amount of all the compounds like Alkaloids, Flavonoids, Glycosides, Steroids, Phenols, Tannins, Saponins and Resins in leaves were present in Methonolic extract than the petroleum ether extract. In leaves more amount of saponins were present than the other compounds.

Chloroform extract of leaves of *Costus speciosus* showed higher antibacterial activity against the gram negative bacteria, *Salmonella typhi* (14mm, 24mm).

The gram negative bacteria *E.coli* showed higher inhibition (11mm) in Chloroform extract of *Costus* leaves. The antifungal activity of *Costus speciosus* leaves against two fugal species namely *Aspergillus oryzae* and *Candida albicans* showed that maximum inhibition zone were observed in high concentration 100 mg (11mm). The minimum inhibition was observed in low concentration 50 mg (6mm) of *Candida albicans*. The yeast *Candida albicans* showed susceptibility to 50 mg and 100 mg of Chloroform extract (6mm and 9 mm). There was no activity in Petroleum ether extract against both bacteria and fungi. In all the three solvents the antimicrobial activity was very high in 100% concentration alone.

Table 2: Antimicrobial activity of leaves of *Costus speciosus* (Koen ex. Retz.) Sm. using different solvents

| S. No | Organism | Diameter of zone of inhibition | | | | | | | | | | | |
|-------|------------------------------|--------------------------------|----|-----|---------|----|-----|------------|----|-----|----------|----|-----|
| | | Petroleum ether | | | Acetone | | | Chloroform | | | Methanol | | |
| | | C | 50 | 100 | C | 50 | 100 | C | 50 | 100 | C | 50 | 100 |
| 1 | <i>Staphylococcus aureus</i> | 25 | - | - | 23 | 8 | 28 | 20 | 12 | 16 | 20 | 12 | 13 |
| 2. | <i>Bacillus subtilis</i> | 25 | - | - | 25 | 14 | 8 | 26 | 10 | 6 | 25 | 10 | 8 |
| 3. | <i>Salmonella typhi</i> | 30 | - | 7 | 26 | 10 | 7 | 26 | 14 | 6 | 24 | 11 | 24 |
| 4. | <i>Escherichia coli</i> | 15 | - | - | 16 | 7 | 9 | 6 | 8 | 11 | 17 | 8 | 6 |
| 5. | <i>Candida albicans</i> | - | - | 8 | - | - | 9 | - | 9 | 11 | - | 8 | 12 |
| 6. | <i>Aspergillus oryzae</i> | - | - | 8 | - | 8 | 10 | - | 6 | 9 | - | 8 | 10 |

C: Control (Streptomycin)

5. DISSUSSION

In the present study petroleum ether, acetone, chloroform and methanol were used as solvent source for the extraction of the metabolites. Since the polarity of methanol is higher, most of the secondary metabolites of *C. speciosus* leaves were dissolved. The curative properties of medicinal plants perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc.^[1] Plant derived natural products such as flavonoids, terpenoids and steroids etc. have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity^[11, 29].

In the present study the maximum amount of all the compounds in leaves were present in methanolic leaf extract than the petroleum ether extract. In leaves more amount of saponins were present than the other compounds. Saponin is a mild detergent used in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hyper cholestrolaemia, hyperglycemia, antioxidant, anticancer, antiinflammatory and weight loss, etc. It is also known to have anti-fungal properties^[12]. Saponins have been implicated as bioactive antibacterial agents of plants^[17, 18].

The gram negative bacteria, *Staphylococcus aureus* was reported to be resistant to acetone extracts of *Solanum tomentosum*^[3]. However, in the present investigation acetone extracts of leaves of *Costus speciosus* suppressed the growth of these bacteria significantly. The methanol and Chloroform extract of leaves of *Costus speciosus* showed higher antibacterial activity against the gram negative bacteria, *Salmonella typhi*. This same results were revealed previously^[7]. Phenolic phytochemicals have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory^[6,24]. The yeast *Candida albicans* showed susceptibility to 50% and 100% of chloroform extracts of *Costus speciosus* leaves (6mm and 9 mm). The susceptibility of this yeast to different plant extract has been documented in previous work.^[2]

The present study is in conformity with the results of Saponin in *Tecoma stans*^[25]. Highest phenolics were found to be in the leaves and inflorescence of *Parthenium hysterophorus* showed highest antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* were reported^[28]. Similarly Tannins are known to possess general

antimicrobial and antioxidant activities^[23]. Recent reports showed that tannins may have potential value as cytotoxic and antineoplastic agents^[4]. Other compounds like saponins also have antifungal properties^[5, 16]^[9]. Antimicrobial activity of Stem bark of *Helicteres isora* showed antimicrobial activity against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli*.

In this study, Petroleum ether extract did not inhibit any tested bacteria however methanol extract inhibited the growth of Gram-positive bacteria such as *S. aureus* and *B. subtilis*.^[26] Methanol and Chloroform extracts of *C. speciosus* rhizome inhibited the growth of *S. aureus* (12 mm). Our study showed that methanol extract inhibited the growth of *S. aureus* (13 mm), and *B. subtilis* (12 mm).^[10] Methanol extract of *C. speciosus* showed activity against *E. coli*, *Salmonella enterica* and *S. aureus*.^[20] Saponins and flavonoids were present in plant extracts have varied uses as antiulcerogenic, anti-inflammatory, fibrinolytic, antipyretic, analgesic and anti-edematous. The activity of the extracts against *S. typhi*, *E. coli* and *V. cholera* which are the potential causative agents of abdominal ailment agreed with previous work^[27]. The stem bark and leaf is used traditionally in treatment of typhoid fever and various stomach related problems^[21]. In this work the extracts of the plant leaf was inhibited the growth of *E. coli*, and *S. typhi* to a high degree. These bacteria were responsible for various stomach related illnesses; *S. typhi* a causative organism of typhoid fever, a systemic infection associated with the consumption of contaminated food^[2].

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