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Evaluation of *in vitro* antimicrobial potential and GC–MS analysis of *Camellia sinensis* and *Terminalia arjuna*

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ABSTRACT

Traditionally, *Camellia sinensis* and *Terminalia arjuna* are being used widely to cure various diseases like cardiovascular diseases, cancer etc. In the present study, extracts of these plants were evaluated for their antimicrobial activities against some human pathogenic bacteria *viz. E. coli*, *P. aeruginosa*, *S. aureus* and fungus *C. albicans*. *In-vitro* inhibition of these pathogenic microorganisms produced inhibition zone ranging from 9 to 18 mm. MIC values of these plant extracts ranged from 6.25 to 12.5 mg/ml. MBC of *C. sinensis* for *E. coli*, *P. aeruginosa* and *S. aureus* was found to be 50 and 12.5 mg/ml, respectively. In case of *T. arjuna*, the MBC of all the tested microorganisms was found to be 25 mg/ml. The MFC of *C. sinensis* and *T. arjuna* against *C. albicans* was observed to be 50 and 25 mg/ml, respectively. GC–MS analysis of *C. sinensis* and *T. arjuna* extract identified 13 and 21 compounds, respectively.

1. Introduction

Medicinal plants are always recognized as rich source of antimicrobial agents and are widely used by different countries for medicinal purposes as they are powerful and potent sources of drugs. Over the years, World Health Organization (WHO) has advocated traditional medicines as safe remedies for ailments of both microbial and non-microbial origins [1]. Now a days synthetic drugs are widely used but their excessive use may cause severe side effects in body and these effects sometimes are more serious than that of disease itself. Hence, in order to overcome this problem, pharmaceutical companies are spending a lot of money and time for the formulation of the natural drugs from the medicinal plant extracts to produce cost effective remedies that are affordable for common person. Recently effectiveness of antimicrobial activity of five medicinal plants was evaluated against 8 multidrug-resistant (MDR) enteropathogenic bacteria that were isolated from clinical samples of under-5 hospitalized children [2]. Due to the rising incidences related to multidrug resistance amongst pathogenic microorganism, there is need to find out new antimicrobial sources. Plants have the ability to produce a number of compounds in the form of secondary metabolites that have diverse biochemical properties. Amount of these secondary metabolites varies species to species and plant to plant accordingly and the variations among different species depend on the age and variations in climates and ecological factors [3]. These secondary metabolites play important role in protection of plants against microorganisms, insects and phytophagous [4]. Many plant extracts of higher plants have been studied under laboratory trails and are found to exhibit antimicrobial properties [5,6]. A number of different solvent system like water, ethanol, chloroform: methanol, petroleum ether have been reported to play important role for extraction of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, phenols and quinones [7]. *Camellia sinensis* is second largest beverage in world and is characterized by the presence of several components with anti-aging, anti-Alzheimer, anti-Parkinson, anti-stroke and anticancer properties [8]. On the other hand *Terminalia arjuna*, traditionally has been used as a cardio tonic and has been designated for instability of three humours *viz*., vata, pitta and kapha in Ayurveda. The philosophy of ayurvedic medicine is based on the principle that health exists due to a balance between three fundamental bodily bio-elements or doshas called Vata, Pitta and Kapha. The doshas derive from the five elements and their related properties. Vata is composed of space and air, Pitta of fire and water, and Kapha of earth and water. Bark of *T. arjuna* has been broadly used in traditional system of medicine for variable purposes [9].

In the present study, an attempt has been made to investigate the antimicrobial activity of *Camellia sinensis* and *Terminalia arjuna* extract against 5 test microorganisms, fungus *Candida albicans*, one

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gram positive bacterium *Staphylococcus aureus* and two gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. Further, these extracts were subjected to GC–MS analysis for the presence of various components that are responsible for their antimicrobial properties.

2. Material and methods

2.1. Collection of plant material

The leaves of *Terminalia arjuna* were collected from Ambala College of Engineering and Applied Research, Ambala, India. Dried leaves of *Camellia sinensis* were purchased from local market of Ambala Cantt., India.

2.2. Test microorganisms

Microbial strains, *C. albicans*, *E. coli*, *P. aeruginosa*, and *S. aureus* used in the present study were purchased from IMTECH, Chandigarh, India. The microbial cultures were maintained in cultural broth (Himedia) at 37 °C and on agar (Himedia) plates at 4 °C.

2.3. Plant extracts preparation

Plant materials were finely grinded to powder by using a blender. Five gram of powdered plant material was kept in 100 ml conical flask and added 50 ml of chloroform: methanol (1:1) solvent. The mouth of the conical flask was enclosed with aluminium foil and kept in a shaker. After 2 days, extract was filtered by using muslin cloth followed by Whatman no. 1 filter paper. The solvent was removed through evaporation by using water bath at 65 °C. Finally, the residues were collected and dissolved in 70% acetone for further use in the experiment [10]. The extracts were stored at 4 °C in the refrigerator until use. Further, all the plant extracts were screened for their antimicrobial activity.

2.4. Screening of antibacterial activities

Antimicrobial activity of the crude extracts was determined by agar well diffusion method [11]. Immediately after autoclaving, the media was allowed to cool at 45 °C to 50 °C. The freshly prepared and cooled media was poured into petri dishes (90 mm in diameter) placed on a level. The agar media was allowed to cool and solidify at room temperature and the plates were incubated at 35 °C for 18–20 h before use to confirm sterility. About 0.1 ml of the test inoculum was evenly spread on the surface of the solidified agar media and spread it on plate evenly by using sterile spreader. Four equidistant wells of 8 mm in diameter and 3 mm in depth were then made on the agar plate. About 100 μl of the each plant extract was filled into the wells. As control, 70% acetone was used. The plates were then incubated for 24 h at 37 °C for bacteria and for 48 h at 30 °C for fungus *Candida*. Antimicrobial activity was determined by measuring the diameters of zones of inhibition. The test was performed in triplicates with controls.

2.5. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

Minimum inhibitory concentration (MIC) of *C. sinensis* and *T. arjuna* extracts was determined using broth dilution method using 96 well plates [12,13]. The wells of each row were filled with 0.5 ml sterilized nutrient broth for *E. coli* and *P. aeruginosa*, Mannitol salt broth for *S. aureus* and malt extract broth for *C. albicans* followed by addition of 0.5 ml of a mixture of culture medium. Each well received plant extract, serially diluted to create a concentration ranging from 50 to 3.125 mg/ml. The plates were incubated aerobically at 37 °C for 24 h (for bacteria) and 25 °C for 48 h (for fungus). The lowest concentration (highest dilution) of the extract that produced no visible growth (no turbidity) in the first 24 h when compared with the control tubes was considered as initial MIC. The dilutions that showed no turbidity were incubated further for 24 h at 37 °C. The lowest concentration that produced no visible turbidity after a total incubation period of 48 h was considered as final MIC.

Minimum bactericidal/Fungicidal concentration (MBC/MFC) value was determined by sub culturing the test dilution that showed no visible turbidity on to freshly prepared respective agar media. The plates were incubated further for 42 h at 37 °C. The highest dilution that yielded no single bacterial colony on the nutrient agar plates was taken as MBC.

2.6. Gas Chromatography–Mass Spectroscopy analysis (GC–MS)

GC–MS analysis of *C. sinensis* and *T. arjuna* extract was carried out on a Trace 1300 GC, Tsq 8000 Triple Quadrupole MS with a column TG 5MS (30 m × 0.25 mm, 0.25 μm). Helium was used as a carrier gas at a flow rate of 1 ml/min. Split/Splitless (S/SL) injector was used with 250 °C injector temperature. 1.0 μl sample injection volume was utilized. Ion source temperature was maintained at 230 °C. The oven temperature was programmed initially at 80 °C for 2 min, then programmed to increase to 280 °C at a rate of 5 °C/min ending with a 5 min isothermal at 280 °C. Total run time was 36.12 min and 36.08 for *C. sinensis* and *T. arjuna*, respectively. The MS transfer line was maintained at a temperature of 250 °C. TSQ 8000 Triple Quadrupole MS detector was used for analysis and data was evaluated using total ion count (TIC) for compound identification and quantification. The mass spectra of the components were matched with the data available in the National Institute of Standards and Technology (NIST) library. Measurement of peak areas and data processing were carried out by XCALIBER software [14].

3. Results

3.1. Determination of antimicrobial activity of plant extracts

In the present study, the antimicrobial activity of *C. sinensis* and *T. arjuna* plant extracts prepared in chloroform: methanol (1:1) was determined against *E. coli*, *P. aeruginosa* (gram negative), *S. aureus* (gram positive) and fungus *C. albicans*. The results presented in Table 1 revealed that both plant extracts displayed potential antibacterial activity against all tested organisms. *C. sinensis* demonstrated highest antibacterial activity against *C. albicans* and *S. aureus* (15 mm). It produced an inhibition zone of 10 mm and 9 mm against *P. aeruginosa* and *E. coli*, respectively. Similarly, *T. arjuna* also exhibited maximum antimicrobial activity against *C. albicans* (18 mm) followed by *E. coli* (14 mm), *P. aeruginosa* and *S. aureus* (12 mm each).

The minimum inhibitory concentration (MIC) of both plant extracts was determined by using 96 well plates through broth dilution method. MIC of *C. sinensis* extract was 12.5 mg/ml against *C. albicans*, *E. coli* and *P. aeruginosa* and 6.25 mg/ml against *S. aureus* whereas, *T. arjuna* had MIC of 12.5 mg/ml against all tested microorganisms (Table 2). After the determination of the MIC, the minimum bactericidal/fungicidal concentration (MBC/MFC) was calculated and the results are presented in Table 3. As per the results, the MBC of *C. sinensis* for *E. coli* and *P. aeruginosa* was found to be 50 mg/ml which is 4 times higher than their MIC. Growth of *S. aureus* was found to be unaffected at its MIC (6.5 mg/ml). However, its growth was inhibited at MBC of 12.5 mg/ml. In case of *T. arjuna*, the MBC of all the tested microorganisms was found to be 25 mg/