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

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**A B S T R A C T**

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 25 and 25 mg/ml, respectively. GC–MS analysis of *C. sinensis* and *T. arjuna* extract identified 13 and 21 compounds, respectively.

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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 antiaging, 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 ground 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 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, Tsg 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. TSG8000 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/
The MFC of *Camellia sinensis* and *Terminalia arjuna* against *C. albicans* was observed to be 50 mg/ml and 25 mg/ml, respectively.

### 3.2. GC–MS Analysis

GC–MS chromatograms of chloroform: methanol extract of *C. sinensis* and *T. arjuna* for different retention time are given in Figs. 1 and 2, respectively. The number and nature of phytochemical constituents as depicted by the peaks were characterized and identified by comparing the mass spectra of the constituents with NIST library. Phytochemical analysis of *C. sinensis* by GC–MS analysis revealed five peaks corresponding to the presence of 13 compounds (Table 4). The most abundant compounds observed were Caffeine (C₈H₁₀N₄O₂), 1,4-Dimethyl-4,5,7,8-tetraydroimidazo-[4,5-E]-1,4- Diazepin-5,8(6H)-dione (C₁₈H₁₈N₄O₂) and 2-Fluorobenzylamine,N,N-dibutyl(C₁₅H₂₆FN) collectively representing 48.21% of the total area. This was followed by 6-Octadecenolic acid, cis-vaccenic acid, trans-13-Octadecenolic acid trans-13-Octadecenoic acid (18.30%), Erucic acid, cis-13-Eicosenoic acid and cis-11-Eicosenoic acid (15.15%), Anethole (14.83%) and cis-13-Eicosenoic acid, cis-11-Eicosenoic acid, cis-10-Nonadecenoic acid (3.50%).

In comparison to *C. sinensis*, GC–MS analysis of *T. arjuna* extract exhibited 13 peaks predicting the presence of 21 compounds (Table 5). Out of these compounds, anethole was the most significant constituent representing 61.41% of the total area. Other major components observed were 1-Monolinoleoylglycerol trimethylsilyl ether (8.13%), 8, 14- Seco-3, 19-epoxycarotane-8, 14-dione, 17-acetoxy-3α-methoxy-4,4-dimethyl (4.40%), Methyl (9Z)-9-hexadecenoate (3.92%), Heptadecyl ester, 3-chloropropanoate and Palmitoleic acid (3.89%). Apart from these compounds, other compounds present in the *T. arjuna* extract were decamethylcyclpentasiloxane, Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester, 2H,1,4-Benzodiazipin-2-one 7-chloro-1,3-dihydro-5-phenyl-1-(trimethylsilyl), Silane, dimethyl(dimethyl(dimethyl (2-isopropylphenoxy)silyloxy)silyloxy)/(2-isopropylphenoxy), tetradecamethylcycloheptasiloxane, methyl (9Z)-9-hexadecenoate, dipentyl phthalate, phthalic acid, butyl 8-chloroocyt ester, dibutyl phthalate, 8,14-Seco-3,19-epoxycarotane-8,14-dione, 17-acetoxy-3α-methoxy-4,4-dimethyl, 1-Monolinoleoylglycerol trimethylsilyl ether and 8,14-Seco-3,19-epoxycarotane-8,14-dione, 17-acetoxy-3α-methoxy-4,4-dimethyl.

### 4. Discussion

Recently, control of the infectious disease in hospitals and societies due to the development of multi drug resistant microorganisms is a serious issue between the world wide scientists. Traditionally, plants have been mentioned for their benefits to treat
various diseases. In this present study we have studied *Camellia sinensis* and *Terminalia arjuna* for their antimicrobial properties that make them suitable for therapeutic treatment. The antimicrobial activity of medicinal plants in chloroform: methanol solvent was determined by agar well diffusion method. The present results are in accordance to Zakir et al. [15] who studied antimicrobial activity of *C. sinensis* against human pathogenic bacteria *E. coli*, *P. aeruginosa*, *S. aureus* and fungal strain *C. albicans*. All the tested bacteria exhibited prominent sensitivity towards the green tea extract. Similarly, Radji et al. [16] also reported significant antimicrobial activity of *C. sinensis* against *S. aureus* ATCC25913, MRSA, *P. aeruginosa* ATCC 27853 and MDR- *P. aeruginosa*. Some authors have also reported antibacterial activity of *C. sinensis* against *S. aureus* and *P. aeruginosa*, separately [17,18].

The results of antimicrobial activity of *Terminalia arjuna* are also in agreement to Jaiswal and Kumar [19] who assessed antibacterial activity of *T. arjuna* against six different pathogenic bacteria, *Escherichia coli* (MTCC 46), *Pseudomonas aeruginosa* (MTCC 1934), *Raoultella planticola* (MTCC 2271), *Enterobacter aerogenes* (MTCC 2822), *Bacillus subtilis* (MTCC 121), *Agrobacterium tumefaciens* (MTCC-431). It was observed that all the extracts showed significant antimicrobial potential against test microbes. Likewise, Mandal et al. [20] also observed significant antimicrobial activity of *T. arjuna* against *S. aureus* and *E. coli*. Results of the present study are also in accordance to Aneja et al. [21] who reported significant antimicrobial activity of *T. arjuna* against *S. aureus*, *P. aeruginosa* and *E. coli*.

Most of the studies in the scientific literature do not demonstrate any antifungal activity of *C. sinensis* and *T. arjuna* against *C. albicans*. However, in the present study both the extracts exhibited significant activity against *C. albicans*. Moreover, there are also few earlier studies in which both the extracts have been found to be effective against *C. albicans*. Zakir et al. [15] reported significant activity of green tea against *C. albicans*. Similarly, Chen et al. [22] and Debnath et al. [23] also observed antifungal activity of *C. sinensis* and *T. arjuna* against *C. albicans*, respectively.

Both extracts had MIC of 12.5 mg/ml against tested microorganism except that of *C. sinensis* against *S. aureus* which was found to be 6.25 mg/ml. MBC values of both extracts were found to be higher than their respective MIC values against tested microorganisms, indicating the bacteriostatic effects of the extracts.

GC–MS analysis of *C. sinensis* and *T. arjuna* detected 13 and 21 components, respectively at different retention time. At a particular retention time, components are separated out according to their mass/charge ratio. GC–MS analysis of *C. sinensis* extract revealed the presence of a number of components with highest peak area at 24.42 RT: Caffeine; 1,4-Dimethyl-4,5,7,8-tetrahydroimidazo-[4,5-E]-1,4-Diazepin-5,8(6H)-dione; and 2-Fluorobenzylamine, N,N-dibutyl and lowest peak area at 33.41 RT: Caffeine, the main component of *C. sinensis* is known to exhibit numerous biological functions. It has been shown to cause inhibition of phosphodiester leading to increased level of intracellular cAMP as well as blockage of enzymes like 5’ nucleotidase and alkaline phosphatase. In the present study, caffeine is presumed to be largely responsible for the antimicrobial activity of *C. sinensis*. Its antimicrobial properties are justified by some previous studies in which it has been shown to exhibit inhibitory effects against *Staphylococcus aureus*, *E. coli* [24,25] as well as *Candida albicans* [26]. The present antimicrobial activity of caffeine may be due to its ability to inhibit synthesis of proteins and DNA by inhibiting the incorporation of adenine and thymidine.

In the present study, nearly all compounds are fatty acids and phenylpropane that are generally utilized as flavour and fragrance agents and in cosmetics also. Similar kind of results have also been observed by Ahmed et al. [27] who reported the presence of methyltetradecanoate/myristic acid, hexadecanoic acid/palmitic acid, octadecanoic acid/stearic acid, 9-octadecenoic acid/oleic acid, 9,12-octadecenoic acid/linoleic acid, 1H-PURINE-2,6-DIONE/caffeine, pthalic acid, diethyl ester and 1H-PURINE-6-AMINE in C.
sinensis extract. Lee et al. [28] identified and quantified 39 volatile compounds by using GC–MS analysis in their study. Some of the compounds (e.g., geraniol, linalool, indole, and jasmine) were similar to those found in brewed Japanese green tea samples [29]. Another report on GC–MS analysis of C. sinensis showed the presence of 60 volatile compounds with terpenes and esters as two major groups representing 33.89% and 15.53% of the total peak area, respectively [30].

GC–MS analysis of T. arjuna extract showed the highest peak area at 9.15 RT with presence of Anethole (61.41%) as detected by using the NIST library as reference. The lowest peak area was observed for Heptadecyl ester, 3-chloropropanoate, Palmitoleic acid at 21.21 RT. This is probably the first report indicating such a high percentage of anethole in case of T. arjuna. The antimicrobial property of this medicinal plant might be attributed to the abundance of anethole since it known to possess potent antimicrobial properties, against bacteria, yeast and fungi. It offers numerous pharmacological properties and was found to inhibit bacteria such as Salmonella typhimurium and Staphylococcus aureus [31]. Anethole has also been reported to enhance the antifungal

![Fig. 2. Total Ion Chromatogram (TIC) of Terminalia arjuna extract.](image)

**Table 4**

| Peak | R. time | Chemical formula         | Compound                                      |
|------|---------|--------------------------|-----------------------------------------------|
| 1    | 9.10    | C10H12O                  | Anethole                                      |
| 2    | 24.42   | C8H10N4O2                | Caffeine                                      |
|      | 24.42   | C15H24FN                 | 1,4-Dimethyl-4,5,7,8-tetrahydromidazo-[4,5-E]-1,4- Diazepin-5,8(6H)-dione |
|      | 24.42   |                          | 2-Fluorobenzylamine, N,N-dibutyl              |
| 3    | 29.94   | C18H34O2                 | 6-Octadecenoic acid                          |
|      | 29.94   | C18H34O2                 | cis-Vaccenic acid                            |
|      | 29.94   | C18H34O2                 | trans-13-Octadecenoic acid                   |
| 4    | 33.41   | C20H38O2                 | cis-13-Eicosenoic acid                       |
|      | 33.41   | C20H38O2                 | cis-11-Eicosenoic acid                       |
|      | 33.41   | C20H38O2                 | cis-10-Nonadecenoic acid                     |
| 5    | 36.60   | C22H42O2                 | Erucic acid                                  |
|      | 36.60   | C20H38O2                 | cis-13-Eicosenoic acid                       |
|      | 36.60   | C20H38O2                 | cis-11-Eicosenoic acid                       |

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activity of polygaloid against Saccharomyces cerevisiae and Candida albicans. It is mostly used as a flavoring agent in the food industry, used in cakes and ice-creams and has also applications in alcoholic beverages.

In another report, Chaudhari and Mahajan [32] carried out GC–MS analysis of the T. arjuna Roxb and revealed the presence of 10 major compounds (Steroid, alcohol, esters, fatty acid esters, saponins, secondary amines, steroidal saponins and alkaliphatic, aromatic, alkane hydrocarbons). Similarly, Ramesh and Dhanraj [33] carried out GC–MS analysis of T. arjuna and reported more than 25 compounds. These compounds were mostly phenolic derivatives and included hydrocarbons, alcholic compounds, flavonoids, alkaloids, ketones, carboxydrates, fatty acid ester, alkenes compounds, fatty acids. Results of this study are further supported by Subramaniam [34] who observed the presence of high amount of plant sterols in GC-MS studies of T. arjuna extracts. In the present report, most of the compounds observed in case of C. sinensis and T. arjuna belong to phenols, fatty acids, steroids, phenylpropene etc. and are generally used as antimicrobial agents, plasticizer, pesticides, flavour and fragrance agents.

5. Conclusion

Camellia sinensis and Terminalia arjuna are traditional medicinal plants and represent rich source of compounds possessing antimicrobial properties. Till now, little work has been carried out on their biological properties and hence extensive research is required to explore and identify the potential biological compounds of medicinal importance. The results of the present study revealed that C. sinensis and T. arjuna could be used as powerful antimicrobial agents for the prevention of many diseases. Further study can be extended to check their ant-oxidative properties. Additionally, these plant extracts could be examined in vivo for better understanding of their safety and efficacy.

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