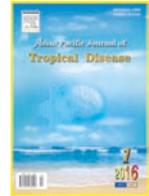


Contents lists available at [ScienceDirect](http://www.sciencedirect.com)

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Original article

doi: 10.1016/S2222-1808(15)60978-1

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Phytochemical analysis and antimicrobial activity of some medicinal plants against selected common human pathogenic microorganisms

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ARTICLE INFO

Article history:

Received 28 Sep 2015

Received in revised form 26 Oct 2015

Accepted 12 Dec 2015

Available online 5 Jan 2016

Keywords:

Antimicrobial activity
Phytochemical analysis
Antibiotics susceptibility
Synergistic activity
Potent plant extracts

ABSTRACT

Objective: To study the antimicrobial activity and phytochemicals of extracts from 5 different medicinal plants, as well as to evaluate the synergistic activity of potent plant extracts with suitable antibiotic discs and antibiotics susceptibility of tested microorganisms.

Methods: The antimicrobial activities of different extracts were evaluated by using agar well diffusion method and antibiotics susceptibility of five selected microorganisms was tested by using disc diffusion method. For determination of synergistic activities of the potent plant extracts along with antibiotic discs, agar well diffusion and disc diffusion methods were combinedly used.

Results: In the present investigation, the maximum *in vitro* inhibition of tested microorganisms, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Staphylococcus aureus* was recorded in chloroform and methanol extracts of *Terminalia arjuna*, ethanol extract of *Camellia sinensis*, and petroleum ether extract of *Polyalthia longifolia* which offered inhibition zone ranged from 11 to 18 mm. The maximum antibacterial efficacy was exhibited by levofloxacin with an inhibition zone of 35 mm against *Escherichia coli*. The potent plant extracts showed positive synergistic effects against *Staphylococcus aureus* with lincomycin. The phytochemical analysis of the potent plant extracts revealed the presence of saponin, tannin, protein, carbohydrate, flavonoid, terpenoids and glycosides.

Conclusions: According to the present study, *Camellia sinensis*, *Terminalia arjuna* and *Polyalthia longifolia* can be used as a potent source of natural antimicrobial agents by replacing commercially available synthetic drug that may have a large number of side effects.

1. Introduction

In the last three decades, a large number of antibiotics and other synthetic drugs were produced in the world with an aim of eradicating the microorganisms which were responsible for many diseases[1]. However, these drugs or antibiotics induced mutations in the genetic composition of these microorganisms rendering them resistant to several drugs or antibiotics[2]. Moreover, the side effects associated with the extensive use of the synthetic medicines may lead to serious damages to many of human organs. Therefore, to overcome this limitation of synthetic drugs, researchers have shifted their focus towards medicinal plants which are recognized as rich sources of antimicrobial agents and are widely used by different countries for medicinal purposes. Traditionally used medicinal plants are known to produce a variety of compounds with therapeutic properties, such as antidiabetic, antioxidant, antibacterial, anti-inflammatory, antipyretic, gastroprotective

effects, etc.[3]. These plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, phenols and quinones[4]. Even World Health Organization has considered plants as the richest source of multiple drugs and has consistently favoured the use of these traditional medicines to cure microbial and non-microbial diseases[5]. Various herbal species have been known to display antimicrobial properties by acting against foodborne pathogens and spoilage bacteria and be used as sources of natural antimicrobial substances for the treatment of infectious disease[6]. Moreover, various bioactive molecules extracted from medicinal plants can be used as antimicrobial and antioxidant additives in the food industry[7]. Today, a number of pharmaceutical companies are investing a lot of money and time to devise cost effective natural drugs from plant extracts[8]. There are several reports on the antimicrobial activity of different herbal extracts[9,10]. Most of the plants possess the ability to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections[11]. Selection of the potent plants and the solvent systems for extraction purpose plays a vital role in the recovery of biomolecules with the desired properties.

In the present study, an attempt has been made to compare the role of different solvent systems like water, chloroform, methanol,

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ethanol and petroleum ether for the extraction of secondary metabolites, such as terpenoids, alkaloids, flavonoids, tannins, phenols and quinones from different medicinal plants [*Tectona grandis* (*T. grandis*), *Camellia sinensis* (*C. sinensis*), *Euphorbia hirta* (*E. hirta*), *Polyalthia longifolia* (*P. longifolia*) and *Terminalia arjuna* (*T. arjuna*)]. These extracts were further investigated for their antimicrobial activity against fungus *Candida albicans* (*C. albicans*), two Gram-negative bacteria *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) and two Gram-positive bacteria *Propionibacterium acnes* (*P. acnes*) and *Staphylococcus aureus* (*S. aureus*).

2. Materials and methods

2.1. Collection of plant materials

Samples of medicinal plants were collected from different regions of Ambala District, Haryana, India. Initially plant material was washed with distilled water and shade dried on paper towels in the laboratory at 37 °C.

2.2. Tested microorganisms

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

2.3. Preparation of plant extracts

Plant materials were finely ground to powder by using a blender. Five grams of powdered plant materials were kept in 100 mL conical flask and 50 mL of solvents such as water, ethanol, petroleum ether and chloroform: methanol (1:1) was added separately. The mouth of the conical flask was enclosed with aluminium foil and kept in a shaker. After 2 days, the extract was filtered through muslin cloth followed by Whatman No. 1 filter paper. The solvent from aqueous extracts was removed by using lyophilization, and solvents from ethanol, petroleum ether and chloroform: methanol (1:1) extracts were removed by using water bath at 65 °C. Finally, the residues were collected and dissolved in sterile distilled water (for aqueous extract) and 70% acetone for others[12]. The extracts were stored at 4 °C in the refrigerator until they were used. Further, all the plant extracts were screened for their antimicrobial activity.

2.4. Screening of antimicrobial activities

The antimicrobial activities of the crude extracts were determined by agar well diffusion method[13]. Immediately after autoclaving, the media was allowed to cool at 45 to 50 °C. The freshly prepared and cooled media was poured into flat-bottomed Petri dishes (90 mm in diameter) and placed on a level and horizontal surface to give a uniform depth of almost 4 mm. 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 they were used to confirm sterility. Then 0.1 mL of the tested inoculum was evenly spread on the surface of the solidified agar by using sterile spreader. Four equidistant wells of 8 mm in diameter and 3 mm in depth were made on the agar plate. About 100 µL of the plant extracts was filled into the

wells. The bacterial agar plates were incubated aerobically for 24 h at 37 °C except *P. acnes* which was incubated under anaerobic condition. The fungal *C. albicans* plates were incubated for 48 h at 30 °C. The antimicrobial activities were determined by measuring the diameters of inhibition zones (mm). The test was performed in triplicates with controls (70% acetone).

2.5. The antimicrobial susceptibility pattern of tested microorganisms

The antimicrobial susceptibility pattern of tested microorganisms was determined by using disc diffusion method[14]. The freshly prepared and cooled media was poured into flat-bottomed Petri dishes, and 0.1 mL of the tested inoculum was evenly spread on the surface of the solidified agar media using sterile spreader. Suitable antibiotics/antifungal discs were placed on the agar plates and incubated for 24 h. The antimicrobial susceptibility was determined by measuring the diameters of inhibition zone (mm).

2.6. The synergistic antimicrobial activities of potent plant extracts with antibiotics

For the synergistic activities of potent plant extracts with antibiotics which have shown the largest zone of inhibition against respective microorganism, levofloxacin (for Gram-negative bacteria) and lincomycin (for Gram-positive bacteria) were used with extracts. About 0.1 mL of the tested inoculum was evenly spread on the surface of the solidified agar media by using sterile spreader. After few minutes, one well of 8 mm in diameter and 3 mm in depth was made in the mid of agar plate. Then 100 µL of the plant extract was filled into the wells followed by placing the antibiotic disc on the well. The plates were incubated at 35 °C for 18–20 h. The synergistic activity was determined by measuring the diameters of inhibition zones (mm) and further comparing with inhibition zones of extracts and antibiotics against respective microorganisms[15].

2.7. Phytochemical analysis

All the potent extracts were subjected to phytochemical analysis by dissolving them in respective solvents. The extracts were screened for the presence of alkaloids, flavonoids, saponins, tannins, carbohydrates, terpenoids, protein, glycosides, diterpenes and steroids by using the standard procedures[16-19].

2.8. Statistical analysis

The data was analyzed by using simple arithmetic means of the different extracts and the SE was compared with the controls.

3. Results

3.1. Determination of antimicrobial activity of different plant extracts

In the present study, the antimicrobial activity of extracts of 5 diverse medicinal plants prepared in different solvents was evaluated against *E. coli*, *P. aeruginosa* (Gram-negative), *P. acnes*, *S. aureus* (Gram-positive) and fungus *C. albicans*. Most of the plant extracts exhibited antimicrobial activity against these pathogenic microorganisms, and the results were summarized in Table 1. Although all the plant extracts revealed antimicrobial

Table 1

The antimicrobial activities of different plant extracts against pathogenic microorganisms. mm.

Plants	Parts used	Solvents used	Gram-negative bacteria		Fungus	Gram-positive bacteria	
			<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>P. acnes</i>	<i>S. aureus</i>
<i>T. grandis</i>	Bark	Aqueous	NA	NA	NA	NA	NA
		C:M	NA	NA	NA	NA	NA
		Ethanol	4.0 ± 0.1	6.0 ± 0.3	5.0 ± 0.2	NA	13.0 ± 0.3
		Petroleum ether	NA	NA	NA	NA	NA
<i>C. sinensis</i>	Leaves	Aqueous	4.0 ± 0.2	8.0 ± 0.3	10.0 ± 0.1	NA	12.0 ± 0.1
		C:M	9.0 ± 0.1	10.0 ± 0.4	15.0 ± 0.1	4.0 ± 0.3	15.0 ± 0.1
		Ethanol	11.0 ± 0.2	13.0 ± 0.3	16.0 ± 0.2	4.0 ± 0.1	17.0 ± 0.3
		Petroleum ether	NA	NA	NA	NA	NA
<i>E. hirta</i>	Leaves	Aqueous	6.0 ± 0.4	NA	12.0 ± 0.3	NA	3.0 ± 0.3
		C:M	NA	NA	NA	NA	NA
		Ethanol	9.0 ± 0.2	4.0 ± 0.3	17.0 ± 0.1	NA	13.0 ± 0.2
		Petroleum ether	NA	NA	NA	NA	NA
<i>P. longifolia</i>	Leaves	Aqueous	NA	NA	8.0 ± 0.5	NA	10.0 ± 0.2
		C:M	NA	NA	14.0 ± 0.3	NA	13.0 ± 0.2
		Ethanol	NA	NA	15.0 ± 0.1	NA	12.0 ± 0.3
		Petroleum ether	NA	NA	18.0 ± 0.2	NA	13.0 ± 0.3
<i>T. arjuna</i>	Leaves	Aqueous	NA	NA	7.0 ± 0.2	NA	NA
		C:M	14.0 ± 0.6	12.0 ± 0.2	18.0 ± 0.2	6.0 ± 0.1	12.0 ± 0.2
		Ethanol	2.0 ± 0.1	7.0 ± 0.1	15.0 ± 0.5	NA	10.0 ± 0.5
		Petroleum ether	8.0 ± 0.3	3.0 ± 0.3	8.0 ± 0.3	NA	7.0 ± 0.3

Values are means of three replicates. NA: No activity exhibited by extracts against microorganism; C:M: Chloroform: methanol (1:1).

activity, they differed in their relative activities against the tested microorganisms. The highest antimicrobial activity was observed with chloroform: methanol extracts of *T. arjuna* and petroleum ether extract of *P. longifolia* against *C. albicans* (18 mm), respectively followed by ethanol extract of *C. sinensis* and *E. hirta* against *S. aureus* and *C. albicans* (17 mm), respectively. No antimicrobial activity was observed in aqueous, chloroform: methanol and petroleum ether extracts of *T. grandis* against any tested microorganisms. The results presented in Table 1 revealed that the studied plant extracts possessed potential antimicrobial activity against all tested organisms except *P. acnes*, though chloroform: methanol and ethanol extracts were found to have the strongest and broadest action spectrum.

3.2. The antimicrobial susceptibility pattern of tested microorganism

The antimicrobial susceptibility pattern of microbial strains was determined by using disc diffusion method. Table 2 revealed the antibiotic susceptibility pattern of Gram-positive bacteria. As per the results, *S. aureus* showed the maximum and the minimum susceptibility to antibiotic lincomycin against tetracycline, producing inhibition zones of 29 mm and 21 mm respectively. On the contrary, *P. acnes* was found to be resistant against cloxacillin and lincomycin. It had the highest inhibition zone of 30 mm against cefotaxime and the lowest of 20 mm against co-trimoxazole. In case of Gram-negative bacteria (Table 3), *E. coli* was found to have maximal susceptibility to levofloxacin and least to aztreonam exhibiting inhibition zones of 35 mm and 15 mm, respectively. *P. aeruginosa* was found to be resistant to aztreonam, ceftazidime, and cefotaxime. However, the maximum inhibition zone of 30 mm of *P. aeruginosa* was observed against levofloxacin. The fungus *C. albicans* was resistant to all the tested antibiotics (Table 4).

Table 2

The antibiotic susceptibility pattern of Gram-positive bacteria.

Antibiotics	Concentration (µg)	Inhibition zone (mm)	
		<i>S. aureus</i>	<i>P. acnes</i>
Co-trimoxazole	25.0	27	20
Cloxacillin	1.0	24	NA
Lincomycin	2.0	29	NA
Cefuroxime	30.0	23	22
Cefotaxime	30.0	25	30
Tetracycline	30.0	21	27

NA: No activity exhibited by extracts against microorganism.

Table 3

The antibiotic susceptibility pattern of Gram-negative bacteria.

Antibiotics	Concentration (µg)	Inhibition zone (mm)	
		<i>E. coli</i>	<i>P. aeruginosa</i>
Levofloxacin	5.0	35	30
Aztreonam	30.0	15	NA
Amikacin	30.0	26	28
Imipenem	10.0	27	26
Ceftazidime	30.0	16	NA
Cefotaxime	30.0	21	NA

NA: No activity exhibited by extracts against microorganism.

Table 4

The antibiotic susceptibility pattern of fungus.

Antibiotics	Concentration (µg)	<i>C. albicans</i>
Nystatin	50.0	NA
Clotrimazole	10.0	NA
Miconazole	30.0	NA
Ketoconazole	50.0	NA

NA: No activity exhibited by extracts against microorganism.

3.3. The synergistic antimicrobial activities of plant extracts with antibiotics

The synergistic activities of the most potent plant extracts (*C. sinensis*, *T. arjuna* and *P. longifolia*) with antibiotics (with the maximum inhibition) were determined to perceive changes in their antimicrobial efficacy (Table 5). When *C. sinensis* and *T. arjuna* were combined

Table 5

The synergistic activities of potent plant extracts with selected antibiotics.

Microorganism	Plant	Z.O.I of plant (mm)	Antibiotic	Z.O.I of antibiotic (mm)	Concentration of antibiotic ($\mu\text{g}/\text{disc}$)	Z.O.I as synergistic activity (mm)	
Gram-negative bacteria	<i>E. coli</i>	<i>C. sinensis</i>	11.0 \pm 0.2	LE	35	5	35.0 \pm 0.2
	<i>P. aeruginosa</i>		13.0 \pm 0.3		30		35.0 \pm 0.3
	<i>E. coli</i>	<i>T. arjuna</i>	14.0 \pm 0.6	LE	35	5	33.0 \pm 0.2
	<i>P. aeruginosa</i>		12.0 \pm 0.2		30		29.0 \pm 0.1
Gram-positive bacteria	<i>S. aureus</i>	<i>C. sinensis</i>	17.0 \pm 0.3	L	29	2	39.0 \pm 0.4
	<i>S. aureus</i>	<i>T. arjuna</i>	12.0 \pm 0.2	L	29	2	37.0 \pm 0.2
	<i>S. aureus</i>	<i>P. longifolia</i>	13.0 \pm 0.2	L	29	2	37.0 \pm 0.3

Z.O.I: Zone of inhibition; LE: Levofloxacin; L: Lincomycin.

with levofloxacin, no synergistic effect was observed in case of Gram-negative bacteria *E. coli* and *P. aeruginosa*, respectively. Combination of lincomycin with all the three potent extracts (*C. sinensis*, *T. arjuna* and *P. longifolia*) significantly inhibited the growth of Gram-negative bacterium *S. aureus* by the increase in the diameter of the zone of inhibition.

3.4. Phytochemical analysis

Phytochemical analysis of the potent plant extracts (ethanol extract of *C. sinensis*, chloroform: methanol extract of *T. arjuna* and petroleum ether extract of *P. longifolia*) was carried out to determine the presence of the various primary and secondary metabolites which were responsible for their numerous antimicrobial properties. Phytochemical screening revealed the presence of saponin, tannin, protein, carbohydrates and glycosides in all the extracts while steroids and alkaloids were limited only to *P. longifolia*. Flavonoids and diterpenes were present only in *T. arjuna* (Table 6).

Table 6

Phytochemical analysis of various compounds presented in potent plant extracts.

Components	Activity	<i>C. sinensis</i>	<i>T. arjuna</i>	<i>P. longifolia</i>
Steroid	Antidiarrhoeal	-	-	+
	Anticancer	+	+	+
Saponin	Antihelminthic			
	Antidiarrhoeal	+	+	+
	Antimicrobial			
Tannin	Antidiarrhoeal	+	+	+
	Antihelminthic			
Protein	Response in stress	+	+	+
	Antimicrobial	-	+	+
Terpenoids	Antidiarrhoeal			
	Antimicrobial			
Carbohydrate	Provide and store energy	+	+	+
	Antidiarrhoeal	-	-	+
Alkaloid	Antihelminthic			
	Antimicrobial			
	Antidiarrhoeal	-	+	-
Flavonoid	Antimicrobial	-	+	-
	Antidiarrhoeal			
Diterpenes	Anti-inflammatory action, antimicrobial	-	+	-
	Antispasmodic			
Glycosides	Antidiarrhoeal	+	+	+

4. Discussion

In the present study, the antimicrobial activity of the studied plants varied with different extraction solvents. Ethanolic extract of *C. sinensis* (4–17 mm) and chloroform: methanol extract

of *T. arjuna* (6–18 mm) displayed the broadest and maximum inhibitory activity as compared to other medicinal plants, whereas ethanol extract of *E. hirta* also exhibited activity in the range of 4–17 mm. Aqueous extracts were found to be the least effective among the different extracts. These results were in accordance to Rawani *et al.* who had reported that chloroform: methanol extract had better antimicrobial activity as compared to aqueous extracts against *E. coli*, *P. aeruginosa*, *Bacillus subtilis*, and *S. aureus*[8]. Similarly, another report has also shown that the organic solvents had better results as compared to water[20]. According to Radji *et al.*[21], the diameter of the inhibition zone of aqueous extracts of *C. sinensis* against *S. aureus* (ATCC 25913) and methicillin-resistant *S. aureus* were (18.970 \pm 0.287) mm and (19.13 \pm 0.25) mm, respectively; whereas the diameter of the inhibition zone against *P. aeruginosa* (ATCC 27853) and multi-drug resistant-*P. aeruginosa* were (17.550 \pm 0.393) mm and (17.670 \pm 0.398) mm, respectively. There have been several previous reports suggesting the antibacterial activity of *C. sinensis* against *S. aureus* and *P. aeruginosa*[22–24]. One of the report suggested that ethanolic extract of *C. sinensis* possessed antibacterial activity even against, namely *Streptococcus mutans* and *Lactobacillus acidophilus*[25]. The antibacterial properties of *C. sinensis* may be attributed to the presence of polyphenolic components including epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate[26,27]. *T. arjuna* extracts have also been shown to possess the antibacterial activity against *E. coli*, *P. aeruginosa*, *Bacillus cereus*, *Bacillus subtilis* and *Lactobacillus bulgaris*[28]. The aqueous and methanolic extracts of *T. arjuna* bark produced significant zones of inhibition against 22 tested bacteria including eight uropathogens. The ethanolic extract of *T. arjuna* exhibited potential antimicrobial activities against the selected strains of human pathogenic microorganisms[29]. The chloroform extract did not exhibit antibacterial activity. The polar extracts of *T. arjuna* also demonstrated strong antifungal effect against eight species of *Candida*[30]. A recent study on the antibacterial and antifungal properties of *Combretum* and *Terminalia* displayed that *Terminalia* extracts had better efficacies than the *Combretum* extracts. Furthermore, the methanol extracts were generally better antimicrobial agents than the water extracts[31]. In another study, the antimicrobial activity of methanolic extract of *T. arjuna* showed larger inhibition zone against Gram-negative bacteria than Gram-positive bacterial[32]. In the present study, no significant antimicrobial activity was observed against *P. acnes*, but these plant extracts displayed significant antimicrobial activity against *C. albicans* and *S. aureus*. Similarly, substantial antimicrobial

activity of different plant ethanolic extracts was observed against *C. albicans* and *S. aureus*. Aneja *et al.* also observed that the organic extracts of *T. arjuna* had better antimicrobial activity against *S. aureus* as compared to aqueous extracts[33]. From the results of antimicrobial activity, it was found that the organic solvent extracts exhibited more antimicrobial activity against the tested microorganisms as compared to aqueous extract. This may be due to the high tendency of the organic solvents to dissolve more organic and active antimicrobial compounds[34]. The antimicrobial action of the aqueous extracts could be attributed to the anionic components such as thiocyanate, nitrate, chlorides and sulfates apart from other water soluble components which were naturally occurring in the plant material[35,36]. Evidences have shown that green tea catechins, such as (-)-epigallocatechin-3-gallate, have strong antioxidant activity and affected several signal transduction pathways relevant to cancer development[37].

The antibiotic susceptibility pattern showed that the Gram-positive bacterium *S. aureus* was susceptible to all the tested antibiotics, whereas *P. acnes* was resistant to cloxacillin and linomycin. *S. aureus* and *P. acnes* had the maximum antibiotic susceptibility to lincomycin and cefotaxime, respectively. Gram-negative bacteria *E. coli* was found to be inhibited by all the tested antibiotics. However, *P. aeruginosa* had resistance to ceftazidime, cefotaxime and aztreonam. The maximum inhibition of Gram-negative bacteria was caused by levofloxacin. Antibiotic susceptibility of fungus exhibited the ineffectiveness of antifungal discs against *C. albicans* with no zone of inhibition. A positive synergistic effect of linomycin with all the three potent extracts (*C. sinensis*, *T. arjuna* and *P. longifolia*) was observed in case of Gram-positive bacterium *S. aureus*. However, no synergistic effect of levofloxacin along with *C. sinensis* and *T. arjuna* was observed for Gram-negative bacteria *E. coli* and *P. aeruginosa*, respectively. *C. sinensis* is a safe, nontoxic, cheap source of beverage which has been reported to have antimicrobial effects on various pathogenic bacteria including *E. coli*. Polyphenolic components of green tea have been shown to possess antibacterial activity. Catechins also have synergistic effect with antibiotics such as chloramphenicol, amoxicillin, sulfamethoxazole, azithromycin, levofloxacin, gentamycin, methicillin, nalidixic acid and ciprofloxacin especially[38].

The phytochemical analysis of the potent plant extracts (ethanol extract of *C. sinensis*, chloroform: methanol extract of *T. arjuna* and petroleum ether extract of *P. longifolia*) confirms the presence of saponin, tannin, protein, carbohydrate and glycosides in all of them. Phenolic compounds such as tannin present in these plant extracts are the potent inhibitors of microbial growth. Some of these phytochemicals may inhibit the attachment of bacteria on host cell surface membranes and act as potential antiadhesive agents. Presence of diterpenes and flavonoids was observed only in *T. arjuna* and alkaloids and steroids were detected only in *P. longifolia*. These results are in agreement with Mandal *et al.* who reported the presence of phytosterol, lactones, flavonoids, phenolic compounds and tannins, glycosides and presence of active compounds in low concentration, such as triterpenoids,

saponins, alkaloids, carbohydrates and proteins in *T. arjuna*[32]. Phytochemical screening of methanolic extracts of *T. arjuna* revealed the presence of active biomolecules in high concentration, such as phytosterol, lactones, flavonoids, phenolic compounds, tannins and glycosides. However, the aqueous as well as methanolic extracts of *C. sinensis* were shown to contain only flavonoids[39]. According to the present results, *P. longifolia* and *T. arjuna* exhibited the presence of most of the components compared to *C. sinensis*. The presence of these components imparts anti-diarrhoeal, anticancer, anti-helminthic and antimicrobial properties. The presence of protein and carbohydrate determines their function in stress responses of plant and its energy sources. Moreover, it is proved experimentally and clinically that crude extracts of *T. arjuna* possess anti-ischemic, antioxidant, hypolipidemic and antiatherogenic activities. Its useful phytoconstituents triterpenoids and flavonoids are considered to be responsible for its beneficial antioxidant cardiovascular properties[40].

It may be concluded that in view of the above results, *C. sinensis*, *T. arjuna* and *P. longifolia* could be used as potent sources of natural antimicrobial agents as a substitute for the commercially available synthetic drugs which may have a large number of side effects. Moreover, these plant extracts should be investigated *in vivo* for better understanding of their safety, efficacy and properties. Further research is also required for isolation and identification of active biomolecules and principles present in these extracts, so that they could be exploited for pharmaceutical use at the industrial scale.

Conflict of interest statement

We declare that we have no conflicts of interest.

Acknowledgments

The authors thank Management of Ambala College of Engineering and Applied Research, Ambala, Haryana, India, for providing necessary laboratory facilities to carry out this work.

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