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Antibacterial and antifungal activity of *Terminalia arjuna* Wight & Arn. bark against multi-drug resistant clinical isolates

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PEER REVIEW

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Comments

The study demonstrated the antimicrobial efficacy of polar extracts of *T. arjuna* bark against ATCC and clinically isolated strains of bacteria and fungi. The observation was commensurate with high polyphenol content of the extract. Prospective application in urinary tract infections, and candidiasis/candidaemia would be a notable outcome of this report.
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ABSTRACT

Objective: To evaluate antimicrobial activity of *Terminalia arjuna* (*T. arjuna*) bark against clinical strains of multi-drug resistant bacteria, and *Candida* spp. isolated from patients, as well as the corresponding reference strains.

Methods: The antimicrobial activity of water, methanol and chloroform extracts of *T. arjuna* bark were evaluated by agar-well diffusion method, followed by determination of minimum inhibitory concentration (MIC) by broth micro-dilution method. The clinical isolates were studied for antibacterial susceptibility by Kirby and Bauer disk diffusion technique.

Results: The water and methanolic extracts of *T. arjuna* bark produced significant zones of inhibition against twenty-two tested bacteria including eight uropathogens. MIC values against the bacteria were found in the range of 0.16 to 2.56 mg/mL. The chloroform extract did not exhibit antibacterial activity. The polar extracts of *T. arjuna* also demonstrated strong antifungal effect against eight species of *Candida*, with MIC between 0.16 and 0.64 mg/mL. The antimicrobial efficacy of the polar extracts was found to be commensurate with high polyphenol content in contrast to the non-polar (chloroform fraction).

Conclusions: This study has revealed the therapeutic prospect of *T. arjuna* bark for the treatment of microbial diseases. The polar fraction of the bark could be used for development of novel antimicrobial agents, particularly against urinary tract infections, and candidiasis/candidaemia.

KEYWORDS

Arjuna, Antimicrobial, Uropathogens, Candidiasis, Agar well diffusion assay

1. Introduction

Infectious diseases continue to be a global concern even in the 21st century. According to the World Health Organization, about 25% of the total 57 million annual deaths that occur worldwide are caused by pathogenic microorganisms, and this proportion is significantly higher in the developing world[1]. Nonetheless, the alarming rise in incidence of microbial diseases has also been documented in Europe and USA[1]. Infectious agents, like other living organisms, are subject to genetic change and evolution as manifested by their ability to infect new hosts, and by changes in their response to host immunity. This threat is likely to intensify further in coming years as the global climate change would inevitably perturb the interrelations between microbes, insect vectors, animal reservoirs and humans, and thereby alter the burden and distribution of infectious diseases of public health importance[1].

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During the last few decades, it has been possible to combat the bacterial and fungal infections through major improvement in early detection techniques, and newly developed antibiotics. Nevertheless, many of the currently used antimicrobials often lead to toxicity and undesirable side effects, or drug-drug interactions. The problem of antibiotic resistance has become particularly alarming due to the emergence of multi-drug resistant strains exhibiting simultaneous resistance to two or more classes of antibiotics[2]. Some of these microbes initially caused nosocomial infections among the immunosuppressed or chronically ill patients in the hospitals. More recently they have spread to the community, causing illness in previously healthy and otherwise non-vulnerable patients, as the available treatments were rather unresponsive as well as expensive. Therefore, the challenge is to develop better understanding of the genetic mechanisms of resistance, control the indiscriminate use of antibiotics, and find newer drugs against microbial diseases[1].

Historically, plants continue to be the invaluable resource for medicinal products, particularly for countries like India. In 1985, WHO estimated that 80% of the people living in developing countries were dependent on traditional herbal health-care, while about 25% of all prescriptions dispensed from community pharmacies in the USA contained either plant extracts, or active principles prepared from higher plants[3]. The use of plant products, either as pure compounds or as standardized plant extracts, could be of great significance in the treatment of microbial diseases. In general, studies on the antimicrobial activity of plant extracts have been restricted to ATCC strains, but not on multi-drug resistant clinical isolates of regional etiology[4]. Presently, our objective was to investigate the antimicrobial potency of an Indian medicinal plant, *viz. Terminalia arjuna* (*T. arjuna*), against several clinical isolates vis-à-vis ATCC strains of bacterial and fungal origin.

T. arjuna, Wight & Arn. (family Combretaceae), is traditionally renowned as a cardiac stimulant, and also recommended by many indigenous systems of medicine to address miscellaneous problems like urinary discharges, anemia, bilious affliction, and as an antidote to poison[5]. The ethnomedical relevance of this plant with reference to cardiovascular ailments has been widely investigated. Experiments revealed that *T. arjuna* bark exerted significant hypolipidaemic activity, produced inotropic and hypotensive effects, and increased coronary artery flow to protect myocardium against ischemic damage[5,6]. However, its antimicrobial profile did not receive adequate attention until recently[7,8], and there is ample scope to undertake further investigation in this regard. Here, we have studied the broad-spectrum antimicrobial efficacy of *T. arjuna* bark extracts on clinical isolates of fifteen bacterial and six fungal species which were found to be multi-drug resistant. The extracts were also evaluated against seven bacteria and two fungi of ATCC strains.

2. Materials and methods

2.1. Plant material

Fresh stem-bark of *T. arjuna* Wight & Arn. was collected from Baruipur, South 24 Parganas, West Bengal, and was duly authenticated at Botanical Survey of India, Shibpur, Howrah. Voucher specimen of a herbarium (JU/BH/020) of the plant was kept in our laboratory for future reference.

2.2. Preparation of extracts

The plant material (500 g) was shade dried and ground to powder. Three portions (about 10 g each) of the same were taken separately in 100 mL each of chloroform, methanol and water, and refluxed for 2 h to obtain the respective extracts. After filtration, the non-polar solvents of the chloroform and methanol extracts were removed in a rotary evaporator. The water extract was filtered through Whatman filter paper No. 1, concentrated and freeze-dried in a lyophiliser. The yield (% w/w) of the crude extracts obtained from chloroform, methanol and water were recorded and named as TAC, TAM and TAW, respectively.

2.3. Phytochemical analysis

Phytochemical analysis of each extract was carried out according to standard procedures[9]. The secondary metabolites present in the extract were detected qualitatively by the characteristic colour changes occurring upon treatment with specific reagents, *e.g.*, alkaloids with Dragendorff reagent, tannins with ferric-chloride solution, flavonoids with sodium hydroxide solution, triterpenoids with acetic anhydride in ethanolic solution, and reducing sugars with Benedict's reagent. Saponins could be detected by the ability to produce stable foam upon intermittent shaking of an aqueous suspension of the extract.

2.4. Total phenolic content

A stock solution of 0.02 mL (10 mg/mL) was taken in 1.58 mL of distilled water, and 0.1 mL of Folin Ciocalteau reagent (0.2 mol/L) was added. The reaction mixture was kept for 3 min at room temperature, Na_2CO_3 solution (7.5%, 0.3 mL) was added, and incubated in the dark for 30 min. Absorbance at 765 nm was measured in a UV-spectrophotometer (Ultrospec 2000, Pharmacia Biotech). A standard curve was prepared using different concentrations of gallic acid (S D Fine Chemicals, Mumbai, India). The determinations were carried out in triplicate, and the total phenolic content was expressed as gallic acid equivalents (GAE, mg/g of sample)[10].

2.5. Total flavonoid content

A stock solution of 0.1 mL (10 mg/mL) was added to 0.3 mL of distilled water containing $NaNO_2$ (5%, 0.03 mL). $AlCl_3$ (10%;

0.03 mL) was added after 5 min of incubation at 25 °C. After another 5 min, the reaction mixture was treated with 0.2 mL of NaOH (1 mmol/L). Finally, the volume was made up to 1 mL with distilled water and the absorbance measured at 415 nm. Quercetin dihydrate (SRL; Mumbai, India) was used to prepare the calibration graph[10]. The test was carried out thrice, and the results were expressed as quercetin equivalent (QE; mg/g of sample).

2.6. Microorganisms for study

A total of thirty pathogenic microbial cultures including nine ATCC strains of bacterial and fungal origin were taken for this study. Fifteen of the bacteria and six fungal strains were isolated from clinical specimen obtained from patient samples (Table 1), and identified by standard laboratory protocol[10,11]. Thus, Gram-positive bacteria were identified by their staining characteristics and morphology, pattern of haemolysis in blood agar, presence of catalase, coagulase and oxidase enzymes, solubility to bile salt, and finally by the pattern of different sugar fermentations. Similarly, Gram-negative isolates were identified by their staining morphology, motility, production of gas and H_2S, indole and acetoin production, utilization of lactose, citrate, presence of oxidase, urease and lysine decarboxylase, metabolism of ortho-nitrophenyl-β-galactoside (ONPG), and the fermentation of different sugars. The fungi of *Candida* spp. were identified by Germ tube test, presence of mycelium and capsule, presence of urease, and fermentation of glucose, sucrose, lactose, maltose, *etc*[10]. All the bacterial strains were maintained in nutrient agar (Hi Media, India), and the fungal cultures in Sabouraud dextrose agar (SDA; Hi Media, India) slants at 4 °C, and subcultured periodically.

Table 1
Antibiotic resistance profile of the clinical isolates.

Bacteria	Source	*In-vitro* antibiotic resistance
Gram Positive		
Bacillus subtilis	Stool	All drug sensitive
Enterococcus faecalis	Urine	Nx, Ca, Cfm
Micrococcus luteus	Throat Swab	All drug sensitive
Staphylococcus aureus	Sputum	Cfx, Cl, Nx, Ca, Cfm, Me
Staphylococcus saprophyticus	Urine	Cp, Ca, Cfm
Gram Negative		
Acinetobacter baumannii	Urine	Cfx, Cl, Ca, Cxm
Citrobacter freundii	Urine	As, Cfx, Cip, Nx, Of, Sfx, Ca, Cfm
Escherichia coli	Urine	As, Cfx, Cf, Cl, Nx, Of, Sfx, Ca, Cfm
Klebsiella pneumoniae	Urine	As, Cfx, Cf, Cl, Mr, Nx, Of, Sfx, Ca, Cfm, Cfs, Ao, Nf
Klebsiella oxytoca	Sputum	As, Cp, Cfx, Cip, Nx, Of, Ca, Cfm, Cfs, Ao
Moraxella catarrhalis	Throat Swab	Cfm
Proteus mirabilis	Pus	Cfx, Do, Lv, Nx, Of, Sfx, Cfm, Ak, Mo
Proteus vulgaris	Urine	As, Amc, Cfx, Do, Nx, Of, Sfx, Ca, Cfm, Ak, Ao
Pseudomonas aeruginosa	Urine	As, Amc, Cfx, Do, Nx, Of, Sfx, Ca, Cfm, Cfs, Nf
Shigella flexneri	Stool	Amp, Ct, T

As: Ampicillin/Sulbactum (10/10 μg), Amc: Amoxy-Clavulanic acid (20/10 μg), Amp: Ampicillin (10 μg), Ct: Co-trimoxazole (Sulfamethoxazole/trimethoprim) (1.25/23.75 μg), Cp: Cephalexin (30 μg), Cfx: Cefuroxime (30 μg), Cip: Ciprofloxacin (5 μg), Cl: Ceftriaxone (30 μg), Do: Doxycycline (30 μg), Lv: Levofloxacin (5 μg), Mr: Meropenem (10 μg), Nx: Norfloxacin (10 μg), Of: Ofloxacin (5 μg), Pt: Piperacillin/Tazobactum (100/10 μg), Sfx: Sparfloxacin (5 μg), Ca: Ceftazidime (30 μg), Cfm: Cefixime (5 μg), Cfs: Cefoperazone/Sulbactum (75/30 μg), Ak: Amikacin (30 μg), Ao: Aztreonam (30 μg), Cxm: Cefotaxime (30 μg), Me: Methicillin (5 μg), Nf: Nitrofurantoin (300 μg), T: Tetracycline (30 μg).

2.7. Antimicrobial activity

2.7.1. Bacterial strains

The plant extracts were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/mL, and tested for antibacterial activity by the agar well diffusion assay[10,12]. The bacterial culture in Muller Hinton broth was adjusted to the final inoculum density of 1×107 CFU/mL (by 0.5 McFarland standard) on molten Muller Hinton agar (MHA) plates. After solidification, wells (diameter 9 mm) were made with a sterile borer in the inoculated MHA plates. About 100 μL solution containing 1 mg of each extract was dispensed in the wells, while DMSO was also tested as the vehicle control. Penicillin G, streptomycin and gentamicin were the standard drugs used as positive controls in this assay. Antibacterial activity was expressed as the diameters of inhibition zones produced around each well by the plant extracts and antibiotics, and was measured after 24 h of incubation at 37 °C (Figure 1). Each test was conducted in triplicate to confirm the reproducibility of the observed data.

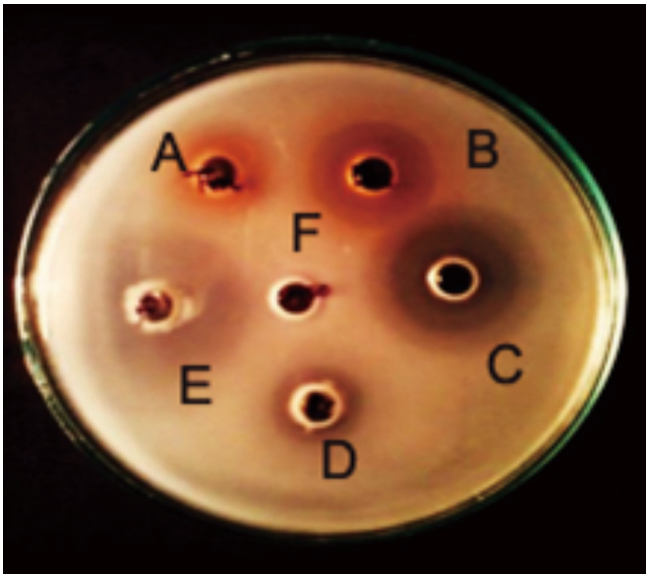

Figure 1. Antibacterial activity of the methanol and water extracts of *T. arjuna* stem-bark against ATCC strain of *Staphylococcus aureus*.
A: TAM; B: TAW; C: Streptomycin; D: Gentamicin; E: Penicillin G; F: DMSO.

2.7.2. Fungal strains

The crude plant extracts as described above were screened for antifungal activity[12]. Fungal culture in sabouraud dextrose broth containing an inoculum density of 0.5 McFarland (1×108 CFU/mL) was diluted at 1:10 ratio in SDA plate to obtain the final inoculum concentration of 1×107 CFU/mL. Wells (diameter 6 mm) were punched on solidified SDA plates and 100 μL solution containing 1 mg of each extract was dispensed in the wells. Amphotericin-B was used as a standard drug for antifungal assay, and DMSO was tested as the vehicle control. The diameter of the inhibition zone was measured after 24 h of incubation at 35 °C (Figure 2). Antifungal activity was expressed as

diameters of inhibition zones produced by the plant extracts and antifungal agent. Each test was conducted in triplicate and the reproducibility of the observations confirmed.

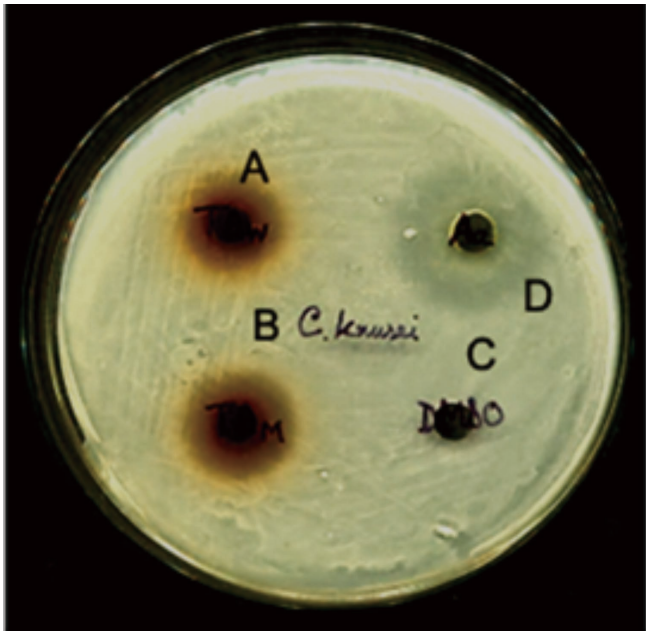

Figure 2. Antifungal activity of the water and methanol extracts of *T. arjuna* stem-bark against clinical isolate of *Candida krusei*.
A: TAW; B: TAM; C: DMSO; D: Amphotericin.

2.8. Determination of minimum inhibitory concentration (MIC)

2.8.1. Bacterial strains

The MIC of the plant extracts against the tested bacteria was determined by broth micro-dilution procedure to find the lowest concentration of the extract at which no growth was visible. Stock solutions (10.24 mg/mL) of TAM and TAW were prepared in DMSO, and serially diluted in Muller Hinton broth at concentrations of 5.12, 2.56, 1.28, 0.64, 0.32, 0.16, 0.08 and 0.04 mg/mL in a 96-well microtitre plate. The broth culture containing 0.5 McFarland (1×108 CFU/mL) inoculum density was then introduced to each of the microtitre wells at 1:10 ratio to maintain final inoculum density of 1×107 CFU/mL. Microtitre plates were incubated for 18 h at 37 °C, and the presence of visible growth in each well was inferred by measuring OD at 630 nm using ELISA reader (Erba LisaScan II, Transasia)[10,11].

2.8.2. Fungal strains

MIC for TAM and TAW against the fungal isolates was determined in a similar procedure, using sabouraud dextrose broth in 96-well microtitre plates incubated for 18 h at 35 °C[13,14]. The presence of visible growth was determined spectrophotometrically as above.

2.9. Determination of drug resistance profile of the isolated bacteria

Antibacterial susceptibility studies were carried out by Kirby and Bauer disk diffusion technique using commercially available CLSI recommended antibiotic disks. Bacterial culture in peptone water (Hi-Media, India) containing 0.5 McFarland turbidity (1×108 CFU/mL) was swabbed in MHA plate. Antibiotic disks were placed on it by maintaining about 20 mm distance with each other[10,11]. Inhibition zone diameter was measured after overnight incubation at 37 °C and results were interpreted as per CLSI guidelines[11].

3. Results

3.1. Total phenolic and flavonoid contents

Phytochemical screening of the bark extracts revealed the presence of alkaloids, tannins, flavonoids, triterpenoids, saponins, and reducing sugars in TAM and TAW. However, tannins, flavonoids and saponins could not be detected in TAC. The content of phenolic compounds in TAM and TAW were estimated to be 289.2±6.9 and 256.2±3.1, respectively, while the total flavonoid contents were found to be 18.0±0.0 and 14.4±0.2 in TAM and TAW, respectively.

3.2. Antibacterial activity of T. arjuna bark extracts

Twenty-two bacterial samples were taken for the study, seven of which were ATCC strains and fifteen were of clinical origin. The latter were tested as per CLSI guidelines and found to be multi-drug resistant with the exception of *Bacillus subtilis* and *Micrococcus luteus* (Table 1), both of which are known to be non-pathogenic contaminants of clinical specimen. The inhibitory activity of *T. arjuna* bark extracts prepared with water/methanol/chloroform (TAW/TAM/TAC) was determined by agar well diffusion assay and expressed as the diameter of inhibition zones in mm (Table 2, Figure 1). It was found that DMSO did not produce any visible zone in the whole study. The plant extracts, *viz.* TAW and TAM could clearly inhibit the growth of all the ATCC and clinical samples of Gram-positive and Gram-negative bacteria. In fact, TAM was found to show a greater potency, particularly in the case of Gram-negative bacteria, as compared to TAW. However, TAC was generally ineffective against all the samples barring a mild response towards *Proteus vulgaris*. Therefore, TAC was not included in the MIC study. It was also interesting to find from Table 2 that the efficacy of the plant extracts (TAM and TAW) was more or less comparable to that of the standard antibiotics. It was also noted that, in some of the cases, either streptomycin or gentamicin were found to be ineffective against clinically isolated bacteria, while penicillin G is not recommended for application against Gram-negative strains[11].

Table 2
Antibacterial activity of the extracts (TAW/TAM/TAC) of the stem-bark of *T. arjuna*.

Bacteria (Source)	Inhibition zones of bacterial growth (mm*)						MIC Values (mg/mL)	
	Extracts (1 mg/well)			Standard antibiotics			Extracts	
Gram Positive Bacteria	TAW	TAM	TAC	P	S	G	W	M
Bacillus subtilis (C.I.)	11	12	–	16	17	24	0.32	0.32
Enterococcus faecalis (ATCC 29212)	15	15	10	22	19	ND	0.64	0.64
Enterococcus faecalis (C.I.)	18	17	–	ND	–	15	0.16	0.32
Micrococcus luteus (C.I.)	16	15	10	19	14	26	0.32	0.64
Staphylococcus aureus (ATCC 25923)	22	15	–	29	25	16	0.16	0.64
Staphylococcus aureus (C.I.)	17	19	–	20	ND	ND	0.32	0.32
Staphylococcus saprophyticus (C.I.)	21	22	–	11	40	36	0.16	0.32
Streptococcus pyogenes (ATCC12384)	14	14	–	20	23	22	0.64	0.64
Gram Negative Bacteria								
Acinetobacter baumannii (C.I.)	17	19	–	ND	–	11	0.32	0.16
Citrobacter freundii (C.I.)	17	17	10	ND	–	19	0.64	0.64
Escherichia coli ATCC 35218	15	16	–	ND	18	18	0.32	0.64
Escherichia coli (C.I.)	15	17	–	ND	13	–	0.64	1.28
Klebsiella pneumoniae ATCC 700603	16	18	–	ND	21	12	0.64	0.32
Klebsiella pneumoniae (C.I.)	–	12	10	ND	12	–	2.56	1.28
Klebsiella oxytoca (C.I.)	14	15	10	ND	–	20	1.28	0.64
Moraxella catarrhalis (C.I.)	15	16	–	ND	13	–	0.32	0.32
Proteus mirabilis (C.I.)	12	13	10	ND	12	–	1.28	1.28
Proteus vulgaris (ATCC 6896)	14	16	13	ND	16	15	0.64	0.32
Proteus vulgaris (C.I.)	16	18	11	ND	14	17	0.64	0.64
Pseudomonas aeruginosa (ATCC 27853)	14	15	10	ND	12	18	0.64	0.32
Pseudomonas aeruginosa (C.I.)	16	17	–	ND	14	–	0.64	0.32
Shigella flexneri (C.I.)	17	19	–	ND	–	18	0.64	0.64

* Including diameter of well (9 mm); ATCC: American Type Culture Collection; C.I.: Clinical isolate –: No activity; ND: Not Determined; P: Penicillin G (0.001 mg/well); S: Streptomycin (0.01 mg/well); G: Gentamicin (0.01 mg/well).

3.3. Antifungal activity of *T. arjuna* bark extracts

TAW and TAM were tested for antifungal activity by agar well diffusion assay against two ATCC strains and six clinical isolates of *Candida* spp. from sputum and blood samples. The results are given in Table 3, which showed significant activity of the extracts against four different pathogenic species of *Candida*. It was also noted that the inhibition was more marked for the clinical strains as compared to their ATCC counterparts. In fact, TAM/TAW showed MIC values in the range of 0.16–0.64 mg/mL, although three out of the six clinical isolates failed to respond to amphotericin B treatment (Figure 2).

4. Discussion

Recently, indigenous medicinal plants are being increasingly explored through scientific validation of different traditional health care systems around the world[15]. India is particularly privileged in this respect, as it is endowed with about 45 000 diversified plant species growing in varied geographic and climatic zones inhabited by 550 tribal communities belonging to 227 ethnic groups with rich heritage of ethno medical wisdom[16]. In view of the emerging menace of adverse side-effects and drug-resistance pathogens all over the world, it is high time to look for novel strategies based on traditional plant-based products to combat microbial infections[10,17].

T. arjuna Wight & Arn. has an age-old reputation as an important medicinal plant. In fact, a number of pharmacological studies have validated the ethnomedical reputation of the bark of *T. arjuna* as an excellent cardiotonic agent[6]. Among the various kinds of secondary metabolites isolated from this plant, the triterpenoids and glycosides were shown to be particularly effective in treatment of cardiovascular diseases, while the flavonoids and polyphenols exerted antioxidant, hypolipidemic, and antiinflammatory properties[5,18]. So far, the antimicrobial potential of *T. arjuna* have been sparsely investigated against only a few selected bacterial and fungal species[7,8]. Aneja *et al*. demonstrated the efficacy of *T. arjuna* leaf/bark extracts on pathogens causing ear infection, but not against *Candida albicans*. However, our results have shown pronounced antifungal activity of TAM/ TAW against several *Candida* species including *Candida albicans*, both of ATCC as well as clinical origin as compared to amphotericin B.

Again, Singh *et al*. had tested the inhibitory effect of triterpenoid constituents of *T. arjuna* bark, *viz*. arjunic

Table 3
Antifungal activity of the extracts (TAW and TAM) of the stem bark of *T. arjuna*.

Fungi (ATCC/C.I.)	Source	Inhibition zones of fungal growth (mm*)		Amphotericin B (0.2 mg/well)	TAW (MIC) (mg/mL)	TAM (MIC) (mg/mL)
		TAW (1 mg/well)	TAM (1 mg/well)			
Candida albicans ATCC10231	ATCC	15	16	30	0.32	0.16
Candida albicans (C.I.#1)	Sputum	21	20	22 (hetero-R)	0.16	0.16
Candida albicans (C.I.#2)	Sputum	12	10	–	0.64	0.64
Candida tropicalis ATCC750	ATCC	18	16	24	0.32	0.32
Candida tropicalis (C.I.#1)	Blood	18	19	–	0.32	0.16
Candida tropicalis (C.I.#2)	Blood	20	19	12	0.16	0.32
Candida glabrata (C.I.)	Blood	20	18	–	0.16	0.32
Candida krusei (C.I.)	Blood	20	21	22	0.16	0.16

*Including diameter of well (6 mm), ATCC: American Type Culture Collection, C.I.: Clinical isolate, hetero-R: hetero resistant, – : No activity.

acid, arjungenin and arjunetin, against etiological agents of infectious endocarditis, with no clear indication about the efficacy against Gram-negative bacteria. Therefore, in the present study it was interesting to observe a broad-spectrum antibacterial potency of *T. arjuna* bark, including the Gram-negative species in particular. Further, the response of the uropathogens isolated from patient samples, *viz. Enterococcus faecalis*, *Staphylococcus saprophyticus*, *Proteus vulgaris*, *Acinetobacter baumannii*, *Citrobacter freundii*, *Escherichia coli* and *Pseudomonas aeruginosa*, was particularly encouraging. Therefore, it would be worthwhile to explore the therapeutic value of *T. arjuna* bark constituents for application on patients of urinary tract infection.

Presently, we have shown that only the polar extracts of *T. arjuna* bark (TAW and TAM) could exhibit significant antibacterial activity, irrespective of the multidrug resistance profile of the individual pathogenic strains, while TAC was mostly inactive. Therefore, the polyphenol content in the extracts was quantitatively estimated by the standard procedure[10], and found to be substantial (about 250-290 mg GAE/g) in the polar extracts, but negligible in the chloroform fraction (TAC; data not shown). In this regard the polar extracts were comparable to *Pinus maritima* with a polyphenol content of 363 mg GAE/g, which is a popular ingredient in nutraceutical formulations[19]. Thus, the strong antimicrobial profile of *T. arjuna* bark might be positively correlated with the high content of polyphenols in the polar extracts (TAM and TAW).

Thus, the sensitive ATCC strains, as well as a couple of isolates of Gram-positive bacteria (*viz. Bacillus subtilis* and *Micrococcus luteus* which were found to be sensitive to all drugs), responded more or less equally as compared to the multi-drug resistant microbes of clinical origin. In fact, the inhibitory response of TAW/TAM was quite notable in the case of a clinical sample of *Staphylococcus aureus*, which was identified to be an MRSA strain by phenotypic confirmation of its resistance to cefoxitin (30 μg) by disc diffusion test[20]. Another Gram-positive strain of *Staphylococcus* also exhibited a high zone of inhibition (21/22 mm). Further, it has been observed that Gram-positive bacteria would be more susceptible to antimicrobials in comparison to the Gram-negative bacteria which possess a thick barrier of lipopolysaccharide layer in the cell surface[7]. However, the Gram-negative species in our study were found to respond consistently to TAM/TAW.

In fact, our revelations on the antimicrobial property of *T. arjuna*, a traditionally reputed cardio-protective plant, assumes added significance in view of the recent concern about the cardiac safety of antibiotics, like fluoroquinolones, macrolides, trimethoprim-sulfamethoxazole, *etc.*, for treatment of elderly patients susceptible to cardiac arrhythmias[21].

Taken together, the present investigation have revealed the broad spectrum antibacterial activity of the polar constituents of *T. arjuna* bark against ATCC as well as multidrug resistant pathogenic strains. The aqueous/methanolic extract also exhibited marked antifungal activity against *Candida* spp. Further studies are envisaged to explore prospective application of *T. arjuna* bark for the treatment of urinary tract infections, and candidiasis/candidaemia.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Currently, antibiotics are often ineffective due to increasing drug resistance in the community. Since herbal medicines are traditionally used by a large section of the population for treatment of various diseases, therefore plant products could be investigated for application against microbial ailments.

Research frontiers

In the present study, the antibacterial and antifungal activities of the water, methanol and chloroform extracts of the stem-bark of *T. arjuna*, against ATCC and drug-resistant strains of bacteria and fungi were evaluated. The extracts were screened for the presence of phytoconstituents to correlate the activities.

Related reports

Previously, *T. arjuna* bark was investigated for cardioprotectant property, and also explored for antimicrobial

efficacy in treating ear infection and endocarditis. Here, some of the clinically isolated bacteria and *Candida* spp., associated with other types of infections, have been evaluated against *T. arjuna* bark extract.

Innovations and breakthroughs

In the present study, the broad-spectrum antimicrobial activity of *T. arjuna* bark extracts on multi-drug resistant clinical isolates of bacteria and fungi was demonstrated. This is a detailed investigation on the antimicrobial potential of this traditional medicinal plant reputed for its antioxidant and hypolipidemic properties.

Applications

T. arjuna bark is an important ingredient of formulations in Ayurvedic system of medicine, mainly for treating heart problems. The present study suggested that the polar extracts of *T. arjuna* bark may be developed into novel antimicrobial agents for the treatment of urinary tract infections and candidiasis.

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The study demonstrated the antimicrobial efficacy of polar extracts of *T. arjuna* bark against ATCC and clinically isolated strains of bacteria and fungi. The observation was commensurate with high polyphenol content of the extract. Prospective application in urinary tract infections, and candidiasis/candidaemia would be a notable outcome of this report.

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