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Antimicrobial Activity of Aqueous Extracts of Bark, Root, Leaves and Fruits of *Terminalia arjuna* Wight & Arn.

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ABSTRACT

The present study was carried out to evaluate the antibacterial activity of bark, stem, root, leaf and fruit extracts from *Terminalia arjuna* on selected Gram positive and Gram negative bacterial strains. Aqueous extracts were used to evaluate the antibacterial activity. Phytochemical extracts from different parts of the plant exhibited significant anti-bacterial activity against tested microbial strains; however, inhibitory activities of the extracts were plant part and test organism dependent. Phytochemical extracts limited the growth of both Gram-positive and Gram-negative bacterial species tested however, *Micrococcus luteus* was less sensitive to the aqueous extracts. The results show that antimicrobial activity of phytochemical extracts of *T. arjuna* were concentration dependent (1.0 mg/disc and 5.0 mg/disc) on the bacterial strains tested. Further, the results depicts that bark extracts of *T. arjuna* could be used as a potential source of antimicrobial agents against the bacterial strains tested.

KEY WORDS: *Terminalia arjuna;* Medicinal Plant; Antibacterial Activity; Disc Diffusion Assay; Bark/ Stem, Leaf/ Fruit extracts.

INTRODUCTION

In India, medicinal plants form the backbone of several indigenous traditional systems of medicine. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds (Prusti *et al*., 2008). Phytochemicals from medicinal plants serve as lead compounds in drug discovery and design (Ebi and Ofoefule, 2000). Medicinal plants are rich source of novel drugs that forms the ingredients in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs (Ncube, 2008).

WHO, report depicts that more than 80% of world's population rely on plants based products to meet their health care needs. Nearly, 25 to 45% of modern prescriptions contain plant derived lead molecules as a basic source in drug formulations. The value of plant based prescribed drugs in 1990 was estimated at $15.5 billon which has been on the raise since then. Furthermore, about 42% of 25 top selling drugs marketed world wide are either directly obtained from natural sources or entities derived from plant products (Ramya *et al*., 2008). Overexploitation of selected medicinal plant species has led to significant reduction in number of plants in the wild. Nevertheless, ruthless hunting has resulted in inclusion of their name in the red data book (Ahmedullah and Nayar 1999).

In recent years, multiple drug resistance in both human and plant pathogens has been developed due to indiscriminate use of synthetic drugs especially in the developing countries (Hart and Karriuri, 1998). Thus, a diverse arsenal of new antibacterial agents is urgently needed to combat the diminishing efficacy of existing antibiotics (Chopra *et al*., 1997). To this emerging problem, phytochemicals obtained from medicinal plants are the sole remedy. This drives the need to screen medicinal plants for novel bioactive compounds as plant based drugs are biodegradable, safe and have fewer side effects (Ramya *et al*., 2008). The demanding healthcare needs and the ability to cure disease with fewer side effects are the driving force behind the resurge of interest world over in the hunt for elite indigenous germplasm of pharmacological prominence.

Terminalia arjuna Wight & Arn., (family: Combretaceae) popularly known as 'Arjuna' (Dwivedi and Udupa, 1989) is a deciduous tree common throughout India. The tree normally grows to a height of 60-90 feet. Traditionally, it has been used as a cardiotonic and has been indicated for derangement of three humours viz., vata, pitta and kapha in Ayurveda. Bark of *T. arjuna* has been widely used in traditional system of medicine for a variety of purposes. Ancient Indian physicians used the powdered bark of *T. arjuna* for alleviating "hritshool" (angina) and other cardiovascular conditions (Dwivedi, 2007). Bark powder used as astringent and diuretic finds mention in works of Carak. Bark of *T. arjuna* has been attributed to possess cardio protective properties as described by Vagbhatta, in 'Astang Hridayam' as early as 500 CE (Dwivedi, 2007). As of now, in many of the traditional medicine, alcoholic extract of bark (asava) is administered with butter (ghrita) or boiled milk (kshirpak) (Warrier *et al*., 1996).

Phytochemical extracts from *Terminalia* species have been known for their antioxidant and antimicrobial properties. They are used in the management of cardiovascular diseases, myocardial infarction, degenerative neurological diseases, cancer, amyloidosis, acute pancreatitis, arthritis, atherosclerosis, inflammatory bowel disease, diabetes, senile dementia, retinal degeneration and senile cataract particularly in humans owing to their antioxidation potential (Dwivedi, 2007). Further, it has been reported that bark of *T. arjuna* exhibits antioxidant activity only in direct aqueous extract as determined in vitro by DPPH radical scavenging and deoxyribose damage protection assay and on lipid peroxidation. Ram *et al*. (1997) reported that ethanolic extract of *T. arjuna* bark at a concentration of 100-500mg/kg significantly reduces total and LDL cholesterol levels in hypercholesterolaemic rabbits. Similarly, it has been reported that bark aqueous extract of *T. arjuna* at a concentration of 50 mg/kg prevented the rise in liver injury enzymes, SGPT, ALP and TBARS and increased the levels of SOD, CAT, and GSH and the results were comparable to vitamin C group mice (Manna et al., 2006).

Recently, Devi *et al*. (2007), evaluated the effect of methanolic extract of *T. arjuna* on diclofenac sodium induced gastric ulcer in experimental rats and concluded that extracts of *T. arjuna* act as gastroprotective agent due to its free radical scavenging activity and cytoprotective nature. Studies have shown that bark of *T. arjuna* contains glycosides, flavonoids, tannins and minerals. Flavonoids have been reported to exert antioxidant, anti-inflammatory and lipid lowering effects while glycosides are cardiotonic, thus making *T. arjuna* unique amongst the medicinal plants (Dwivedi, 2007). Major phytochemicals present in different parts of *T. arjuna* is listed in Table 1 (Dwivedi, 2007).

However, only little work has been carried out with the phytochemical extracts as far as the antimicrobial an activity is concerned. Valsaraj *et al*. (1997) reviewed the antibacterial activity of some of the Indian medicinal plants. In a study, Perumalsamy *et al*., (1998) showed that aqueous extracts bark of *T. arjuna* holds significant antibacterial activity against *Escherichia coli, Klebsiella aerogenes*, *Proteus vulgaris*, and *Pseudomonas aerogenes*. Recently, Singh et al., (2008) indicated the presence of antibacterial principles in the bark of *T. arjuna* with arjunetin

particularly exhibiting selectively higher activity against *S. epidermidis*. However, not much of work has been done on the antibacterial activity of root, leaves and fruits of *T. arjuna*. In the present work, we describe antibacterial activity of aqueous extracts of bark, root, leaves and fruits of *T. arjuna*.

MATERIALS AND METHODS

Collection of Plant Material

Mature bark, root, leaves and fruits of *T. arjuna* were collected from the wild in Vellore District, Tamilnadu, India during Apr – Jun 2008. The Flora of Presidency of Madras (Gamble, 1935) and The Flora of Tamil Nadu Carnatic (Matthew, 1983) were used for identification and authentication of the plants. Collected material was washed thoroughly in running tap water, rinsed in distilled water and shade dried in open air and ground to powder.

Preparation of Phytochemical Extracts

The powder was extracted by maceration in double distilled water. The plant extracts were concentrated using rotary evaporator (Buchi, Switzerland) and stored at 4 until used in the assay.

Test Organisms

Eight strains of Gram-positive bacteria - *Micrococcus glutamicus, Lactobacillus bulgaris, Streptococcus faecalis, Staphylococcus aureus, Bacillus stearothermophilus, Staphylococcus pyogenes, Micrococcus luteus, Bacillus cereus* and two strains of Gram negative bacteria - *Escherichia coli* and *Pseudomonas aeruginosa* were used to evaluate the antibacterial activity (Table 2). All bacterial cultures were maintained in NA slants/ plates; stored at 4 and periodically sub-cultured.

Antimicrobial Activity Test

Antimicrobial activity was tested using a modified disc diffusion assay (DDA) method originally described by Bauer (1966) and Ncube *et al* (2008). Plant extracts were dissolved in 20% DMSO treated water. The inoculums for each microorganism were prepared from broth cultures (10^5 CFU/ml). A loop of culture from the NA slant stock was cultured in LB medium overnight and spread with a sterile swab into Petri-plates. Sterile disc (6 mm dia, Hi-media, Mumbai, India) impregnated with the plant extracts (1.0 mg/disc and 5.0 mg/disc) were placed on the cultured plates and incubated for 24 h at 37 . The solvent loaded disc without extracts in it served as control in the study. The results were recorded by measuring the zones of growth inhibition. Clear inhibition zones around discs indicated the presence of antimicrobial activity. All data on antimicrobial activity were average of triplicate.

RESULTS AND DISCUSSION

Plants are known to have beneficial therapeutic effects documented in Traditional Indian System of Medicine. Though bioactive products of Arjuna have been used in treatment of various aliments since time immemorial, role of phytochemical in inhibition of growth of microorganisms has gained less prominence (Sasidharan *et al*., 1998). In the present study, aqueous extracts of bark/ stem, root, leaves and fruits of *T. arjuna* were tested against selected Gram positive and Gram negative bacterial species (Table 3). Different extracts of *T. arjuna* exhibited significant anti-bacterial activity against all test organisms. Bark extracts limited the growth of both Gram-positive and Gram-negative bacterial species tested. However, inhibitory role of leaf extracts was organism

dependent. Aqueous extracts of leaves and the fruits were active towards the Gram negative strains and less active towards the Gram positive stains used in the study (Table 3). Among the different microorganisms tested maximum inhibition was found in *E. coli* followed by *P. aeruginosa, B. cereus, B. subtilis* and *L. bulgaris*. However, *M. glutamicus* and *M. luteus* remained less sensitive to aqueous extracts of *T. arjuna*. Of the different extracts tested, bark extract exhibited significantly higher activity towards all the strains expect *M. glutamicus*. However, leaf and fruit extracts were not active towards any of the Gram positive strains used in the study.

CONCLUSION

Arjuna, the versatile traditional medicinal plant of India, is the rich source of bioactive compounds with diverse chemical structure. As of now, little work has been done on the biological activity and plausible medicinal applications of the phytochemical compounds and hence extensive investigation is needed to exploit the bioactive principles of Arjuna for therapeutic utility. In the present study antibacterial activity of *T. arjuna* extracts towards drug resistant/ clinically significant microbes has been investigated. Further investigations may lead to development of new antibiotic (s) of high potency.

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Table 1. Major chemical constituent in different parts of *T. arjuna*.

Compound	Stem/ bark	Root	Leaf/fruit
Triterpenoids	Arjunin, Arjunic acid, Arjunolic acid, Arjungenin, Terminic acid	Arjunic acid, Arjunolic acid, Oleanolic acid, Terminic acid	
Glycosides	Arjunetin, Arjunaphthanoloside, Arjunoside I, II and Terminoside A	Arjunoside I-IV Glucopyranoside	
Sitosterol	Sitosterol	Sitosterol	
Flavonoids	Arjunolone, Arjunone , Bicalein Luteolin, Gallic acid, Ethyl gallate Kempferol, Proanthocyanidins, Quercetin, Pelorgonidin,		Luteolin
Tannins	Pyrocatechols, Casuarinin, Casurin, Punicallin, Punicalagin, Castalagin, Terchebulin, Terflavin C,		
Trace elements	Calcium, Aluminium, Magnesium, Silica, Zinc, Copper		

(Source: Dwivedi S (2007), *Journal of Ethnopharmacology*, 114:114-129).

Table 2. Bacterial strains used in the present study.

S. No	Bacterial strain	Gram (+/-)

1	*Escherichia coli*	-
2	*Pseudomonas aeruginosa*	-
3	*Bacillus cereus*	+
4	*Bacillus subtilis*	+
5	*Lactobacillus bulgaris*	+
6	*Micrococcus glutamicus*	+
7	*Micrococcus luteus*	+
8	*Staphylococcus aureus*	+
9	*Staphylococcus pyogenes*	+
10	*Streptococcus faecalis*	+

Table 3. Anti-microbial activity of *T. arjuna* aqueous extracts.

Organism	**Stem/ bark**		**Root**		**Leaf/ fruit**	
	1 mg/ml	5 mg/ml	1 mg/ml	5 mg/ml	1 mg/ml	5 mg/ml
Escherichia coli	+	++	+	++	+	++
Pseudomonas aeruginosa	+	++	+	+	+	++
Bacillus cereus	+	++	+	+	-	-
Bacillus subtilis	+	++	+	+	-	-
Lactobacillus bulgaris	+	++	+	+	-	-
Micrococcus glutamicus	-	-	-	-	-	-
Micrococcus luteus	-	+	-	-	-	-
Staphylococcus aureus	-	+	-	+	-	-
Staphylococcus pyogenes	-	+	-	+	-	-
Streptococcus faecalis	+	++	+	+	-	-

(Growth analysis: ++ =more; + =less; - = no)