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Research Article

Biological activity of *Terminalia arjuna* on Human Pathogenic Microorganisms.

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ABSTRACT

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World's population relies chiefly on traditional medicinal plants, using their extracts or active constituents. Terminalia arjuna of family Combretaceae reported to be effective as aphrodisiac, expectorant, tonic, styptic, anti-dysenteric, sweet, acrid, purgative, laxative, diuretic, astringent, cirrhosis, cardio protective and cancer treatment. In present study, antibacterial, antifungal, brine shrimp lethality and phytotoxic effect of Terminalia arjuna was performed. Our results showed that methanolic extract of Terminalia arjuna leaves has moderate antifungal effect against Microsporm canis and fruit extract possess good antibacterial activity against Staphylococcus aureus and Pseudomonas aeruginosa. Moreover, Dichloromethane extract of Terminalia arjuna bark and fruit possess moderate phytotoxic activity.

Keywords: Antibacterial, Antifungal, Phytotoxicity, *Terminalia arjuna*

INTRODUCTION:

The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Ayurveda, Unani and Siddha. In India, it is reported that traditional healers use 2500 plant species and more than hundred plant species serve as regular sources of medicine (Sheng-Ji, 2001). Even in the developed countries, like USA 25% of prescription for pharmaceutical drugs contains one or more substances of plant origin (Dev *et al.*, 1997). A large number of plants with therapeutic properties are quite astonishing. It is estimated that around 70,000 plant species, from lichens to trees, have been used at one time or another for medicinal purposes. (Das *et al.*, 2003). The value of ethno-medicine and traditional pharmacology is gaining recognition because the search for potential medicinal plants is successful if the plants are chosen on ethno-pharmacological basis. Phytochemical components are important sources of potential pharmacological, chemotherapeutic and toxic activities (Lawal *et al.*, 2005).

The continuous development of antibiotically resistant strains of microbial pathogens, such as methicillin resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP) and vancomycin- resistant enterococci (VRE), is a growing problem and it is therefore, extremely important to discover and develop new antimicrobial compounds (Tally *et al.*, 1999). The antimicrobial compounds from plants may inhibit bacteria through different mechanisms than conventional antibiotics and could therefore be of clinical value in the treatment of resistant microbes (Eloff *et al.*, 1998a). The selection of the plant group for this investigation was based on uses in traditional medicine, since many *Combretum* and *Terminalia* species are well known medicinal plants both in Africa and Asia (Watt and Breyer-Brandwijk, 1962; Hedberg *et al.*, 1982).

Alcoholic extract of *Terminalia arjuna* increased the force of contraction of frog heart (Gupta *et al.*,

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1974) and had a marked reduction in total cholesterol level in rabbits (Tiwari *et al.,* 1990). The aqueous extract of *Terminalia arjuna* resulted in dose-dependent decrease in blood pressure (Srivastava *et al.,* 1992) and produced hypotensive effects (Takahashi *et al*, 1997). Another study reveals that an Injection of bark extract in isolated rabbit heart preparation increased the coronary flow (Bhatia *et al.,* 1998) and tannins of the leaves have anticancer activity (Kandil and Nassar, 1998). The bark powder is reported to exert hypocholesterolaemic and antioxidant effect in humans (Gupta *et al.,* 2001), antimutagenic effect (Kaur *et al.,* 2001) and decrease in total cholesterol and triglycerides (Dwivedi *et al.,* 2000; Dwivedi and Gupta, 2002). While, the bark extract has antioxidant compounds that prevent from oxidative stress (Gauthaman *et al.,* 2001) and to exert free radical scavenging activities in human polymorpho-nuclear cells (Pawar and Bhutani., 2005). Furthermore, the aqueous extract of *Terminalia arjuna* bark protected the oxidant damage to the liver and kidney following carbon tetrachloride challenge in mice (Manna *et al.,* 2006).

Terminalia arjuna extract prevails significant improvement in diastolic dysfunction (Dwivedi *et al.,* 2002) and exhibit an outstanding antioxidant activity (Sultana *et al.,* 2007). Oral administration of abana, a compound formulation containing *Terminalia arjuna,* 30 mg per tablet, resulted in significant reduction of the systolic blood pressure; echocardiographic left ventricular internal diameter, posterior wall thickness and interventricular septal thickness (Dwivedi *et al.,* 2007). An oleanane triterpenoid, arjunolic acid, exhibited cardiac protective action in isoproterenol-induced myocardial necrosis in rats (Sumitra *et al.,* 2001), has profound effects on human hepatoma cell line (HepG2) for cytotoxicity (Sivalokanathan *et al.,* 2006).

MATERIALS AND METHODS

Plant Material Collection and Extraction

Plant material was collected in the vicinity of Bahauddin Zakariya University, Multan and identified by Dr. Mumtaz Bokhari (Taxonomist), Department of Biological Sciences, Bahauddin Zakariya University, Multan. Different parts (fruit, leaves, bark) of *Terminalia arjuna* were collected and kept under shade for drying. The plant material was ground in a grinding mill and weighed. Extraction from the different parts of *Terminalia arjuna* was carried out by simple maceration process. 100 g of the ground parts of *Terminalia arjuna* was taken in extraction bottle and 250 ml of Dichloromethane (DCM) was added. The mixture was occasionally shaken and homogenized using ultrasonic bath. After 24 hours, the mixture was filtered and the marc was again macerated by the solvent using the same procedure. Extracts of DCM and methanol were concentrated by using the Rota vapor (Buchi-rotavapor R-200) and weighed.

Agar Diffusion Assay

Test sample was dissolved in sterile DMSO to serve as stock solution. Sabouraud dextrose agar was prepared by mixing sabouraud 4% glucose agar in distilled water. Test tubes containing media were autoclaved at 121 °C for 15 minutes. Tubes were then allowed to solidify in slanting position at room temperature. All culture containing tubes were inoculated at optimum temperature of 28-30 °C for growth for 7-10 days. Humidity (40% to 50%) was controlled by placing an open pan of water in incubator. After the incubation for 7-10 days, the test tube with no visible growth of the microorganism was taken to represent the minimum inhibitory concentration (MIC) of the test sample **(**Okeke *et al.,* 2001; Aneja *et al*, 2009).

Brine-Shrimp Lethality Assay

Bioactive compounds are often toxic to brine shrimp larvae *Artemia salina* (Leech). Artificial "Sea water" was prepared by dissolving by *ca.* 3.8 g sea salt per liter and filtered. "Sea water" was placed in a small unequally divided tank and

shrimp eggs added to the larger compartment of tank which was darkened by covering it with aluminum foil. After 2 days (when the brine shrimp larvae had matured), added 5 ml Sea water to each vial and added 10 shrimps per vial with the help of Pasteur pipette (30 shrimps per dilution). The vials were maintained under illumination. After 24 hours had elapsed, counted and recorded the number of surviving shrimps, with the aid of a 3 x magnifying glass (Rahman *et al.*, 2001).

Lemna Bioassay for Phytotoxicity

Inorganic medium (E-Medium) was prepared: add KOH pellets to attain pH 5.5-6.0. Prepared vials for testing: 10 vials per dose (500, 50, 5 ppm, control). (a)Weighed 15 mg of compound or extract and dissolved 15 ml of solvent. (b) Added 1000, 100, and 10 µl solutions of vials for 500, 40, and 5 ppm; allowed solvent to evaporate overnight. (c) Added 2 ml of E-Medium and then a single plant containing a rosette of the fronds to each vials (Rahman *et al.*, 2001). Vials were placed in a glass dish filled with about 2 cm water, seal container with stopcock grease and glass plate. Placed dish with vials in growth chamber for seven days at 26 °C under fluorescent and incandescent light. Counted and recorded number of fronds per vial on day 3 and day 7.

Results and Discussion

A wide variety of medicinal plants used traditionally have not yet been scientifically investigated against diverse microbial pathogens. Previous reports reveal only few studies on antibacterial and antifungal activity of various parts of *Terminalia arjuna*, such as bark (Kadam and Ghosh, 2008), leaves, root and fruit (Dwivedi *et al.*, 2007; Chouksey *et al.*, 2001). The present study was carried out to validate the antimicrobial potential of *T. arjuna* leaves, fruit and bark against the bacterial and fungal isolates. It was found that methanol extract of *T. arjuna* fruit showed good antibacterial activity against *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Table 2). The DCM extract of different plant parts like leaves, bark and fruit showed no antibacterial activity. As it was evident from the biological screening results of methanol extract of *T. arjuna* fruit showed good antibacterial activity against *Staphylococcus aureus* (Gram positive) and *Pseudomonas aeruginosa* (Gram negative) has a potential to combat the bacterial pathogens in human and animals (Table 2). Therefore, substitute the findings of some (Cowan *et al.*, 1999) but concords with findings of (Aneja *et al.*, 2012)

Furthermore, the samples were tested for antifungal activity of *T. arjuna* and found that methanol extract of *T. arjuna* leaves have moderate activity against *Microsporum canis* and non-significant activity against *Aspergillus flavus, whereas T. arjuna* fruit (methanol) extract showed non-significant activity against *Aspergillus flavus, Microsporum canis* and *Fusarium solani* (Table 2). *T. arjuna* bark (methanol) showed no antifungal activity. *T. arjuna* leaves, stem bark and fruit (DCM) extract showed no antimicrobial activity (Table 2). The narrow spectrum of antimicrobial activity may be due to active compound present in insufficient amount in the crude extract to show activity with the dose level employed or present in high quantities in the extract exerting antagonistic effects of the bioactive compounds of leaves (Nair *et al.*, 2005). Antimicrobial activity of *T. arjuna* is reported to be present due to of secondary metabolites such as arjunic acid, arjungenin, arjunetin and luteolin (Kadam and Ghosh, 2008; Dwivedi *et al.*, 2007).

Moreover, the samples were also tested for phytototoxicity by using lemna minor bioassay and found that methanol extract of *Terminalia arjuna* showed good phytotoxic activity while leaves and bark (Methanol) showed moderate phytotoxic activity. *Terminalia arjuna* fruit (DCM) showed moderate phytotoxic activity while leaves and bark (DCM) showed no phytotoxic activity. However, the samples tested for brine shrimp lethality bioassay showed no

cytotoxic activity (Table 2). The extraction of whole plant by using with dichloromethane and methanol is shown in Table 1.

CONCLUSION

Terminalia arjuna fruit extract (methanol) has shown good antimicrobial activity against Gram positive and Gram negative bacteria and inhibition is more for Gram positive than Gram negative bacteria. Extensive investigations are required on antimicrobial activity and plausible medicinal applications of phytochemicals. Hence, more studies on this plant are necessary to explore the bioactive principles for therapeutic efficacy.

Tables

Table 1: Extraction of plant material with dichloromethane and methanol

Plant Name	Part used	Solvent	Extract (Wt)
Terminalia arjuna	Leaves (100 g)	Dichloromethane	2.38 gm
		Methanol	14.70 gm
	Bark (100 g)	Dichloromethane	0.40 gm
		Methanol	12.20 gm
	Fruit (100 g)	Dichloromethane	0.30 gm
		Methanol	8.90 gm

Table 2: Results of biological activities of DCM and Methanol extracts of *Terminalia arjuna*

***Terminalia arjuna* Extract**	**Antibacterial Activity**		**Antifungal Activity**			**Lemena**	**Brine shrimp lethality**
	Staphylococus aureus (NCTC # 6571) (Gram-positive)	*Preudomonas aeruginosa* (NCTC # 10662) (Gram-negative)	*Microsporm canis* (ATCC # 11622)	*Aspergillus Flavus* ATCC # 32611)	*Fusarium solani* (ATCC # 9846)	**Phytoto-xicity**	
	Bioassays						
TALD	-	-	-	-	-	-	-
TALM	-	-	+	-	-	+	-
TABD	-	-	-	-	-	-	-
TABM	-	-	-	-	-	+	-
TAFD	-	-	-	-	-	+	-
TAFM	+	+	-	-	-	+	-

List of Abbreviations

TALD: Dichloromethane extract of *T. arjuna* leaves

TALM: Methanol extract of *T. arjuna* leaves

TABD: Dichloromethane extract of *T. arjuna* stem bark

TABM: Methanol extract of *T. arjuna* stem bark

TAFD: Dichloromethane extract of *T. arjuna* fruit

TAFM: Methanol extract of *T. arjuna* fruit

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