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ORIGINAL RESEARCH ARTICLE

Antidermatophytic and Antioxidant Activity of *Terminalia arjuna* (roxb.) Wight & Arn. Bark

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ABSTRACT

Bark of *Terminalia arjuna*, a well-known medicinal plant, has extensively been used in ayurvedic medicines to treat various diseases. The present study was carried out to evaluate the antidermatophytic and antioxidant activity of the crude extract of bark of *Terminalia arjuna*. The antagonistic activity of the crude extract was tested against five clinically significant antidermatophytic fungi i.e., *Trichophyton mentagrophytes, T. rubrum, T. tonsurans, Microsporum gypseum* and *M. fulvum* using agar cup diffusion method. Acetone, 95 % alcohol and methanol were used as solvents for the extraction of crude from bark. The methanol extract was most effective against *T. tonsurans* (zone of inhibition 25 mm) and least effective against *M. gypseum* (zone of inhibition 3 mm). The minimum inhibitory concentration of the crude ranged between 2000 µg/ml to 75 µg/ml. Antioxidant activity was determined using scavenging activity of DPPH (1, 1-diphenyl-2-picrylhydroxyl) radical method. The methanolic bark extracts of 50, 100 and 200 µg/ml concentrations exhibited high free radical scavenging activity respectively. The phytochemical screening showed the presence of alkaloids, tannins, saponins in the extract which could be responsible for the antidermatophytic and antioxidant activities.

Key words: Agar cup diffusion method, DPPH radical method, minimum inhibitory concentration, photochemical screening.

INTRODUCTION

The incidence of microbial skin infections in humans have increased worldwide. In Northeast India, almost fifty percent of the total infectious skin diseases are of fungal origin of which dermatophytoses, caused by a group of keratinophilic fungi, dermatophytes are the most predominant [16]. These infections are problematic, causing extreme discomfort and sometimes may cause even death. Immunocompromised people and people taking a broad spectrum antibiotic have higher risk of such fungal skin infections. Treatments of these highly infectious diseases with currently available drugs are quite challenging, since, infections are often associated with frequent relapse. In recent years, the problem has become more serious due to multi drug resistance developed due to indiscriminate use of synthetic drugs. Therefore, new antidermatophytic agents are urgently needed to combat the diminishing efficacy of existing antidermatophytic drugs. The approach to develop new drugs through natural products might be the single most successful strategy [10, 17]. Natural products like; plant extract, can provide unlimited opportunities for new drug discoveries because of their unmatched chemical diversity. Medicinal plants provide important ingredients in modern medicines, nutraceuticals, food supplements, folk medicines, and lead compounds for synthetic drugs [19, 23]. Several studies indicated that medicinal plants contain compounds like peptides, unsaturated long chain fatty acids, aldehydes, alkaloids, essential oils, phenols and water or ethanol soluble compounds, which are significant in therapeutic applications against the pathogens like; bacteria, fungi and viruses [22]. Screening medicinal plants for novel bioactive compounds are the sole remedy since, plant based drugs are biodegradable, safe and have fewer side effects [25]. Thus, the therapeutic potential of the herbs opens up new vista in the future pharmacological research of herbal drug development.

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Besides, in the last few years, interest in the antioxidant activity of the plant extracts has increased tremendously which is very important due to the fact that free radicals can be responsible for various diseases like; heart diseases, strokes, arteriosclerosis, cancer as well as for ageing processes. Potent sources of natural antioxidants and antimicrobials can reduce the use of synthetic forms such as Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT). Since, many antioxidants are of plant origin, they can react rapidly with these free radicals thereby causing retardation in the extent of oxidative deterioration [3]. Moreover, consumption of antioxidant or addition of antioxidant to food materials could protect the body against these harmful effects of free radicals [8].

In modern days, the antioxidants and antimicrobial activities of plant extracts have formed the basis of many applications in pharmaceuticals, alternative medicines and natural therapy [1]. Antimicrobial and free radical scavenging activities of aqueous extracts of leaves of an antidiabetic plant *Gymnema montanum* have been largely exploited in the management of various infectious diseases caused by the pathogenic microorganisms like; *Salmonella typhi*, *Pseudomonas aeruginosa* and *Candida albicans* [24]. Promising free radical scavenging activity of some plant extracts against food spoilage and antimicrobial activity against pathogenesis have also been well reported [7]. The antifungal activity of methanol extracts of some *Cassia*, *Detarium* and *Ziziphus* species against dermatophytes have been well studied [2]. In vitro antifungal activity of ethanolic leaf extract of *Salvadora persica* was also assessed against *Aspergillus niger, A. zylinium, A. flavus* and *Candida albicans* [20]. The basic screenings for antibacterial activity of solvent fractions of crude water decoction of apical twigs and latex of *Calotropis propera* has also been well investigated [21]. Different solvent extracts of bark, fruits and leaves of *Mimusops elengi* has been screened for their antibacterial and antifungal activities against some pathogenic bacteria and fungi [4]. Recently, it has been proved that ethanol and methanol extracts of *Tecoma stans* were highly effective against pathogenic bacteria like; *Pseudomonas fluorescens, Clavibacter michiganensis, Xanthomonas axanopodis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and pathogenic fungi belongs to all species of *Aspergillus* and *Alternaria* [12]. The organic extracts of these medicinal plants could be a possible source to obtain new and effective drugs to treat severe infections in modern times [25].

The North eastern region in India has been in focus for its high biodiversity and was identified as Indo-Burma mega biodiversity hot spot [18]. Screening for antidermatophytic activity as well as assessment of antioxidant activity of the vast plant resources of this biologically diverse region is important for its optimum utilization to solve the new and emerging disease problems causing advancement in modern biotechnology since; little scientific research has been carried out to investigate the biological and pharmaceutical importance of the medicinal plants of this practically unexplored zone.

Terminalia arjuna Wight & Arn., (family: Combretaceae) popularly known as 'Arjuna' is a large deciduous tree common throughout India, especially in the sub Himalayan tracts and North-eastern India. It is traditionally used in the management of cardiovascular diseases, myocardial infarction, amyloidosis, acute pancreatitis, arthritis, atherosclerosis, inflammatory bowel disease, diabetes, senile dementia, and retinal degeneration [11]. *In vitro* antioxidant and antibacterial properties of *T. arjuna* has been well investigated [29]. However, almost no work has been carried out concurrently on both antidermatophytic and antioxidant activities of this important medicinal plant. Keeping this point in view, the present investigation was undertaken to (i) evaluate the antidermatophytic activity of the crude extract obtained from the bark of *T. arjuna* (ii) assess its antioxidant activity (iii) identify the major chemical constituents through phytochemical screening.

MATERIALS AND METHODS

Collection of the plant material:

Terminalia arjuna bark was collected from North Brahmaputra valley ($26^0 40^/$ N and $92^0 58^/$ E), Assam, India during 2010. The herbarium specimen was made following standard herbarium techniques [14]. The specimen was identified with the help of references and herbarium specimen of Botanical survey of India, Shillong (India) and voucher No. 099 has been added and the specimen was deposited in Gauhati University Botanical Herbarium (GUBH).

Preparation of the bark extract:

The collected bark was shade dried and ground into coarse powder. Extraction was done at room temperature by simple extraction method [9] using three different solvents namely, acetone, 95 % ethanol, and Methanol. Dried powdered bark (15 g) was mixed separately with 100 ml of each solvent in 500 ml conical flasks. The flasks were sealed tightly and kept on orbital shaker for 24 h. Then the flasks were taken out and allowed to stand for 5 h. The supernatant was filtered and evaporated using a rotary evaporator and freeze dryer, respectively to obtain the crude dried extract. The crude so, obtained was dissolved in 1 ml Dimethylsulphoximide (DMSO) and used for antidermatophytic assay.

Test organisms:

Antidermatophytic activity of *T. arjuna* crude bark extract was tested against five potent dermatophytes namely; *Microsporum fulvum* (MTCC8478) *M. gypseum* (MTCC8469) *Trichophyton mentagrophytes* (MTCC8476), *T. tonsuarans* (MTCC8475) and *T. rubrum* (MTCC8477) obtained from Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology (IMTECH), Chandigarh, India. All the test fungi were maintained on freshly prepared Sabouraud's agar (SA) medium, procured from Hi Media Laboratories Pvt. Ltd, Mumbai, India.

Antidermatophytic assay:

The antidermatophytic activity of the crude bark extract was determined by agar cup diffusion method [13]. Sabouraud's agar plates were inoculated with 0.2 ml of overnight grown culture of each test fungal suspension containing 1.0 X 10^9 cells. The plates were evenly spread out with the help of a sterile cotton swab. Agar cups were prepared by scooping out the media with a sterile cork borer (7mm in diameter). Each cup was loaded with 100 µl of the crude extract dissolved in DMSO. Fluconazole disc was used as positive control while, agar cups filled with only DMSO was used as negative control. The plates were incubated at 37±1 °C for 5 days and the zone of inhibition was measured and compared with the control (i.e. both positive and negative). Three replicates were maintained in each case.

The activity index of the crude was determined by using following formula [15].

Activity index = Zone of inhibition of the extract / Zone of inhibition of the standard

Percent of inhibition was calculated [30].

% of inhibition = Inhibition zone in mm / *****control x 100

*****Growth zone is equal to the plate diameter i.e., 80 mm, as growth occurs all over the agar plate.

Determination of minimum inhibitory concentration:

The minimum inhibitory concentration (MIC) was determined by micro broth dilution assay as recommended and described [27]. The assays were performed in sterile 96-well plates and total volume per well was maintained to 100 µl. The crude was tested at concentrations ranging from 2000 µg/ml to 75 µg/ml. Three wells were inoculated for a given concentration. The MIC was determined as the lowest concentration of the crude extract that reduced and inhibited the growth of the test organisms.

Determination of antioxidant activity:

Antioxidant activity was determined using DPPH free radical scavenging assay [6] that measures the free radical scavenging capacity of test extract. For this purpose, the bark of *T. arjuna* was dried under sunlight for two weeks. The dried barks were then ground to get the bark powder. 1 g of the bark powder was extracted with 200 ml of methanol by refluxing. The methanolic extract was redissolved in methanol and various concentrations like; 200, 100, 50, 25, and 12.5 µg/ml were prepared. The extract was then used for determination of antioxidant activity. During present investigation, gallic acid was used as standard.

The free radical scavenging activity was calculated using the following formula**:**

RSA (%) = ($Abs_{control} - Abs_{sample}$) /$Abs_{control}$ x 100.

Phytochemical screening of the methanolic extract:

The screenings of chemical constituents were carried out with the methanol extract using chemical methods and thin-layer chromatography as mentioned [31].

Statistical analysis:

The statistical processing of the data obtained from all the studies are expressed as means ± standard deviation (SD) of three separate experiments using the computer programme Excel.

RESULTS AND DISCUSSION

In the present investigation, out of three different solvents used for crude extraction, methanol extract of *T. arjuna* showed efficient antagonistic activity against all the test fungus except *M. gypseum* at almost all the concentrations (5 % 10 % 15 % 20 %) (**Table 1**). Acetone extract also showed considerable antagonistic activity. No

significant activity, however, was noticed in case of 95 % ethanol bark extract. Overall *In vitro* screening of the crude extract *T. arjuna* bark revealed maximum antagonistic activity against *T.tonsurans* (Zone of inhibition 25 mm) followed by *T. mentagrophytes* (14 mm), *T. rubrum* (13 mm), *M. fulvum* (10 mm) and *M. gypseum* (3 mm) respectively (**Table 2**). The reasons accounting for higher antidermatophytic activity of methanol extract in present investigation, might be due to enhanced production of biologically active compounds like; alkaloids, tannins, saponins, and flavonoids etc. in this particular solvent [28]. Besides, the better solubility of these active components in methanol [5] might be another reason responsible for its good antagonistic activity. Since, methanolic bark extract showed most potent antidermatophytic activity; hence further experiments on antidermatophytic activities were carried out with this solvent extract.

The minimum inhibitory concentrations of the extract was determined (Table 2). It was recorded as 75 µg/ml in *T. tonsurans,* 125 µg/ml in *T. mentagrophytes,* 250 µg/ml in *T. rubrum*, and *M. fulvum* and 2000 µg/ml in *M. gypseum*. Zone of inhibition (ZI), activity index (AI) and inhibition percentage (I %) of the crude extract were also evaluated (**Table 3**). Maximum activity index (1.32) and inhibition percentage (31.3 %) of the extract was obtained in case of *T. tonsurans* while, minimum was in case of *M. gypseum* (activity index =0.3; inhibition percentage = 3.75). Antidermatophytic activity of the crude extract was compared to the standard antifungal disc, Flucanazole.

DPPH free radical assay is a method used to evaluate the antioxidant activities in a relatively short time compared with other methods. This method is not affected by side reactions such as metal chelation and enzyme inhibition which is a major disadvantage associated with laboratory generated free radicals. The methanolic bark extract of *T. arjuna* showed effective free radical scavenging activity in DPPH radical assay. It exhibited remarkable antioxidant effect even at very low concentration. Free radical scavenging activity of methanolic bark extract of *T. arjuna* was evaluated in comparison with gallic acid on DPPH-free radical (**Table 4 & Fig 1**). Inhibition of DPPH free radical (above 92 %) was observed for all the concentrations of our standard reference compound (gallic acid). When methanol bark extract was tested for DPPH radical scavenging activity, it was found that four concentrations such as; 25, 50 100 and 200 µg/ml lowered the DPPH radical levels above 56, 72, 81 and 87 % respectively. Inhibition of DPPH radicals above 50 % is considered to be significant for antioxidant properties of any compound [26]. The free radical scavenging activity of the extract shows not only the antioxidant properties but the concentrations, which brought these changes, were high as compared to standard reference used. The phytochemical study showed the presence of alkaloids, tannins, saponins in the extract and absence of triglycerides.

The results of the present study are in good agreement with the Ayurvedic application of this plant. This is the first report on the antioxidant and antidermatophytic properties of bark of this potential medicinal plant *T. arjuna* from Northeast India. Further investigations on purification and structure elucidation of the compounds are in progress, to facilitate its screening as therapeutic agent. The immense potentiality of this plant would raise the international attention towards this planet as the results of this study authenticated the pharmaceutical potential of this plant. Selection and careful screening of the medicinal plants of this practically unexplored Northeast region having immense antioxidant and antimicrobial properties would prove a potential area of research in modern biotechnology.

Table 1. Antidermatophytic activity of the bark extract of *T. arjuna*; compared with standard antibiotic disc (Flucanozole) using agar cup diffusion method

Plant used : *Terminalia arjuna* (bark)
Zone of inhibition (mm) using agar cup diffusion method

Test Organism	Flucanazole (10mcg/disc)	Solvents used											
		Methanol				Acetone				95% ethanol			
		5%	10%	15%	20%	5%	10%	15%	20%	5%	10%	15%	20%
T. mentagrophytes	18	6	8	**12**	**14**	-	4	6	**11**	-	-	-	6
T. rubrum	16	5	7	**11**	**13**	-	-	5	7	-	-	-	4
T. tonsurans	19	8	**12**	**15**	**25**	-	6	10	**13**	-	-	4	5
M. gypseum	10	-	-	-	3	-	-	-	3	-	-	-	-
M. fulvum	13	4	4	7	10	-	4	5	7	-	-	-	-

*****Zone of inhibition ≥ 10 mm is indicated in bold; - indicates no activity.

Table 2. Zone of inhibition (in mm) and minimum inhibitory concentration (MIC) of the crude extract of the bark of *T. arjuna* against the dermatophytes

Test organisms	Zone of inhibition (mm)	MIC (µg/ml)
T. mentagrophytes	14.0 ± 0.9	125 ± 0.4
T. rubrum	13.0 ± 1.4	250 ± 1.2
M. gypseum	3.0 ± 0.471	2000 ± 0.4
M. fulvum	10.0 ± 1.4	250 ± 1.2
T. tonsurans	25 ± 1.4	75 ± 0.9

*****The datas are the standard deviations of three replicates; expressed as mean ± SD.

Table 3. Zone of inhibition (ZI), activity index (AI) and inhibition percentage (I %) of *T. arjuna* bark extract *against* the dermatophytes in comparison to both positive and negative controls

Bark extract and controls	Dermatophytes														
	T. mentagrophytes			*T. rubrum*			*T. tonsurans*			*M. gypseum*			*M. fulvum*		
Bark extract	14	0.78	17.5	13	0.81	16.3	25	1.32	31.3	3	0.3	3.75	10	0.77	12.5
Flucanazole (10 mcg/disc)	18	ND	ND	16	ND	ND	19	ND	ND	10	ND	ND	13	ND	ND
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

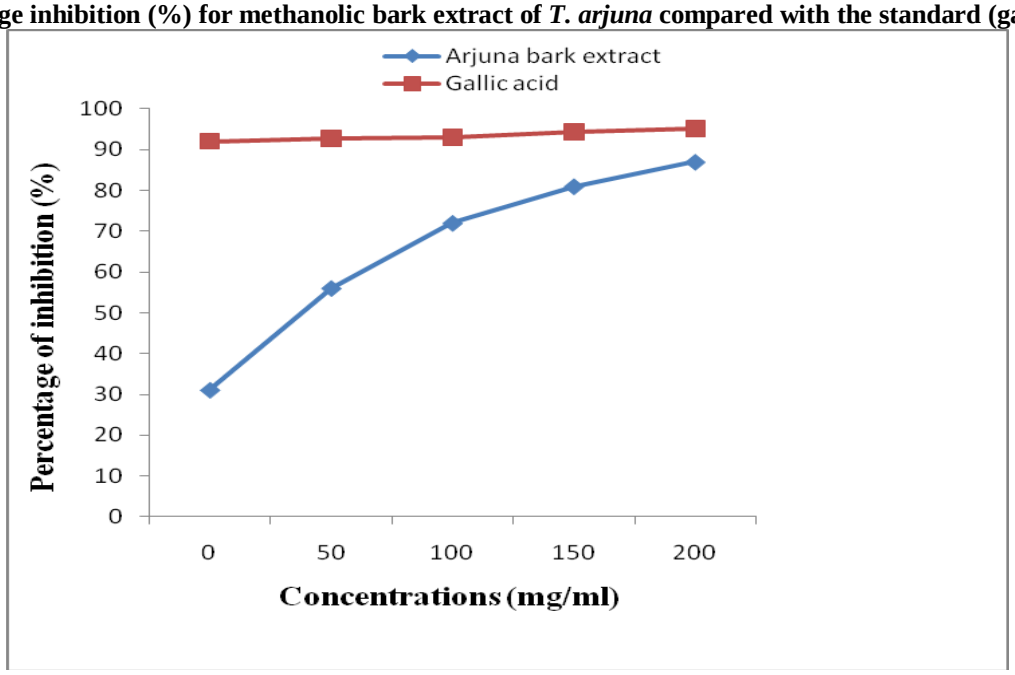
*ND indicates not determined; - indicates no activity.

Table 4. Free radical scavenging activity of gallic acid and methanolic bark extract of *T. arjuna* on DPPH-Free radical.

Concentrations (µg/ml)	DPPH free radical scavenging activity (%)	
	T. arjuna	Gallic acid
12.5	31.20 ± 0.23	92.03±0.12
25	56.05 ± 0.08	92.68 ± 0.09
50	72.33 ± 0.13	93.05 ± 0.03
100	81.12 ± 0.07	94.34 ± 0.16
200	87.1 ± 0.04	95.12 ± 0.11

* The datas are the standard deviations of three replicates, expressed as mean ± SD.

Fig 1. Percentage inhibition (%) for methanolic bark extract of *T. arjuna* compared with the standard (gallic acid)



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