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Terminalia Arjuna: Phytochemical Screening and Anti Bacterial Activity

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Abstract
In present investigation, the detailed physicochemical, Phytochemical and screening for antibacterial activity of stem bark of *Terminalia Arjuna* belonging to the family *combretaceae* is carried out to lay down the standards which could be useful in future experimental studies. The study includes physicochemical evaluation, preliminary Phytochemical screening, HPTLC determination and antibacterial activity. The plant is a large evergreen deciduous tree common in plains and foothills of Himalayas. Stem Bark have major therapeutic clams as antihyperlipidemic, antihypertensive and in heart diseases in traditional medicine.

Keywords: *Terminalia Arjuna*, Phytochemical, Antibacterial activity, *Arjuna*, HPTLC

Introduction

The spread of multi-drug-resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages, medicinal plants have evoked interest as sources of natural products for their potential uses as alternative remedies to heal many infectious diseases (Parekh *et al*., 2005) [1]. Herbal plants play an important role in the development of potent therapeutic agents. Over the years, various medicinal plants and their extracts have been reported to be effective in the treatment of diseases [Ramesh and Karuna 2012] [2]. *T. arjuna* is distributed throughout India, Burma and Sri-Lanka. It mainly grows along the banks of the river and streams. *Terminalia Arjuna* Roxb. (Combretaceae) commonly known as A*rjuna* is one of the medicinally important evergreen trees [Kumar and Prabhakar 1987] [3] which possesses antimicrobial, cytotoxic, antidiabetic [Raghwan and Krishankumari 2007][4], antidiarrheal, antidysentery [Alawa 2002] [5] and hepatoprotective [Doorika and Ananthi 2012] [6] activities.

Terminalia Arjuna stem bark is reported to contain different groups of chemical constituents *viz*. hydrolysable tannins, (Kandil 1998) [7] triterpene (arjunetin, arjunic acid, arjunolic acid, and arjungenin (Roa 1978) [8], Arjunolic acid (2,3,23-trihydroxyolean-12-en-28-oic acid); Flavanoids (arjunolone, arjunon, luteolin), phenolics, and phyto sterols (Kandil 1998) [7], gallic acid, ellagic acid, oligomeric proanthocyanidins (OPCs), calcium, magnesium, zinc and copper (Miller, 1998) [9]. *Arjuna* is a good source of phytosterol, namely, sitosterol which lowers down the cholesterol in blood serum mediated through inhibition of cholesterol absorption resulting from the higher solubility of phytosterols than of cholesterol in bile salt micelles (Anjaneyulu and Prasad, 1982; Ghani, 2003) [10, 11].

Traditionally the drug is widely used in the preparations of Ayurvedic and Unani formulations used in cardio protection (Nandkarni and Linn 2006, 1996) [12]. Bark powder is useful to cure headache and to kill worms in the teeth and treatment of heart troubles (Mutthu 2006) [13] (Yashodharan and Sujaana 2007) [14]. Juice is used as antacid (Prusti 2007) [15] and fruits are found useful as tonic. Bark ash is used in the treatment of the snakebite and scorpion sting in rural/tribal peoples.

In the present study an attempt has been made to investigate physicochemical, Phytochemical and antibacterial activity of *T. arjuna* to explore its therapeutic potential.

Materials and Methods

Plants collection and identification

The stem bark of *Terminalia Arjuna* was collected from the forest of Chitrakoot region, Satna. The herbarium of the plant was also prepared for their correct identification. After proper collection, the plant was washed with water to remove dust and after washing this plant was dried, grinded and sieved (sieve no.100) and finally the plant sample was stored in airtight container.

Physico-chemical analysis [16, 17]

1. Total Ash

Accurately weighed 2g of powdered drug was taken in a tarred silica dish and ignited at temperature not exceeding 450°C until it became white (carbon free). Cooled in desiccator and weighed. Finally, percentage of total ash content was calculated with reference to air dried drug.

2. Acid Insoluble Ash

The total ash obtained from 2g of bark and leaf sample were boiled with 25ml of dilute hydrochloric acid for 5 minutes. The insoluble matter was collected on Gooch crucible, washed with hot water and ignited to obtain constant weight. The percentage of amount of acid insoluble ash was calculated with reference to air dried drug.

3. Alcohol Soluble Extractive value

2 g powdered drug was placed in a conical flask and macerated with 100 ml of Alcohol (90% v/v) for 6 hours, with frequent shaking and then allowed to stand for 18 hours. Filtered through Whatman filter paper. 10 ml of filtrate was transferred to flat bottom dish and solvent was evaporated on a water bath. Cooled it in desiccators for 30 minutes and finally weighed. The content of extractable matter air-dried material was calculated.

4. Water Soluble Extractive Value

2 g powdered drug was placed in a conical flask and macerated with 100 ml of water for 6 hours, with frequent shaking and then allowed to stand for 18 hours. filtered through Whatman filter paper. 10 ml of filtrate was transferred to an evaporating dish and solvent was evaporated on a water bath. Cooled it in desiccators for 30 minutes and finally weighed. The content of extractable matter air-dried material was calculated.

5. Loss on drying

Weighed 2 gm of the drug powder in a dried petri dish. The sample was heated in an oven at a temp. 1050C and this procedure was repeated until constant weight of sample was obtained. After drying was completed, the petri dish was allowed to cool in desiccators. The loss on weight in percentage of air-dried material was calculated.

Preliminary Phytochemical screening

Preliminary qualitative phytochemical analysis of Aqueous and alcoholic extracts of *T. arjuna* was carried out by employing standard protocols [18, 19] for determining the presence and/or absence of phytochemicals *viz*: Alkaloids: (Dragendorff's, wagner, mayer; s test), flavonoids (Shinoda test), Carbohydrate (fehling, molisch, Anthrone test), Protein (Biurate test, millons test), Resin, saponins (Foam tests), Tannins (ferric chloride), starch and steroids (Salkowski tests).

High Performance Thin-layer Chromatography (HPTLC) [20,21]

HPTLC of Methanolic extract of stem bark were carried out by using Toluene: ethyl acetate (9:1) as solvent system. 5 ul of the test solutions were spotted on pre-coated silica gel aluminum plate 60F-254 (5cm×10cm) using Camag Linomat V sample applicator fitted with 100ul Hamilton syringe. Plats were developed in Camag development chamber followed by photo documentation in Camag photoreprostar with CATS software. Visualization was done under UV 254nm, 366nm and white R and after derivatization with 5% Methanolic sulphuric acid reagent. Calculated the Rf value and color of the resolved bands.

Screening of antimicrobial activity [22]

The *in vitro* sensitivity of the Pseudomonas aeruginosa isolates to the crude extracts of *T arjuna* was determined by disc diffusion method. Dried and sterilized paper discs were treated separately with desired concentration of previously prepared methanol and aqueous solution of the crude extract using a micropipette dried in air under aseptic condition and placed at equidistance in a circle on the seeded plate. The concentrations of crude extract used were 1 mg/ml. These plates were kept for 4-6 hours at low temperature and the test materials diffuse from disc to the surrounding medium by this time. The plates were then incubated at 37^{o} C for 24 hours and zone diameter was measured in mm.

Results and Discussion

In present study the bark of *T. arjuna* Linn. was evaluated for its physicochemical, Phytochemical along with HPTLC determination and antibacterial activity against *Pseudomonas aruginosa.*

Physico-chemical analysis

Physicochemical and phytochemical analysis are used to check the genuine nature of the crude drug; thus, it plays an important role in preventing the possible steps of adulteration [23]. Quality tests for drug powder were performed for moisture content, ash content, acid insoluble ash, water soluble extractive, alcohol soluble extractive, and were compared to API standards and are found to be within ranges. The Results of physicochemical analysis are given in (Table -1). The results are expressed as mean (n=3) ± Standard deviation (SD).

Preliminary Phytochemical screening

The results of preliminary phytochemical screening showed the presence of carbohydrates, phenolic compounds, flavonoids, alkaloids, proteins, saponins, and tannins, in the methanolic extract of the drugs (Table -2). The medicinal properties of plants material are mainly due to the presence of various phytoconstituents [24]. The presence of different phytoconstituents in studied drugs justifies their therapeutic potential [25, 26]. These phytoconstituents have been reported to have multiple biological effects such as anti-inflammatory, anti-allergic, antioxidant, antidiabetic, analgesic, antispasmodic, antibacterial, anti-viral, anti-cancer and aldose reductase inhibitory activities. It is also used for the treatment of diarrhea and dysentery [27].

High Performance Thin-layer Chromatography (HPTLC)

Fingerprinting analysis of sample was done through HPTLC method, and the data were presented in the (Table-3) (Figure-1,2). The result indicates the presence of various spots with different color at different rf values. HPLTC is an important analytical tool in the separation, detection and estimation of different classes of natural products. Developed fingerprint profile would serve as a reference standard for quality evaluation and standardization of the formulation with same drug. In the last few decades, an HPTLC technique has gained much popularity for standardization of the herbal drugs and formulations. Analysis of several samples simultaneously using a small quantity of marker compound and mobile phase with very less time is the major advantage of HPTLC [28]. TLC and HPTLC techniques have been used as important analytical tools in pharmaceuticals, medicine, chemistry, food analysis, toxicology and environmental science [29].

Susceptibility against T. *arjuna* extract

The bark of the *Terminalia Arjuna* constitutes an important crude drug, which contains tannins, triterpenoids saponins, flavonoids, sterols, calcium salts, alkaloidal and glycosidal substances, arjunine and arjunglyciside etc. It stops bleeding and pus formation in the gums and is useful in asthma, dysentery, menstrual problems, pains, leucorrhea, wounds and skin eruptions [11]. Due to increased awareness of the importance of traditional medicine in human and animal health care, research into the efficacy of some of the herbs

used in the treatment of some illness would be worthwhile [30].

Arjun bark extract was used as a biological tool to resolve the antibiotic-resistant Pseudomonas aeruginosa problem. Arjun extract showed promising effect against the isolated Pseudomonas aeruginosa at concentration of 1 mg/mL. The zone diameter of the extract is given in (Table 4). This study revealed that *Terminalia Arjuna* would be a good antibacterial drug in the treatment of Pseudomonas aeruginosa infections, provided if it is found effective and nontoxic through *in vivo* study. Further study is needed to understand the molecular mechanism of the extract that will help us to make more effective therapeutics to combat Multi-Antibiotic Resistant (MAR) pathogen. The decrease in percent was found to be more potent in aqueous extract of T. arjuna which is 33% high as compared to other solvents. Hence, it was found that people used chloramphenicol in their dose as an antibiotic is more beneficial for them.

Conclusion

Quality evaluation of medicinal plants is a fundamental requirement of industry and other organization dealing with ayurvedic and herbal products. The bark of *Terminalia Arjuna* contains appreciable amount of secondary metabolite. These phytoconstituents may acts as resource of pharmacologically active agents and natural antioxidants. The present investigations will be helpful while standardizing the drug for its various pharmacological potentials and to check the adulteration in natural valuable drug at the time of consumption for desire pharmacological effect.

The additional antibiotic resistance might have contributed to the initial selection of the new strain, since these antibiotics are used to treat patients. Antibiotics clearly influence the prevalence of novel antibiotic-resistant clones in asthmatic areas. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent anti-microbial drugs of natural origin against *Pseudomonas aeruginosa*.

Table 1: Physicochemical analysis

S. No	Name of the experiment	Result (w/w)	Standard (API)
1.	Loss on drying 105^0c	3.4%	
2.	Water soluble extractive value	54%	Not less than 17%
3.	Alcohol soluble extractive value	18.5%	Not less than 16%
4.	Total ash	16.35%	Not more than 17%
4	Acid insoluble ash	1.8%	Not more than 2%

*Results are average of three values

Table 2: Preliminary Phytochemical screening

S. No	Phyto constituents	Observation	Methanol extract	Aqueous extracts
1	Alkaloids Dragendorff's test Wagner's test Mayer; s test	Orange colour appears Brown colour appears Pale yellow colour appears	+ve +ve +ve	-ve -ve -ve
2.	Carbohydrate Anthrone's test Fehling's test Molisch test	Brown colour appears Brick red colour appears Ring of red violet colour appears	+ve +ve +ve	+ve +ve +ve
3.	Flavonoids	Light brown colour appears	+ve	+ve
4.	Protein Biuret's test Millon's test	Brown colour appears Orange colour appears	-ve -ve	-ve -ve
5.	Resins	Turbidity appears	+ve	+ve
6.	Saponin	Honey comb like froth forms	+ve	+ve
7.	Steroid	Red violet colour ring appears	+ve	+ve
8.	Tannin	Brown colour appears	+ve	+ve
9.	Starch	Yellow colour appears	-ve	-ve

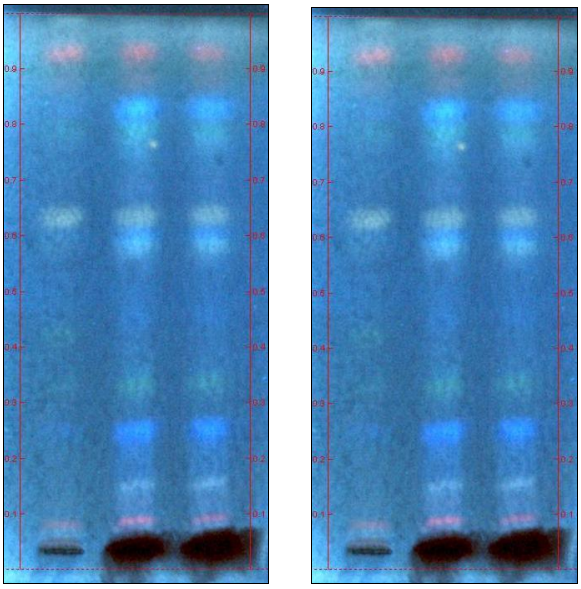
Table 3: R_f values color of the bands resolved in test solutions of *T. arjuna* Linn.

Mobile phase: Tolune: Ethyl Acetate: Formic acid (7:2.5:0.5)		
	Standard	**Test sample**
366nm	0.07(pink),0.11(pink),0.22(light blue), 0.31 (light yellow), 0.54 (light pink),0.59(light blue), 0.77(blue),0.86 (pink),0.91(pink)	0.07(pink), 0.11 (pink), 0.20 (blue), 0.24 (blue), 0.31 (light yellow),0.43(sky blue), 0.54(light pink), 0.54(light pink), 0.66(reddish blue), 0.77(sky blue), 0.82(blue), 0.86(pink), 0.91(pink)
366nm AD	0.08(light pink), 0.63(light brown), 0.92 (light orange)	0.08 (light pink), 0.15(faint blue), 0.23 (blue), 0.59(sky blue), 0.63 (light brown), 0.83 (blue), 0.92(orange color)

Table 4: Zone diameter of *Terminalia Arjuna* extracts

Pathogen	Zone of inhibition			
	Chloramphenicol)	**Methanol extract**	**Aqueous extract**	**Antibiotics (chloramphenicol)**
Pseudomonas aeruginosa	14 mm	37mm	36mm	20mm

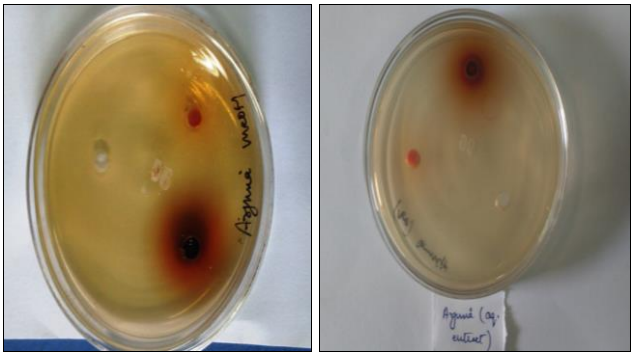
Plate 1 HPTLC Chromatograph



T1 T2 T3
(I) 366nm

T1 T2 T3
(II) 366nm AD

Plate 2: Zone diameter of *Terminalia Arjuna* extracts



(**I**) (**II**)

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