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**RESEARCH ARTICLE**

# EVALUATION OF ANTIMICROBIAL AND PHYTOCHEMICAL PROPERTIES OF *TERMINALIA ARJUNA* LINN. EXTRACTS AGAINST RESPIRATORY TRACT PATHOGENS

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**ABSTRACT**

The aim of this research was to evaluate the antimicrobial activity of *Terminalia arjuna* bark extracts was aimed to investigate against three gram-positive, two gram-negative bacteria and one fungi causing respiratory infections. Dried plant materials were crushed and extracted in petroleum ether, acetone, methanol and water by using Soxhlet apparatus. The agar well diffusion method was adopted to examine antimicrobial activity of extracts against test organisms. Phytochemical analysis was done for plant extract also. The results showed that methanol extract was most active as comparison to other extract. The maximum inhibition was found against *S. pneumoniae* (17.3±0.57 mm) followed by *H. influenzae* (16.6±0.57mm), *P. aeruginosa* (14.6±0.76 mm) and *S. pyogenes* (13.6±0.28 mm), *S. aureus* (12.6±0.28 mm) and minimum against *C. albicans* (11.3±0.28 mm) respectively. Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, steroids, saponins and tannins in plant extracts. The results signify traditional values of *T. arjuna* in treatment of respiratory diseases which might be accountable for its antimicrobial potential.



## INTRODUCTION

Herbal medicines have been used for the treatment and cure of various diseases in Indian traditional practiced methods such as Ayurveda, Unani and Siddha (Dahanukar *et al.,* 2000; Kumar *et al*., 2006). *Terminalia arjuna* (Combretaceae) is commonly called arjuna in Hindi. It is a deciduous and evergreen tree distributed throughout India and also found in Burma, Srilanka and Mauritius, growing up to a height of 60 to 90 feet. Leaves of arjuna are simple, oblong or elliptic with pale and dark green upper surface and pale brown lower surface. Flowers are bisexual, sessile and white arranged in short axillary spikes or in terminal pannicule. The bark is smooth, pinkish-grey from outside and flakes off in large, curved and rather flat pieces (Dwivedi *et al*., 2007; Jain *et al*., 2009). The bark and leaves of this plant have been used in indigenous system of medicine for curing different diseases, the bark in the treatment for angina (hritshool), expectorant, antidysentric, purgative, laxative, leucoderma, anaemia, hyperhidrosis, asthama, tumors and other cardiovascular disorders (Udupa, 1986). Literature search revealed that bark possesses good anticancer, antiviral and antimicrobial activities (Tripathi and Singh, 1996; Cooper, 2005; Singh *et al*., 2008).

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Plants have unlimited capacity to synthesize secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides and phenols which have been found to have antimicrobial properties (Cowan, 1999; Sher, 2009; Das *et al*., 2010). The purpose of this research was to determine the antimicrobial and phytochemical aspects of *T. arjuna* bark extracts against respiratory pathogens that usually cause infections in upper and lower respiratory tract region.

## MATERIALS AND METHODS

### Plant collection

Plant was collected from Srinagar, Uttarakhand and authenticated at Department of Botany and Microbiology, H.N.B. Garhwal University, Srinagar. Collected roots were dried under shade at room temperature and crushed to small pieces by using pestle and mortar and powdered in an electric grinder.

### Extraction of plant material

Plant extracts were prepared by immersing 200 g of powdered plant material in 600 ml of four different solvents i.e. petroleum ether (PET), acetone (ACE), methanol (MeOH) and water ($H_2O$), loaded in Soxhlet assembly and extracted for 72 h through successive method (Ahmed *et al*, 1998). Plant extracts were filtered through Whatman No. 1 filter paper and crude

extracts obtained by removing solvent in rotary vacuum evaporator. Residues were stored at 4°C until further use.

**Test Microorganisms**

Five standard bacterial strains (*Haemophilus influenzae* MTCC 3826, *Pseudomonas aeruginosa* MTCC 2474, *Staphylococcus aureus* MTCC 1144, *Streptococcus pneumoniae* MTCC 655, *Streptococcus pyogenes* MTCC 442) and one fungi *Candida albicans* MTCC 227 and their isolates were subjected for antimicrobial study. The selected microbial standard strains were procured from Institute of Microbial Technology (IMTECH), Chandigarh.

**Screening for antimicrobial activity**

The antimicrobial activity of different extracts was determined by agar well-diffusion method (Perez *et al.,* 1990). 0.1 ml of 12-16 h incubated cultures of microbial species were mixed in molten Mueller Hinton Agar (MHA) medium and poured in pre-sterilized Petri plates. A cork borer (6 mm diameter) used to punch wells in solidified medium and filled with extracts of 45 µl of 200 mg/ml final concentration of extracts. DMSO was used as negative control. The efficacy of extracts against test organisms was compared with broad spectrum antibiotic erythromycin (positive control). The plates were incubated at 37°C for 24 to 48 h in BOD incubator and the diameter of the zone of inhibition was measured in millimetre. Each sample was assayed in triplicate and mean values were observed. The antimicrobial activity was interpreted from size of diameter of zone of inhibition measured to nearest millimetre (mm) as observed from clear zones surrounding wells.

**Phytochemical screening**

Major phytoconstituents present in *T. arjuna* extracts were subjected to phytochemical analysis to determine the presence of bioactive components by using standard qualitative methods (Trease and Evans, 1996).

**Test for flavonoids**

On addition of conc. HCl in MeOH extract of material, a red colour appeared which indicated the presence of flavonoids.

**Test for glycosides**

Plant extract was filtered and sugar was removed by fermentation with baker's yeast. The acid was removed by precipitation with $Ba(OH)_2$. The remaining extract contained the glycosides. The hydrolysis of solution was done with conc. $H_2SO_4$ and after hydrolysis the presence of sugars was determined with help of Fehling's solution.

**Test for Steroids**

Extracts were mixed with 3 ml $CHCl_3$ and 2 ml conc. $H_2SO_4$ was poured from side of test tube and colour of the ring at junction of two layers was noted. A red colour showed the presence of steroids.

**Test for Saponins**

Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

**Test for Tannins**

Extract was added in 1% ferric chloride and observed the colour. Bluish black colour appeared which disappeared on addition of dilute $H_2SO_4$ follow a yellow brown precipitate indicates the presence of tannins.

## RESULTS AND DISCUSSION

The results for antimicrobial activity are depicted in Table 1. MeOH extract was found most active against all test pathogens in comparison to other extracts.

**Table 1. The percentage of potency of *Terminalia arjuna* extracts against respiratory tract pathogens**

| S. No. | Pathogens | Diameters of inhibition zone (mm) | | | |
|---|---|---|---|---|---|
| | | PET | $CHCl_3$ | MeOH | $H_2O$ |
| 1. | *H. influenzae* | 9.0±0.50 | 13.3±0.57 | 15.3±0.28 | 11.0±0.50 |
| 2. | *H. influenzae* (MTCC 3826) | 10.3±0.57 | 12.3±0.57 | 16.6±0.57 | 12.3±0.28 |
| 3. | *P. aeruginosa* | 8.3±0.28 | 12.3±0.28 | 14.0±0.50 | 11.3±0.28 |
| 4. | *P. aeruginosa* (MTCC 2474) | 9.6±0.76 | 11.3±0.57 | 14.6±0.76 | 11.0±0.50 |
| 5. | *S. aureus* | 8.6±0.28 | 11.3±0.28 | 12.3±0.28 | 12.3±0.57 |
| 6. | *S. aureus* (MTCC 1144) | 9.6±0.28 | 10.6±0.28 | 12.6±0.28 | 13.6±0.28 |
| 7. | *S. pneumoniae* | 9.6±0.76 | 13.6±0.76 | 17.3±0.57 | 11.3±0.57 |
| 8. | *S. pneumoniae* (MTCC 655) | 9.3±0.57 | 15.0±0.50 | 16.3±0.57 | 10.3±0.28 |
| 9. | *S. pyogenes* | 10.0±0.50 | 11.3±0.57 | 13.6±0.28 | 12.6±0.76 |
| 10. | *S. pyogenes* (MTCC 442) | 9.3±0.28 | 10.3±0.28 | 12.6±0.76 | 13.0±0.50 |
| 11. | *Candia albicans* (MTCC 227) | 7.6±0.28 | 9.3±0.57 | 11.3±0.28 | 9.6±0.76 |

*Values are means of three replicates, Cork borer diameter: 6 mm

**Test for alkaloids**

Test solution was acidified with acetic acid and a drop of Mayer's reagent was added. A white precipitate indicated the presence of alkaloids.

MeOH extract showed maximum activity followed by PET, ACE and $H_2O$ extract. The maximum inhibition was found against *S. pneumoniae* (17.3±0.57 mm) followed by *H. influenzae* (16.6±0.57mm), *P. aeruginosa* (14.6±0.76 mm) and *S. pyogenes* (13.6±0.28 mm), *S. aureus* (12.6±0.28 mm) respectively. The minimum inhibition was noted against *C.*

*albicans* (11.3±0.28 mm). So far, antimicrobial potential of *T. arjuna* has been found effective against selected bacterial and fungal species. *T. arjuna* ACE leaf extract was found most effective against *S. aureus* (28 mm) followed by *Proteus mirabilis* (27.6 mm), *Acinetobacter* sp. (16.6 mm) and *P. aeruginosa* (16 mm) (Aneja *et al*., 2012). Different pathogenic bacteria such as *Escherichia coli, Klebsiella pneumoniae,* and *Salmonella* spp. are widely distributed in the hospitals and in community thus creating serious health problems (Khan, 2004; Akram *et al*., 2007).

**Table 2. Phytochemical screening of various extracts of *T. arjuna* bark**

| S. No. | Phytoconstituents | Solvents | | | |
|---|---|---|---|---|---|
| | | PET | ACE | MeOH | $H_2O$ |
| 1. | Alkaloids | + | + | + | + |
| 2. | Flavonoids | - | + | + | + |
| 3. | Glycosides | - | + | - | + |
| 4. | Steroids | + | + | + | + |
| 5. | Saponins | - | - | + | + |
| 6. | Tannins | + | + | + | + |

+ = Present, - = Absent

The phytochemical screening of *T. arjuna* extract has shown that plant contains alkaloids, flavonoids, glycosides, steroids, saponins and tannins which are very important constituent when looking for pharmacologically active phytochemical in the plant (Table 2). According to Akhter *et al*. (2012), extracts of *T. arjuna* (bark) contain phenols, flavonoids, tannin, saponin, alkaloids, glycosides, phytosterols and carbohydrate. Doorika *et al.* (2012), reported *T. arjuna* were screened of biologically active compounds like steroids, tannins, phenolics compound, quinone, terpinoids, sugar, alkaloids and flavonoids. By this study, it is concluded that *T. arjuna* can be used as herbal medicine to treat respiratory infections caused by tested pathogens as comparative to synthetic chemotherapeutic agents. It is urged that further research should be carried out to expose the bioactive constituents present in *T. arjuna*.

## Conclusion

The investigation for antimicrobial activity of *T. arjuna* bark extracts revealed that plant has broad spectrum activity against selected pathogens which explain the basis for its use in traditional medicines. The significant activity was exhibited by MeOH extract against test respiratory pathogenic microorganisms. The study supported the usefulness in term of availability of phytoconstituents. By results, it can be concluded that *T. arjuna* can be helpful as an alternative source of medicine and new drug discovery.

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