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Original Article

Comparative evaluation of antimicrobial and antioxidant potential of ethanolic extract and its fractions of bark and leaves of *Terminalia arjuna* from north-western Himalayas, India

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ABSTRACT

The present study was aimed to evaluate the antimicrobial and antioxidant potential of ethanolic extract and its different solvent fractions (chloroform, ethyl acetate, n-butanol and aqueous fraction) of bark and leaves of *Terminalia arjuna.* The antimicrobial activity was determined by disc diffusion and minimum inhibitory concentration (MIC) methods against six bacterial stains. The antioxidant activity was evaluated by using DPPH, FRAP and Nitric oxide (NO) scavenging assay. The total phenolics and flavonoid content were found to be higher in n-butanolic fraction of bark (294.6 ± 8.1 mg/g GAE and 168.6 ± 12.3 mg/g RE, respectively) and leaves (203.7 ± 7.0 mg/g GAE and 144.8 ± 11.1 mg/g RE, respectively). The maximum antimicrobial activity was shown by n-butanolic fraction of bark and leaves. The zone of inhibition of 15.0 ± 0.7 mm, 15.5 ± 0.7 mm, 15.0 ± 1.5 mm, 15.5 ± 0.7 mm, 15.0 ± 0.7 mm, 15.0 ± 0.7 mm was observed against *Bacillus subtilis*, *Staphylococcus aureus, Eschericia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhi* respectively. In case of leaves extract, zone of inhibition of 13.5 ± 0.7 mm, 16.5 ± 0.7 mm, 14.0 ± 0.5 mm, 15.0 ± 0.5 mm, 13.5 ± 0.7 mm, 14.0 ± 0.7 mm was observed against *B. subtilis*, *S. aureus, E. coli, K. pneumoniae, P. aeruginosa, S. typhi,* respectively. The n-butanol fraction of bark [IC_{50}-4.1 μg/ml (DPPH), 21.0 μM (FRAP), 3.3 μg/ml (NO)] and leaves [IC_{50}-4.8 μg/ml (DPPH), 28.9 μM (FRAP), 3.2 μg/ml (NO)] showed more antioxidant potential as compared to that of crude ethanolic extract, ethyl acetate fraction, chloroform fraction, aqueous fraction and even ascorbic acid. These results clearly indicated comparative antioxidant potential and antimicrobial activity in extracts of bark and leaves of *T. arjuna.*

1. Introduction

Medicinal plants are the important constituents of both traditional and conventional medicine preparations from ancient times. Majority of people prefer herbal-based medicines as compared to that of conventional medicine.[1] Safe, effective and inexpensive indigenous remedies have become more popular among the people of both the urban and rural areas of India and China.[2] Therefore, medicinal plants have become the essential part of human health care system.[3] Moreover, medicinal plants have attained more attention because of their effectiveness, increased cost of current medicines and cultural preferences.[4,5] Large number of plants have been reported to possess antimicrobial and antioxidant potential.[6,7] The search for plant-based potent antimicrobials has dramatically increased because of the emergence of multiple drug resistance.[8] Identification of plant based antioxidants is another aspect which has gained immense importance to protect the cell/tissues from the damage caused by free radicals. Phenolic compounds present in plants act as powerful antioxidants which can protect the cellular machinery from free radicals by acting as hydrogen donors and radical scavenger.[9] Antioxidants act as free radical scavengers and

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are thus helping to mitigate the effect of oxidative stress in a variety of diseases such as cardiovascular diseases, Parkinson's disease, Alzheimer's disease, cancerogenesis, Neuro-degenerative, nephrotoxicity, diabetes and the ageing.[10] Many studies have demonstrated the efficacy of plant derived products as a good source of antioxidants against various diseases induced by reactive oxygen species. Several studies have reported that phenolic compounds, such as flavonoids and phenolic acids present in plants are responsible for their antioxidant nature.[11–14]

Therefore, there is need to carry out a screening of the plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their active constituents. *Terminalia arjuna* belonging to family combretaceae is a potent cardioprotective agent from ancient times. The bark of *T. arjuna* is used in the treatment of fractures, ulcers, hepatic and also showed hypocholesterolemic, antibacterial, antimicrobial, antitumoral, antioxidant, anti allergic and anti feedant, anti fertility and anti-HIV activities.[15–17] The use of bark in traditional medicine may lead *T. arjuna* to become endangered. Therefore, the current study was focused to compare the antimicrobial and antioxidant potential of leaves and bark and to promote the utilization of leaves (non-destructive method) in therapeutics.

2. Material and methods

2.1. Processing of bark and leaves of T. arjuna

The bark and leaves of *T. arjuna* were collected from Dharamshala region of District Kangra of Himachal Pradesh (30°22′40″–33°12′40″ N to 75°45′55″–79°04′20″ E), India. The collected samples were thoroughly washed with running tap water followed by distilled water. The samples were completely dried in hot air oven at 40 °C and ground to fine powder and stored in airtight jars.

2.2. Extraction and fractionation

The dried powder of bark and leaves (50 g) of *T. arjuna* were mixed with 500 ml ethanol in a conical flask plugged with cotton wool and incubated on a rotary shaker at 120 rpm for 5 days to ensure complete extraction.[18] The extracts were filtered through Whatmann No. 1 filter paper and then centrifuged at 4000 g for 5 min. The solvent phase was collected and evaporated at 40 °C. The dried crude extracts were stored at 4 °C in airtight bottles till further use. The process of extraction was repeated three times to ensure complete extraction. The crude ethanolic extract was dissolved in distilled water and successive fractionation was done using chloroform, ethyl acetate, and n-butanol and remaining aqueous fraction as shown in Fig. 1.[19,20]

2.3. Qualitative analysis of phytochemicals

The ethanolic extract and its various fractions (chloroform fraction, ethyl acetate fraction, n-butanol fraction and aqueous fractions) of bark and leaves of *T. arjuna* were subjected to different chemical tests for the detection of various phytocomponents such as phenolics, tannins, flavonoids, phytosteroids and saponins as per standard methods.[21–23]

2.4. Quantification of total phenolic content (TPC) and total flavonoid content (TFC)

TPC of the ethanolic extract and its fractions of bark and leaves of *T. arjuna* was quantified using Folin-Ciocalteu reagent according to the method described by Singleton et al[24] TPC was calculated from calibration curve of gallic acid (25–200 μg) and expressed in terms of GAE per gram of dry extract (see Fig. 2A and B).

TFC in ethanolic extract and its fractions of bark and leaves of *T. arjuna* were assessed by using aluminum chloride ($AlCl_3$) method as described by Zhishen et al[25] and was quantified from the standard curve of rutin (25–200 μg/ml) and expressed as RE per gram of dry extract (see Fig. 2C and D).

2.5. Microbial strains

The six bacterial strains (two Gram's positive, viz. *Staphylococcus aureus* and *Bacillus subtilis* and four Gram's negative, viz. *Eschericia coli, Salmonella typhi, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and a fungal strain (*Candida albicans*) were used to study antimicrobial activity of ethanolic extract and its fractions of bark

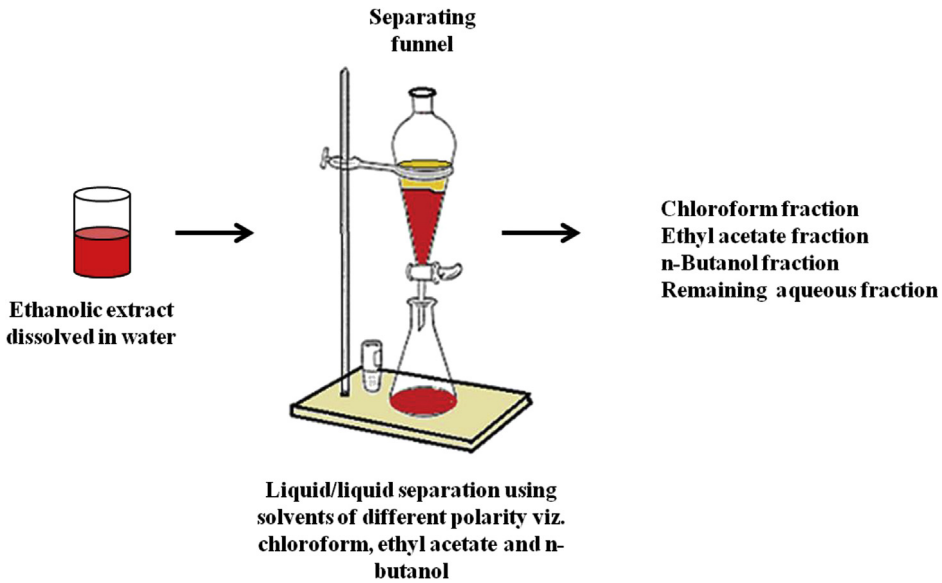

Fig. 1. Schematic diagram showing liquid–liquid fractionation of the crude ethanol extract.

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