



Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: <http://www.elsevier.com/locate/jtcme>

## 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, IndiaVikas Kumar<sup>a,\*</sup>, Nitin Sharma<sup>a</sup>, Anuradha Sourirajan<sup>a</sup>, Prem Kumar Khosla<sup>b</sup>, Kamal Dev<sup>a,\*\*</sup><sup>a</sup> Faculty of Applied Sciences and Biotechnology, Shoolini University, Post Box No. 9, Head Post Office, Solan, H.P., India<sup>b</sup> Faculty of Biological Sciences and Environmental Sciences, Shoolini University, Post Box No. 9, Head Post Office, Solan H.P., India

## ARTICLE INFO

## Article history:

Received 23 January 2017

Received in revised form

4 April 2017

Accepted 5 April 2017

Available online 23 April 2017

## Keywords:

*Terminalia arjuna*

Antimicrobials

Antioxidants

IC<sub>50</sub>

Medicinal trees

## 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-butanol fraction of bark ( $294.6 \pm 8.1$  mg/g GAE and  $168.6 \pm 12.3$  mg/g RE, respectively) and leaves ( $203.7 \pm 7.0$  mg/g GAE and  $144.8 \pm 11.1$  mg/g RE, respectively). The maximum antimicrobial activity was shown by n-butanol fraction of bark and leaves. The zone of inhibition of  $15.0 \pm 0.7$  mm,  $15.5 \pm 0.7$  mm,  $15.0 \pm 1.5$  mm,  $15.5 \pm 0.7$  mm,  $15.0 \pm 0.7$  mm,  $15.0 \pm 0.7$  mm was observed against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhi* respectively. In case of leaves extract, zone of inhibition of  $13.5 \pm 0.7$  mm,  $16.5 \pm 0.7$  mm,  $14.0 \pm 0.5$  mm,  $15.0 \pm 0.5$  mm,  $13.5 \pm 0.7$  mm,  $14.0 \pm 0.7$  mm was observed against *B. subtilis*, *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. typhi*, respectively. The n-butanol fraction of bark [IC<sub>50</sub>–4.1 µg/ml (DPPH), 21.0 µM (FRAP), 3.3 µg/ml (NO)] and leaves [IC<sub>50</sub>–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*.

© 2018 Center for Food and Biomolecules, National Taiwan University. Production and hosting by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 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.<sup>1</sup> Safe, effective and inexpensive indigenous remedies have become more popular among the people

\* Corresponding author. Faculty of Applied Sciences and Biotechnology, Shoolini University, Bajhol, Solan H.P., India.

\*\* Corresponding author. Faculty of Applied Sciences and Biotechnology, Shoolini University, Bajhol, Solan, H.P., India.

E-mail addresses: [vikaskmr59@gmail.com](mailto:vikaskmr59@gmail.com) (V. Kumar), [kamaldevbhardwaj1969@gmail.com](mailto:kamaldevbhardwaj1969@gmail.com) (K. Dev).

Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

of both the urban and rural areas of India and China.<sup>2</sup> Therefore, medicinal plants have become the essential part of human health care system.<sup>3</sup> Moreover, medicinal plants have attained more attention because of their effectiveness, increased cost of current medicines and cultural preferences.<sup>4,5</sup> Large number of plants have been reported to possess antimicrobial and antioxidant potential.<sup>6,7</sup> The search for plant-based potent antimicrobials has dramatically increased because of the emergence of multiple drug resistance.<sup>8</sup> 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.<sup>9</sup> Antioxidants act as free radical scavengers and

<http://dx.doi.org/10.1016/j.jtcme.2017.04.002>

2225-4110/© 2018 Center for Food and Biomolecules, National Taiwan University. Production and hosting by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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.<sup>10</sup> 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.<sup>11–14</sup>

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.<sup>15–17</sup> 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.<sup>18</sup> 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.<sup>19,20</sup>

### 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 phytochemicals such as phenolics, tannins, flavonoids, phytosteroids and saponins as per standard methods.<sup>21–23</sup>

### 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.<sup>24</sup> 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<sub>3</sub>) method as described by Zhishen et al.<sup>25</sup> 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. *Escherichia 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

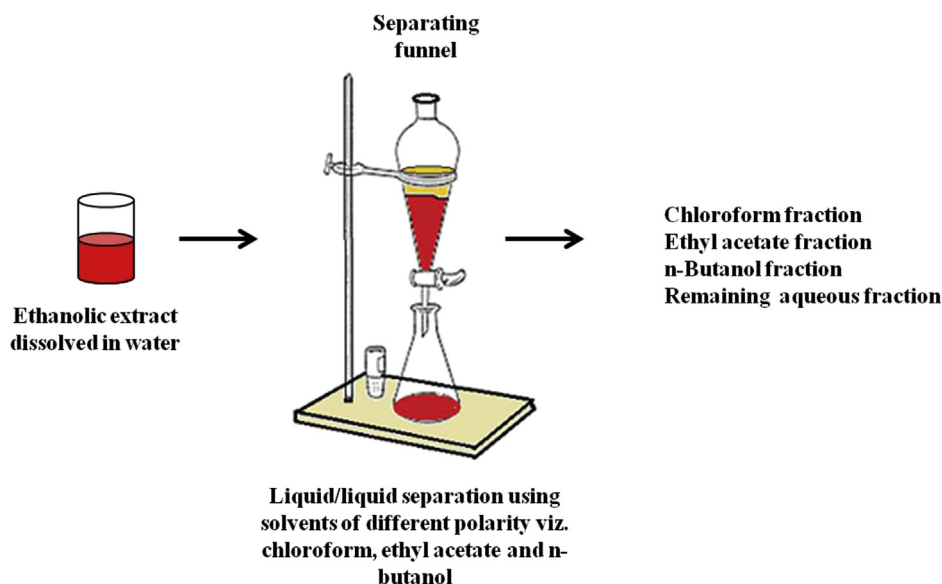
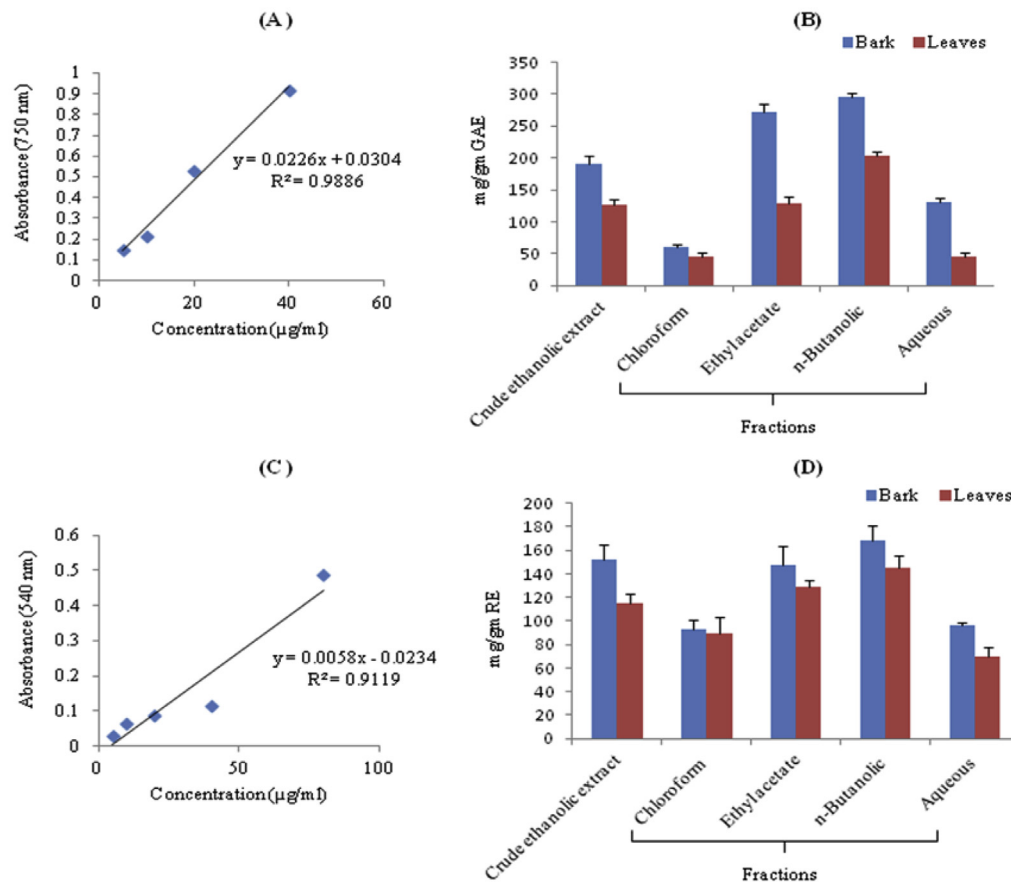


Fig. 1. Schematic diagram showing liquid–liquid fractionation of the crude ethanol extract.



**Fig. 2.** Quantification of total phenolics and flavonoids content in crude ethanolic extract of bark and leaf and their fractions. A) Standard curve of Gallic acid (5–40 µg/ml). B) Total phenolic contents of crude ethanolic extract of bark and leaf and their fractions. C) Standard curve of Rutin (5–80 µg/ml). D) Total flavonoid content of crude ethanolic extract of bark and leaf and their fractions. Total Phenolic content was expressed as mg/gm gallic acid equivalents (GAE), whereas flavonoid content was expressed in terms of mg/gm rutin (RE).

and leaves of *T. arjuna*. All the strains were obtained from Yeast Biology Lab, Shoolini University, Solan, Himachal Pradesh, India. The bacterial strains were grown in nutrient broth at 37 °C, whereas *C. albicans* was grown in YPD broth at 30 °C. Bacterial strains were maintained on nutrient agar (NA) slants, whereas, PDA slants were used for storage of *C. albicans* at 4 °C.

## 2.6. Antimicrobial activity using agar well diffusion method

The antimicrobial activity of ethanolic extract and its various fractions such as chloroform fraction, ethyl acetate fraction, n-butanol fraction and aqueous fractions dissolved in dimethyl sulfoxide (DMSO) at concentration of 50 mg/ml of bark and leaves of *T. arjuna* was evaluated by agar well diffusion method.<sup>26</sup> The bacterial and fungal culture of 0.5 McFarland Standard ( $\sim 2 \times 10^8$  colony forming units (CFU)/ml) was uniformly spread on the surface of the nutrient agar plates using sterile cotton swabs.<sup>27</sup> The wells were punched with the cork borer (6 mm) in the agar. Approximately 50 µL of the crude extract and its fractions (50 mg/ml) of bark and leaves were added into the wells, allowed to stand at room temperature for about 2 h and incubated at 37 °C. After 24 h of incubation, the zone of inhibition was measured using a HiAntibiotic Zone scale-C (Himedia Biosciences, Mumbai (India)). Amoxycylav (10 µg) and Fluconazole (25 µg) were used as a positive control in case of bacterial strains and fungus strain respectively. DMSO

(solvent) was used as solvent control. The tests were performed in duplicates and results were recorded as mean  $\pm$  SD.

## 2.7. Determination of MIC by broth dilution method

The minimum inhibitory concentration (MIC) of the extract and fractions was evaluated by broth dilution method described under CLSI<sup>28</sup> guidelines using 2,3,5-triphenyl tetrazolium chloride. The ethanolic extract and its fractions were dissolved in DMSO and geometric dilutions ranging from 25–48 µg/ml of each extracts were prepared in a 96-welled micro titer plate, including one growth control (nutrient broth (NB) containing DMSO) and a positive control (NB inoculated with bacterial culture and containing Amoxycylav). Plates were incubated under normal atmospheric conditions at 37 °C for 24 h for bacteria and 30 °C for *C. albicans*. The color change was then observed visually. The growth was indicated by changes in color from purple to pink or colorless. The lowest concentration at which color change appeared was taken as the MIC value.

## 2.8. In-vitro antioxidant activity

Different antioxidant methods were employed to characterize the antioxidant potential of the ethanolic extract and its subsequent fractions.<sup>29,30</sup> Ethanolic extract and its various fractions (chloroform fraction, ethyl acetate fraction, n-butanol fraction and

aqueous fraction) were dissolved at a concentration of 1 mg/ml in ethanol and then diluted in order to prepare different concentrations (2.5–10 µg/ml) for antioxidant assays. Ascorbic acid was used as a standard antioxidant compound for comparative analysis in all assays.

### 2.8.1. DPPH radical scavenging activity

DPPH radical scavenging activity of the extract was determined by the method described by Barros et al.<sup>31</sup> The percentage of inhibition activity was calculated using the following equation:

$$\% \text{ Inhibition} = (Ac - As/Ac) \times 100$$

where Ac is the absorbance of the control and As is the absorbance of the extract/standard. The free radical scavenging activity of samples was expressed as IC<sub>50</sub> value, which represented the effective concentration of extract/standard required to scavenge 50% of DPPH radicals.

### 2.8.2. FRAP assay

The ability to reduce ferric ions was measured using the method described by Benzie and Strain.<sup>32</sup> The antioxidant capacity based on the ability to reduce ferric ions of extract was calculated from the linear calibration curve of FeSO<sub>4</sub> (2.5–20 µM) and expressed as µM FeSO<sub>4</sub> equivalents per gram of extract.

### 2.8.3. NO scavenging assay

The nitric oxide scavenging assay was carried out using sodium nitroprusside method as described by Sreejayan and Rao<sup>33</sup> with ascorbic acid as positive standard.

## 2.9. Statistical analysis

Total phenolic content, total flavonoid content and half of minimum inhibitory concentration (IC<sub>50</sub>) was determined by linear regression analysis method. Each sample was analyzed individually in triplicates and the results are expressed as the mean value (n = 3) ± standard deviation.

## 3. Results and discussions

### 3.1. Quantitative analysis of phytochemicals in bark and leaves of *T. arjuna*

Phytochemical components such as phenolics, flavonoids, tannins, terpenoids and saponins were determined in ethanolic extract and its fractions as shown in Table 1. Phytochemical analysis revealed that flavonoids and phenolics were present in both crude ethanolic extract and their n-butanolic fractions. Tannins and saponins were found to be present in crude ethanolic extract, n-butanolic fraction of bark and leaves. Terpenoids were present in ethanolic extracts of bark and leaves and their fractions except ethyl acetate fraction of leaves. In agreement with our studies, phenolics, tannins, flavonoids, phytosteroids and saponins from bark and leaves of *T. arjuna* were also reported previously.<sup>34–36</sup>

### 3.2. Quantification of TPC and TFC

TPC was calculated from the standard curve of gallic acid using equation:  $y = 0.0226x + 0.0304$  while TFC was calculated using standard curve of rutin using equation:  $y = 0.0058x - 0.0234$ . The phenolic content was found to be more in n-butanolic fraction as compared to that of crude ethanolic extract of both bark and leaves. The order of TPC in bark extract was: n-butanolic fraction (294.6 ± 8.1 mg/g GAE) > ethyl acetate fraction (270.8 ± 14.8 mg/g GAE) > crude extract (189.9 ± 12.7 mg/g GAE) > aqueous fraction (131.9 ± 5.8 mg/g GAE) > chloroform fraction (59.9 ± 4.3 mg/g GAE). In case of leaves, the order was n-butanolic fraction (203.7 ± 7.0 mg/g GAE) > ethyl acetate fraction (129.0 ± 11.5 mg/g GAE) > crude extract (125.8 ± 9.9 mg/g GAE) > chloroform fraction (46.4 ± 4.84 mg/g GAE) > aqueous fraction (44.7 ± 6.1 mg/g GAE). The order of TFC in bark extract was: n-butanolic fraction (168.6 ± 12.3 mg/g RE) > crude extract (152.6 ± 11.8 mg/g RE) > ethyl acetate fraction (147.3 ± 16.0 mg/g RE) > aqueous fraction (96.2 ± 2.7 mg/g RE) > chloroform fraction (92.9 ± 7.7 mg/g RE). In case of leaves, the order was n-butanolic fraction (144.8 ± 11.1 mg/g RE) > ethyl acetate fraction (129.1 ± 5.7 mg/g RE) > crude extract (114.7 ± 8.5 mg/g RE) > chloroform fraction (89.3 ± 14.6 mg/g RE) > aqueous fraction (69.7 ± 8.0 mg/g RE). TPC and TFC of ethanolic extract of bark of same tree collected from different location<sup>36</sup>, i.e. Kangra of Distt. Kangra was more than that of sample reported in the present study, which was collected from Dharmshala located at altitude of 1500 m above the sea level. However, TPC and TFC of ethanolic extract of leaves were found to be more in the present study. This variation in TPC and TFC clearly indicate that the environmental factors have some effect on the phytochemical content of the same plant in different location as also shown by studies of Bhakta and Ganjewala on *Lantana camara*<sup>37</sup> and Fonseca et al.<sup>38</sup>. Ambika and Chauhan<sup>44</sup> found that gallic acid, apigenin, luteolin, quercetin, epicatechin, ellagic acid and 1-O-β-galloyl glucose were the major phenolics compounds which are responsible for the antioxidant potential of leaves extract of *T. arjuna*.

### 3.3. Antimicrobial activity of crude extract and their fractions

The ethanolic extract and its fractions exhibited good antimicrobial activity against all the tested bacterial strains. The results from the agar well diffusion method, followed by measurement of minimum inhibitory concentration (MIC), indicated that n-butanol fraction of both bark and leaves exhibited more antimicrobial activity as compared to that of ethyl acetate and ethanolic extract with diameter of zone of inhibition were 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 in bark, whereas zone of inhibition diameters were 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 ± 0.71 mm in leaves and MIC values for bark were 0.4 mg/ml, 0.4 mg/ml, 0.4 mg/ml, 0.8 mg/ml, 0.2 mg/ml, 0.2 mg/ml and for leaves were 0.8 mg/ml, 0.2 mg/ml, 0.2 mg/ml, 1.6 mg/ml, 0.4 mg/ml, 0.8 mg/ml against *B. subtilis*, *S. aureus*, *E. coli*,

**Table 1**

Quantitative analysis of phytochemicals in ethanolic extract and its fraction of bark and leaves of *T. arjuna*.

S. No.	Phytoconstituents	Tests	Crude extract		Chloroform fraction		Ethyl acetate fraction		n-butanol fraction		Aqueous fraction	
			B	L	B	L	B	L	B	L	B	L
1.	Phenolics and Tannins	Ferric chloride test	+	+	–	–	+	+	+	+	+	+
		Gelatin test	+	+	–	–	–	–	+	+	–	–
2.	Flavonoids	Lead acetate test	+	+	–	–	+	+	+	+	+	+
3.	Phytosteroids	Liebermann–Burchard's test	+	+	+	+	+	–	+	+	+	+
4.	Saponin	Foam test	+	+	–	–	–	–	+	+	–	–

'+' represented the presence, whereas '–' indicates the absence, B & L stands for bark and leaves

**Table 2**  
Antimicrobial activity of crude ethanolic extract and its fractions. Antimicrobial activity of ethanolic extract and its fractions from bark and leaves were evaluated against Gram's positive (*B. subtilis*, *S. aureus*) and Gram's negative (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. typhi*) bacterial strains and fungal strain (*C. albicans*).

	Extract and fractions												DMSO
	Ethanolic extract		Chloroform fraction		Ethyl acetate fraction		n-Butanol fraction		Aqueous fraction		Amoxycylav (10 µg)/ Fluconazole (25 µg)		
	B	L	B	L	B	L	B	L	B	L			
Zone of inhibition (mm)													
<i>B. subtilis</i>	12.5 ± 0.6	11.5 ± 0.7	7.0 ± 0.5	6.0 ± 0.7	13.5 ± 0.7	12.5 ± 0.5	15.0 ± 0.7	13.5 ± 0.7	9.5 ± 0.5	8.0 ± 0.5	17.5 ± 0.6	6.0 ± 0	
<i>S. aureus</i>	13.0 ± 0.6	14.5 ± 1.2	6.0 ± 0.6	6.0 ± 0.5	14.5 ± 1.5	15.5 ± 0.5	15.5 ± 0.7	16.5 ± 0.7	9.0 ± 1.0	7.5 ± 0.7	18.0 ± 0.7	6.0 ± 0	
<i>E. coli</i>	12.5 ± 0.7	11.5 ± 0.6	6.0 ± 0.6	6.0 ± 0.7	13.0 ± 0.7	12.0 ± 1.5	15.0 ± 1.5	14.0 ± 0.5	8.5 ± 0.5	8.5 ± 0.7	20.5 ± 1.0	6.0 ± 0	
<i>K. pneumoniae</i>	12.0 ± 1.2	11.0 ± 0.7	6.0 ± 0.6	6.0 ± 0.7	14.5 ± 0.7	13.5 ± 0.5	15.5 ± 0.7	15.0 ± 0.5	9.5 ± 1.0	7.5 ± 0.5	18.0 ± 0.7	6.0 ± 0	
<i>P. aeruginosa</i>	13.5 ± 0.6	12.0 ± 0.6	6.0 ± 0.6	6.0 ± 0.7	13.5 ± 1.5	12.5 ± 0.5	15.0 ± 0.7	13.5 ± 0.7	9.0 ± 0.5	7.0 ± 1.0	21.5 ± 0.7	6.0 ± 0	
<i>S. typhi</i>	12.5 ± 0.7	11.5 ± 0.7	6.0 ± 0.6	6.0 ± 0.7	14.0 ± 0.7	13.0 ± 0.5	15.0 ± 0.7	14.0 ± 0.7	9.0 ± 1.0	8.5 ± 0.5	18.5 ± 1.5	6.0 ± 0	
<i>C. albicans</i>	–	–	–	–	–	–	–	–	–	–	15.0 ± 1.0	6.0 ± 0	
MIC (mg/ml)													
<i>B. subtilis</i>	6.3	12.5	12.5	25	1.6	1.6	0.4	0.8	12.5	25	0.3	–	
<i>S. aureus</i>	3.1	6.3	25	12.5	1.6	0.8	0.4	0.2	25	12.5	0.08	–	
<i>E. coli</i>	6.3	3.1	12.5	25	3.1	6.3	0.4	0.2	12.5	25	0.04	–	
<i>K. pneumoniae</i>	3.1	6.3	12.5	12.5	1.6	3.1	0.8	1.6	12.5	12.5	0.08	–	
<i>P. aeruginosa</i>	3.1	6.3	6.3	12.5	1.6	3.1	0.2	0.4	6.3	12.5	0.08	–	
<i>S. typhi</i>	3.1	6.3	12.5	12.5	1.6	6.3	0.2	0.8	12.5	12.5	0.08	–	

*K. pneumoniae*, *P. aeruginosa*, *S. typhi* respectively. However, chloroform fraction did not showed antimicrobial potential in both bark and leaves. This may be explained by the presence of higher amount of phenolics and flavonoids in n-butanolic fraction as compared to that of ethanol extracts and other fractions. The antibacterial activity of *T. arjuna* were in agreement with study of Aneja et al<sup>39</sup> in which ethanolic extract of both bark and leaves showed comparative antibacterial activity against *S. aureus* and *Acitobacter* sp. However, ethanolic extract and their fractions did not exhibit any effect on the growth of *C. albicans* as shown by Aneja et al<sup>39</sup> and Sharma et al<sup>40</sup> Both the positive controls-amoxycylav and fluconazole showed greatest inhibitory activity against all bacterial and fungal strain respectively, while DMSO alone had no antibacterial/antifungal activity (Table 2).

### 3.4. In vitro antioxidant activity

The antioxidant potential was determined by various methods such as DPPH radical scavenging assay, FRAP and NO scavenging assay.

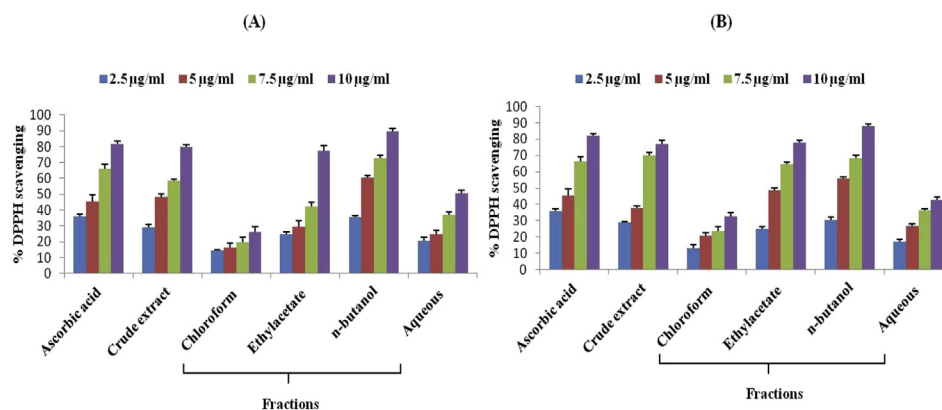
#### 3.4.1. DPPH radical scavenging assay

Fig. 3 (A & B) shows a dose-response curve of DPPH radical scavenging activity of the ethanolic extracts and its fractions of *T. arjuna* bark and leaves in comparison to ascorbic acid. DPPH

scavenging activity was found to be highest in n-butanolic fraction of both bark and leaves extract (IC<sub>50</sub>-1.6 and 1.9 µg/ml, respectively) as compared to that of ascorbic acid (IC<sub>50</sub>-2.0 µg/ml) and crude ethanolic extract of bark (IC<sub>50</sub>-2.2 µg/ml) and leaves (IC<sub>50</sub>-2.3 µg/ml). The least DPPH scavenging was observed in chloroform fraction of both bark and leaves (IC<sub>50</sub>-10.5 and 7.0 µg/ml, respectively) (Table 3).

#### 3.4.2. FRAP assay

The ethanolic extract and its fractions (ethyl acetate and n-butanolic fraction) showed more FRAP activity as compared to that of standard ascorbic acid (Fig. 4A and B). The IC<sub>50</sub> values was found to be lowest in n-butanolic fraction (20.7 and 32.5 µM Fe (II) equivalents in bark and leaves, respectively) indicating its more antioxidant activity as compared to that of standard ascorbic acid (IC<sub>50</sub>-33.3 µM Fe (II) equivalents) (Table 3). In case of bark, the order of FRAP activity was n-butanol fraction [20.7 µM Fe (II) equivalents] > crude ethanolic extract [24.4 µM Fe (II) equivalents] > ethyl acetate fraction [25.8 µM Fe (II) equivalents] > chloroform fraction [50.9 µM Fe (II) equivalents] > aqueous fraction [62.0 µM Fe (II) equivalents]. In case of leaves, the order was n-butanol fraction [22.5 µM Fe (II) equivalents] > crude ethanolic extract [29.1 µM Fe (II) equivalents] > ethyl acetate fraction [43.7 µM Fe (II) equivalents] > aqueous fraction [66.4 µM Fe (II) equivalents] > chloroform fraction [73.1 µM Fe (II) equivalents].



**Fig. 3.** DPPH radical scavenging activity of ethanolic extract and its different fractions. % DPPH activity was determined for bark extract (A) and leaves extract (B) of *T. arjuna* and ascorbic acid. The values represent mean ± S.D. of three independent experiments.

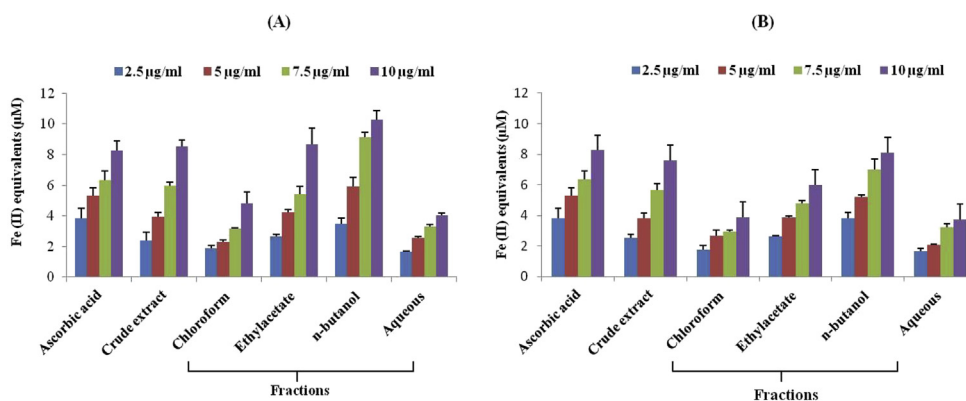
**Table 3**  
IC<sub>50</sub> values of ethanolic extract and its fractions of bark and leaves of *T. arjuna*.

Antioxidant assay	Half maximal inhibitory concentration (IC <sub>50</sub> ) (μg/ml)										
	Ascorbic acid	Crude ethanolic extract		Chloroform fraction		Ethyl acetate fraction		n-Butanol fraction		Aqueous fraction	
		B	L	B	L	B	L	B	L	B	L
DPPH <sup>a</sup>	5.1	4.9	5.8	26.3	17.5	7.2	5.7	4.1	4.8	10.3	11.8
FRAP <sup>b</sup>	40.8	28.2	29.3	50.0	53.1	25.8	44.7	21.0	28.9	62.7	69.8
NO <sup>c</sup>	5.6	3.9	3.7	6.0	7.1	3.9	4.0	3.3	3.2	6.4	6.0

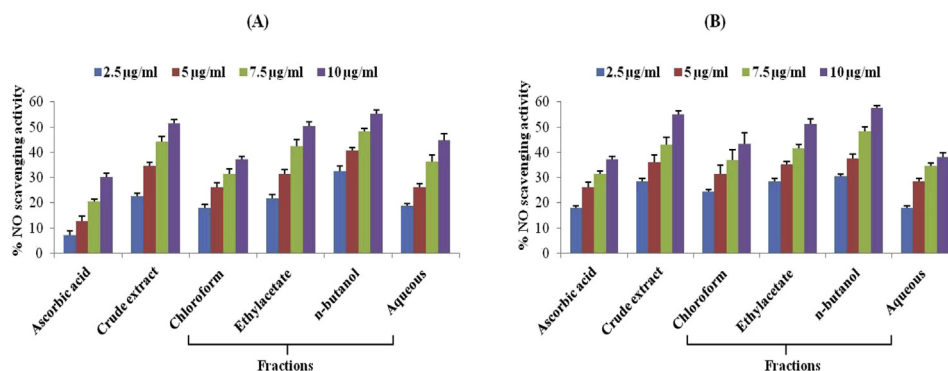
<sup>a</sup> DPPH (2, 2-diphenyl-1-picrylhydrazyl) in μg/ml.

<sup>b</sup> Ferric Reducing Antioxidant Power assay (FRAP) in μM.

<sup>c</sup> Nitric oxide assay (NO) in μg/ml. Lower the value of IC<sub>50</sub>, more is antioxidant capacity.



**Fig. 4.** FRAP assay of ethanolic extract and its different fractions. FRAP assay was performed for bark extract (A) and leaves extract (B) of *T. arjuna* along with standard, ascorbic acid. The values represent mean ± S.D. of three independent experiments.



**Fig. 5.** Nitric oxide (NO) scavenging assay of ethanolic extract and its different fractions. NO assay was performed for bark extract (A) and leaves extract (B) of *T. arjuna*. Ascorbic acid was used as control. The values represent mean ± S.D. of three independent experiments.

### 3.4.3. NO assay

The ethanolic extract and its different fractions of bark and leaves showed inhibition of nitric oxide in a dose dependent manner. However, highest NO scavenging activity was observed in n-butanol fraction in both bark (IC<sub>50</sub>-3.3 μg/ml) and leaves extract (IC<sub>50</sub>-3.1 μg/ml) in comparison to crude ethanolic extract of bark (IC<sub>50</sub>-3.7 μg/ml) and leaves (IC<sub>50</sub>-3.6 μg/ml) and ascorbic acid (IC<sub>50</sub>-6.7 μg/ml). Chloroform (IC<sub>50</sub>-6.0 μg/ml for bark and 7.1 μg/ml for leaves) and aqueous fractions (IC<sub>50</sub>-6.4 μg/ml for bark and IC<sub>50</sub>-5.6 μg/ml for leaves) showed comparatively less NO scavenging activity (Table 3). However, ethanolic extract of leaves (IC<sub>50</sub>-3.1 μg/ml) showed more NO scavenging activity than that of bark extract (IC<sub>50</sub>-3.7 μg/ml) which is in agreement with study of Kumar et al<sup>36</sup> (see Fig. 5).

## 4. Conclusions

Phenolic compounds or polyphenols are presently a major axis of research, because they are considered to be powerful chain-breaking antioxidants, anti-inflammatory, antibacterial, antiviral, and anticancer therapeutics.<sup>41,42</sup> Phenolic content present in the plants are directly correlated with their antioxidant properties.<sup>43</sup> Ambika et al<sup>44</sup> found that gallic acid, apigenin, luteolin, quercetin, epicatechin, ellagic acid and 1-O-β-galloyl glucose were the major phenolics compounds which are responsible for the antioxidant potential of leaves extract of *T. arjuna*. In the present study, antimicrobial activity antioxidant activity of different solvent extracts was compared in leaves and bark. The partition of the ethanolic extract enhanced its antimicrobial and antioxidant potential

specially in n-butanol fraction, indicating that the active principles might be more concentrated in n-butanol as compared to all other fractions. The total phenolic content was comparatively higher in bark extract as compared to that of leaves. On the other hand, flavonoid content was comparable in bark and leaves extracts. These differences in total phenolic content may be due to varied efficiency of the extracting solvents. Thus, the presence of high phenolic and flavonoid content in the various fractions has contributed directly to the antioxidant activity by neutralizing the free radicals. Results obtained in the present study strongly suggest that the polyphenols are important constituents of *T. arjuna* for the antioxidants potential as well as antimicrobial activity. Chloroform fraction as well as aqueous fraction showed negligible activity due to lesser amounts of such compounds. Furthermore, the phytochemical investigations may possibly bring new natural antioxidants in foodstuff that might contribute excellent defence against the oxidative damage to the cellular system. New bioactive compounds may contribute to better antimicrobial activity. In conclusion, antimicrobial and antioxidant potential of bark and leaves extract were comparable. There was marginal increase in antimicrobial and antioxidant in some extracts for bark and leaves. Therefore, the current study recommends the use of leaves of *T. arjuna* as substitute of bark for antioxidant and antimicrobial therapeutics.

### Conflict of interest

The authors declare no competing financial interest.

### Acknowledgement

The authors acknowledge Shoolini University, Solan, for providing infrastructure support to conduct the research work. Authors also acknowledge the support provided by Yeast Biology Laboratory, School of Biotechnology, Shoolini University, Solan, India.

### References

- Azaizeh H, Fulder S, Khalil K, Said O. Ethnobotanical knowledge of local Arab practitioners in the Middle Eastern region. *Fitoterapia*. 2003;74(1):98–108.
- Katewa SS, Chaudhary BL, Jain A. Folk herbal medicines from tribal area of Rajasthan, India. *J Ethnopharmacol*. 2004;92(1):41–46.
- WHO World Health Organization. *Traditional Medicine Strategy Report*. Document WHO/EDM/TRH/2002.1. 2002.
- Heinrich M. Ethnobotany and its role in drug development. *Phytother Res*. 2000;14:479–488.
- Tabuti JR, Lye KA, Dhillon SS. Traditional herbal drugs of Bulamogi, Uganda: plants, use and administration. *J Ethnopharmacol*. 2003;88:19–44.
- Bharti R, Ahuja G, Sujana GP, Dakappa SS. A review on medicinal plants having antioxidant potential. *J Pharm Res*. 2012;5(8):4278–4287.
- Namita P, Mukesh R. Medicinal plants used as antimicrobial agents: a review. *IRJP*. 2012;3:31–40.
- Sharma SK, Singh L, Singh S. A review on medicinal plants having antioxidant potential. *IJRPB*. 2013;1(3):404.
- Orhan DD, Ozelik B, Hosbas S, Vural M. Assessment of antioxidant, antibacterial, antimycobacterial, and antifungal activities of some plants used as folk remedies in Turkey against dermatophytes and yeast-like fungi. *Turk J Biol*. 2012;36:672–686.
- Chew YL, Chan EW, Tan PL, Lim YY, Stanslas J, Goh JK. Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia. *BMC Complement Altern Med*. 2011;11(1):1.
- Pukumpuang W, Thongwai N, Tragoolpua Y. Total phenolic contents, antibacterial and antioxidant activities of some Thai medicinal plant extracts. *J Med Plants Res*. 2012;6:4953–4960.
- Kulisic T, Radonic A, Katalinic V, Milos M. Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chem*. 2004;85:633–640.
- Katalinic V, Milos M, Kulisic T, Jukic M. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chem*. 2006;94:550–557.
- Letelier ME, Molina-Berrios A, Cortes-Troncoso J, et al. DPPH and oxygen free radicals as pro-oxidant of biomolecules. *Toxicol In vitro*. 2008;22:279–286.
- Junaid S, Rakesh KN, Dileep N, Poornima G, Kekuda TRP, Mukunda S. Total phenolic content and antioxidant activity of seed extract of *Lagerstroemia speciosa* L. *Chem Sci Trans*. 2013;2:75–80.
- Jain S, Yadav PP, Gill V, Vasudeva N, Singla N. *Terminalia arjuna* a sacred medicinal plant: phytochemical and pharmacological profile. *Phytochem Rev*. 2009;8:491–502.
- Dwivedi S, Chopra D. Revisiting *Terminalia arjuna*-an ancient cardiovascular drug. *J Tradit Complement Med*. 2014;4:224–231.
- Amalraj A, Gopi S. Medicinal properties of *Terminalia arjuna* (Roxb.) Wight & Arn.: a review. *J Tradit Complement Med*. 2017;7:65–78.
- Mehmood N, Zubair M, Rizwan K, Rasool N, Shahid M, Ahmad V. Antioxidant, antimicrobial and phytochemical analysis of *Cichorium intybus* seeds extract and various organic fractions. *Iran J Pharm Res*. 2012;11:1145–1151.
- Ghosh G, Sahoo S, Das D, Dubey D, Padhy RN. Antibacterial and antioxidant activities of methanol extract and fractions of *Clerodendrum viscosum* Vent. leaves. *Ind J Nat Prod Res*. 2015;5:134–142.
- Harborne JB. *Introduction to Ecological Biochemistry*. 3rd edition. London: Academic Press; 1988.
- Khandelwal KR. *Practical Pharmacognosy: Techniques and Experiments*. 17th ed. Nirali Prakashan: Pune, India; 2007.
- Guleria S, Dev K, Khosla PK. Comparative analysis of phytochemicals and antioxidant activities of fruit and leaves of *Terminalia chebula* from Himachal Pradesh. *IJBPA*. 2016;5(6):1195–1206.
- Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-ciocalteu reagent. *Methods Enzymol*. 1999;299:152–178.
- Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*. 1999;64:555–559.
- Perez C, Paul M, Bazerque P. Antibiotic assay by agar-well diffusion method. *Acta Biol Med Exp*. 1990;15:113–115.
- Tamokou JD, Mpetga Simo DJ, Lunga PK, et al. Activities of ethyl acetate extract, fractions and compounds from the stem bark of *Albizia adianthifolia* (Mimosoideae). *BMC Complement Altern Med*. 2012;12:99.
- CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard*. 9th ed. 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA: Clinical and Laboratory Standards Institute; 2012. CLSI document M07–A9.
- Chanda S, Dave R. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: an overview. *Afr J Microbiol Res*. 2009;3:981–996.
- Chanda S, Amrutiya N, Rakholiya K. Evaluation of antioxidant properties of some Indian vegetable and fruit peels by decoction extraction method. *Am J Food Technol*. 2013;8:173–182.
- Barros L, Ferreira MJ, Queiros B, Ferreira ICFR, Baptista P. Total phenols, ascorbic acid,  $\beta$ -carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chem*. 2007;103:413–419.
- Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. *Anal Biochem*. 1996;239:70–76.
- Sreejayan N, Rao MNA. Nitric oxide scavenging by cucumins. *J Pharm Pharmacol*. 1997;49:105–107.
- Mandal S, Patra A, Samanta A, et al. Analysis of phytochemical profile of *Terminalia arjuna* bark extract with antioxidative and antimicrobial properties. *Asian Pac J Trop Biomed*. 2013;3:960–966.
- Parveen T, Sharma K. Phytochemical profiling of leaves and stem bark of *Terminalia arjuna* and *Tecomella undulate*. *IJBPA*. 2014;1(1):1–7.
- Kumar V, Dev K, Sourirajan A, Khosla PK. Comparative antioxidant potential of bark and leaves of *Terminalia arjuna* (Roxb) Wight & Arn from Himachal Pradesh. *Int J Pharm Phytopharm Res*. 2016;6:27–33.
- Bhakta D, Ganjewala D. Effect of leaf positions on total phenolics, flavonoids and proanthocyanidins content and antioxidant activities in *Lantana camara* (L.). *J Sci Res*. 2009;1(2):363–369.
- Fonseca JM, Rushing JW, Rajapaske NC, Thomas RL, Riley MB. Potential implications of medicinal plant production in controlled environments: the case of feverfew (*Tanacetum parthenium*). *HortScience*. 2006;41(3):531–535.
- Aneja KR, Sharma C, Joshi R. Antimicrobial activity of *Terminalia arjuna* Wight & Arn.: an ethnomedicinal plant against pathogens causing ear infection. *Braz J Otorhinolaryngol*. 2012;78:68–74.
- Sharma V, Sharma HV, Mehta D, et al. Comparative analysis of antibacterial and antifungal properties of traditional medicinal plants of Shimla and Solan, Himachal Pradesh. *IJPPR*. 2014;6:18–26.
- Kahkonen MP, Hopia AI, Vuorela HJ, et al. Antioxidant activity of plant extracts containing phenolic compounds. *J Agric Food Chem*. 1999;47:3954–3962.
- Chen CY, Blumberg JB. *In vitro* activity of almond skin polyphenols for scavenging free radicals and inducing quinone reductase. *J Agric Food Chem*. 2008;56:4427–4434.
- Orak HH, Yagar H, Isbilir SS. Comparison of antioxidant activities of juice, peel and seed of pomegranate (*Punica granatum* L.) and inter-relationships with total phenolic, tannin, anthocyanin, and flavonoid contents. *Food Sci Biotech*. 2012;21:373–387.
- Ambika SP, Chauhan SM. Activity-guided isolation of antioxidants from the leaves of *Terminalia arjuna*. *Nat Prod Res*. 2014;28:760–763.