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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT AND ITS FRACTIONS FROM FRUIT AND LEAVES OF *TERMINALIA CHEBULA* FROM HIMACHAL PRADESH, INDIA

Shikha Guleria[1], Vikas Kumar[1], Shikha R. Chandel[1], Nitin Sharma[2], Anuradha Sourirajan[1], Prem Kumar Khosla[1] and Kamal Dev[1]*

[1*]Faculty of Applied sciences and Biotechnology, Shoolini University of Biotechnology and Management Sciences, Bajhol, PO Sultanpur, Distt. Solan-173229 (HP), India.

[2]Department of Biotechnology, Chandigarh Group of Colleges, Landran, Mohali (Punjab), India.

Abstract

The present study was undertaken to compare the antimicrobial and antioxidant activity of ethanolic extracts and its fractions of fruits and leaves of *Terminalia chebula*. Total phenolic (TPC) and flavonoid (TFC) content was quantified using spectrophotometric method. Antimicrobial activity was done using agar well diffusion and broth dilution method. DPPH radical scavenging and FRAP assay was used to evaluate antioxidant potential in ethanolic extracts and its different solvent fractions (*viz.* chloroform (CF), ethyl acetate (EA), n-Butanol (BuOH), and aqueous fraction (AF). TPC was found to be higher in ethyl acetate fraction of ethanolic extracts of fruits (359±12.73 mg/g gallic acid equivalents, GAE) and leaves (54.77±1.12 mg/g GAE). Similarly, TFC was also found to be higher in the ethyl acetate fraction of ethanolic extract of fruits (112.65±1.28 mg/g rutin equivalents, RE) and leaves (72.35±5.05 mg/g RE). Among all extracts and fractions, ethyl acetate fraction of fruits [IC_{50}-6.05±0.35 µg/ml (DPPH), 8.95±0.21µM (FRAP)] and leaves [IC_{50}-8.1±0.28 µg/ml (DPPH), 10.83±0.61µM (FRAP)] showed more antioxidant potential. Similarly, antibacterial activity was also higher in ethyl acetate fraction of ethanolic extracts of fruits of *T. chebula* with zone of inhibition of 12.5±1.14mm, 12±0.70mm, 13.5±0.75mm, 12.5±0.71mm and 13±1.12mm and MIC value of 6.25 mg/ml, 6.25 mg/ml, 0.78 mg/ml, 0.39 mg/ml and 3.12 mg/ml against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhi*, respectively. In case of leaves extracts, ethyl acetate showed higher antibacterial activity with zone of inhibition of 11±0.80mm, 10.5±0.70mm, 9±1.25mm, 10±1.17mm and 10±0.78mm and MIC value of 3.125 mg/ml, 12.5 mg/ml, 1.56 mg/ml, 0.78 mg/ml and 6.25 mg/ml against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhi*, respectively. The results of this study showed that the solvent fractions of both fruits and leaves of *T. chebula* possess antioxidant and antimicrobial activities, hence justifying the folkloric use of this plant for the treatment of various ailments in traditional medicine.

Key words : *Terminalia chebula*, antioxidant, antimicrobial, IC_{50} value.

Introduction

Medicinal plants have played an important role in the treatment of various diseases in humans as well as in animals from the beginning of civilization. Medicinal plants are a rich source of antimicrobial compounds and used in different countries as a source of many potent and powerful drugs (Srivastava *et al.,* 1996). Large number of plants has been reported to possess antimicrobial and antioxidant potential (Namita and Mukesh, 2012; Bharti *et al.,* 2012; Udd and Rao, 2016; Dubey *et al.,* 2018). The search for potent plant-based antimicrobials has dramatically increased because of the emergence of multiple drug resistance (Orhan *et al.,* 2016). The increasing failure of chemotherapies and antibiotic resistance exhibited by pathogenic microbial infection has led to the screening of several medicinal plants for their potential antimicrobial activity (Ritch-Karc *et al.,* 1996;

***Author for correspondence :** E-mail: kamaldevbhardwaj1969@gmail.com

Martins *et al.,* 2001; Chandra *et al.,* 2017). Phenolic compounds present in plants act as powerful antioxidants, which can protect the cellular system from free radicals by acting as hydrogen donors and radical scavengers (Chew *et al.,* 2011). Antioxidants act as free radical scavengers and are thus 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 (Pukumpuang *et al.,* 2012). Antibacterial properties of various tree plants parts have been well documented during the past two decades (Khare, 2008; Khan, 2017). Many Indian tree plants such as *Terminalia chebula*, *T. bellerica*, *T. muelleri* and *Phyllanthus emblica* have been reported potent sources of antioxidant compounds and antimicrobial compounds. *Terminalia chebula,* which is a member of Combretaceae family, enjoys the prime place among medicinal tree plants not only in India, but also in other countries of Asia and Africa. The fruits of *T. chebula* have been used in the treatment of dental caries, bleeding gums, ulcer of oral cavity, and also have anti-diabetic, anti-immunomodulatory, antioxidant, and antimicrobial properties (Varier, 2002 Venkatesan *et al.,* 2017; Kher *et al.,* 2018). So, due to its high medicinal properties, fruits of *T. chebula* are in high demand and becoming expensive. Antimicrobial activity of fruits has been reported in several studies (Bag *et al.,* 2013; Dhiman *et al.,* 2015; Rani *et al.,* 2016; Datta *et al.,* 2017). The leaves of *T. chebula* have strong phytochemical properties and thus can be utilized as an effective source of functional food material (such as natural antioxidants) and even in some medicinal preparations (Kathirvel and Sujatha, 2012; Guleria *et al.,* 2016). Therefore, the current study was focused to compare the antimicrobial and antioxidant potential of leaves and fruit, so that leaves could also be exploited as a cheaper source of such phytocompounds.

Materials and Methods

Chemicals and Reagents

Ascorbic acid, aluminum chloride, 2, 2'-diphenyl-2-picrylhydrazyl (DPPH), nutrient agar, sodium nitrite ($NaNO_2$), 2, 4, 6-tripyridyl-s-triazine (TPTZ) were purchased from Sigma Chemical Co., U.S.A. Ferric chloride, Folin-Ciocalteau reagent, gallic acid and rutin were procured from Loba Chemicals, Pvt. Ltd, Mumbai, India. All the chemicals and reagents used in this study were of analytical grade.

Collection of plant material

Fruits and leaves of *T. chebula* were collected in the month of August (2017) from Kangra, Himachal Pradesh, India. The collected plant materials were washed twice with tap water followed by surface sterilization with 70% ethanol for 2 minutes. After surface sterilization, plant materials were subjected to drying in hot air oven at 40°C. The dried fruit and leaves were grinded to fine powder with the help of a mixer grinder and then stored in air tight bottles in dark until use.

Extract preparation and fractionation of prepared extract

Ethanolic extract of fruits and leaves samples of *T. chebula* was prepared after defatting with petroleum ether (60-80°C) using cold maceration method. The collected extracts were concentrated to dryness with the help of water bath at 40 °C. The crude ethanolic extract (5 g) of both fruits and leaves of *T. chebula* were sequentially partitioned with chloroform, ethyl acetate, n-butanol and remaining water (WF) to obtained chloroform (CF), ethyl acetate (EF), n- butanol (BF) and aqueous fractions (AF) (Kumar *et al.,* 2018; Chandel *et al.,* 2019). These fractions were evaporated to dryness at 40 °C in a water bath. The crude extract and its fractions were stored in tightly sealed collection bottles at -20 °C until use.

Stock preparations and dilutions

Stock solutions of crude ethanolic extract and its fractions at 50 mg/ml concentration were prepared using dimethyl sulphoxide (DMSO) as solvent. For the antibacterial minimum inhibitory concentration (MIC) assay, the stock solutions were serially diluted with nutrient broth (NB) to obtain concentrations between 25 to 0.05 mg/ml. For antioxidant activity, ethanolic extract and its various fractions such as chloroform, ethyl acetate, n-butanol and aqueous were dissolved at a concentration of 1 mg/ml in ethanol and then diluted in order to prepare different concentrations for antioxidant assays (2.5 to 10 µg/ml).

Bacterial Strains and inoculum preparation

The following Gram's positive bacterial strains such as *Bacillus subtilis* and *Staphylococcus aureus*, whereas *Gram's negative* bacterial strains such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Salmonella typhi were used in this study. All the bacterial strains were obtained from Yeast Biology Lab, Shoolini University, Solan, Himachal Pradesh, India.* The bacterial cultures were maintained on NA slant at 4 °C, and were sub-cultured on fresh agar plate for 24 h before the antibacterial assay.

A loopful of bacteria was inoculated on NB and was incubated for 24 h at 37 °C. Then, the turbidity of the

bacterial suspension was adjusted to 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/ml). 10µl of diluted bacterial suspension was used for analysis of antibacterial activity. The solvents used for dissolving the extracts (DMSO) served as negative controls, while amoxyclav (10 µg) was used as positive control.

Qualitative analysis of phytochemicals in *T. chebula* extracts

Crude extract and its subsequent solvent fractions of both fruits and leaves were tested for the presence of various phytocompounds (*viz.*, phenols, flavonoids, tannins, saponins, alkaloids, glycosides, phytosterols and carbohydrates) using standard methods (Harbourne, 1988; Khandelwal, 2007; Guleria *et al.*, 2016).

Quantification of total phenolics and flavonoids

The total phenolic content (TPC) in the ethanolic extract and its fractions of fruits and leaves was estimated as described by Singleton *et al*. (1999) using gallic acid as standard. Total phenolic content was calculated from the calibration curve of gallic acid (5-100 µg/ml) and expressed as mg per gram gallic acid equivalents.

Total flavonoid content (TFC) in ethanolic extract of fruits and leaves of *T. chebula* were determined by aluminium chloride ($AlCl_3$) method (Zhishen *et al.*, 1999). Rutin was used as standard. The total content of flavonoid was calculated from calibration curve of rutin (5-100 µg/ml) and expressed as mg per gram rutin equivalents.

***In vitro* antioxidant assays**

The principle of the antioxidant activity is the availability of electrons to neutralize any so-called free radicals. Different antioxidant methods were employed to characterize the antioxidant potential of the ethanolic extract and its various fractions (Chanda and Dave, 2009; Chanda *et al.*, 2013). Ascorbic acid was used as a standard antioxidant compound for comparative analysis in all the assays. Antioxidant activity was expressed in terms of IC_{50} value obtained from the linear regression equation prepared from the concentrations of the different extracts and the absorbance values (Bourgou *et al.*, 2008).

DPPH radical scavenging assay

To assess the scavenging ability on 2, 2-diphenyl-2-picrylhydrazyl (DPPH), 100 µl of each extract (2.5–20 µg/ml) in ethanol was mixed with 900 µl of ethanolic solution containing DPPH radicals (4mM) (Barros *et al.*, 2007; Rolta *et al.*, 2018a). The mixture was shaken vigorously and left to stand for 30 min in the dark before measuring the absorbance at 517 nm against a blank. Scavenging ability was calculated using the following equation-

$$\% \text{ scavenging ability} = (OD_{control} - OD_{sample}) - OD_{control} \times 100$$

Ferric Reducing Antioxidant Power (FRAP) assay

The ferric reducing ability of ethanolic extract and its fractions of fruits and leaves of *T. chebula* were analysed by the method described by Benzie and Strain (1990). Briefly, the FRAP reagent contained 2.5 ml of 10 mM of 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) solution in 40 mM HCl plus 2.5 ml of 20 mM $FeCl_3$ and 25 ml of 0.3 M acetate buffer, pH 3.6, which was freshly prepared and warmed prior to the analysis. The antioxidant capacity of the extracts was calculated from the linear calibration curve of $FeSO_4$ (2.5-20 µM) and expressed as µmol of Fe (II) equivalents per gram of the extract.

Antibacterial agar well diffusion method

The antibacterial activity of ethanolic extract (fruit and leaves) and its different solvent fractions such as chloroform (CF), ethyl acetate (EF), n-butanol (BF) and aqueous (AF) fractions dissolved in dimethyl sulfoxide (DMSO) at concentration of 50 mg/ml was evaluated by agar well diffusion method (Perez *et al.*, 1990). The bacterial culture of 0.5 McFarland standards (~2×10^8 colony forming units (CFU) / ml) was uniformly spreaded on the surface of the nutrient agar plates using sterile cotton swabs. The wells were punched with the cork borer (6 mm) in the agar. Approximately 50 µl of the crude extracts and its different solvent fractions of fruit and leaves (50 mg/ml) 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 zones of inhibition diameter were measured using a HiAntibiotic Zone scale-C (Himedia Biosciences, Mumbai (India). The tests were carried out in triplicate, and results were recorded as mean± S.D.

Minimum inhibitory concentration (MIC) determination

Broth dilution method was used for the determination of minimum inhibitory concentration, according to CLSI methodology (CLSI, 2015; Rolta *et al.*, 2018b) using 2, 3, 5-tripheny tetrazolium chloride. MIC was determined by observing the change in color from purple to pink or colorless.

Statistical Analysis

Total phenolic content, total flavonoid content and half maximal inhibitory concentration (IC_{50}) 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.

Results

Phytochemical analysis of crude extract and its fractions of fruits and leaves of *T. chebula*

Phytochemical compounds such as phenolics, flavonoids, tannins, terpenoids and saponins were determined in both fruit and leaf and their fractions and results are shown in table 1. Qualitative assay revealed that important phytocompounds such as phenolics and flavonoids were present in both the crude extract and their n-butanol fractions whereas tannins were found to be present in crude extract of ethanol and butanol and aqueous fractions of fruit extract. Terpenoids were present in both the extracts and their fractions except ethyl acetate fraction of leaves. Saponins were present in the both crude extract and n-butanol fractions of fruit and leaves (Table 1).

Quantification of total phenolic content in crude extract and its fraction

Total phenolic content was calculated using standard curve of gallic acid using equation, y=0.0117x-0.0098. Total phenolic content in fruits was found to be higher in the ethyl acetate fraction (359±12.73 mg/g gallic acid equivalents) among all fractions and crude ethanolic extract of fruits. Order of total phenolic content was n-butanol fraction (359±12.73 mg/g GAE) followed by ethyl acetate fraction (311±2.83 mg/g GAE), crude extract (261±1.91 mg/g GAE), chloroform fraction (95.95±1.91 mg/g GAE) and aqueous fraction (35.74±7.16 mg/g GAE). Similarly in case of leaves, highest phenolic content was found in ethyl acetate fraction. Order of total phenolic content was ethyl acetate fraction (54.77±1.12 mg/g GAE)> crude ethanolic extract (51.62±1.80 mg/g GAE) > n-butanol fraction (50.23±3.34 mg/g GAE)> aqueous fraction (24.89±1.42 mg/g GAE)> chloroform fraction (24.66±1.4 mg/g GAE). (Fig. 1).

Quantification of flavonoid content in crude extract and its fraction

Total flavonoid content was calculated by the standard curve of rutin using equation: y=0.0117x-0.0098. Highest flavonoid content was observed in ethyl acetate fraction of both fruits and leaves. The order of flavonoid content in the fruits was ethyl acetate (112.65±1.28 mg/g RE) > n-butanol fraction (103.29±1.24 mg/g RE) > crude extract (82.82±3.96 mg/g RE) > chloroform fraction (51.74±2.43 mg/g RE) > aqueous fraction (28.1±3.11 mg/g RE). In case of leaves, the order was ethyl acetate (72.35±5.05 mg/g RE) > n-butanol fraction (64.15±1.77 mg/g RE) > crude extract (50.1±1.27 mg/g RE) > chloroform (36.44±2.06 mg/g RE) > aqueous fraction (22.2±2.2 mg/g RE) (Fig. 2).

In vitro antioxidant activity

The antioxidant potential was determined by various methods such as DPPH radical scavenging assay, FRAP and expressed in terms of IC_{50}.

DPPH radical scavenging assay

The result showed that the DPPH radical scavenging activity was dose dependent in both fruits and leaves extracts (Fig. 3A & B). However, ethyl-acetate fraction of both fruit and leaves showed strongest antioxidant activity. The order of DPPH activity in case of fruit was ethyl acetate fraction (6.05±0.35 µg/ml) > n-butanol (6.95±0.08 µg/ml) fraction > crude ethanolic extract (8.65±0.64 µg/ml) > chloroform fraction (21±0.28 µg/ml)> aqueous fraction (21.51±1.97µg/ml). In case of leaves, the order was ethyl acetate (8.1±0.28 µg/ml > crude

Table 1: Qualitative analysis of phytochemicals in crude extract and its fractions of fruits and leaves of *T. chebula*.

Sr. No.	Phytoconstituents	Tests	EE		CF		EF		BF		AF	
			F	L	F	L	F	L	F	L	F	L
1.	Alkaloids	Dragendroff test	+	+	-	-	-	-	+	+	-	+
2.	Phenolics and Tannins	Ferric chloride test	+	+	-	-	+	+	+	+	+	+
		Gelatin test	+	-	-	-	-	-	+	-	+	-
3.	Phytosteroids	Liebermann-Burchard test	+	+	-	-	+	-	+	+	+	+
4.	Phytosterol	Salkowski test	+	+	-	-	+	-	+	+	-	-
5.	Carbohydrates	Fehling test	+	+	+	+	-	-	+	-	+	-
6.	Glycosides	Borntrager test	+	-	-	-	-	-	+	-	+	-
7.	Proteins/amino acid	Millon test	+	+	-	-	-	-	+	+	+	-
		Ninhydrin test	-	-	-	-	-	-	-	-	-	-
8.	Flavonoids	Lead acetate test	+	+	-	-	+	+	+	+	+	+
9.	Saponin	Foam test	+	+	-	-	-	-	+	+	-	-

(+) indicated the presence; whereas (-) indicated the absence of the phytocompounds. EE- Ethanolic extract; CF-Chloroform fraction, EF- Ethyl acetate fraction; BF- n-Butanol fraction; AF- Aqueous fraction. L- Leaves; F-Fruits.

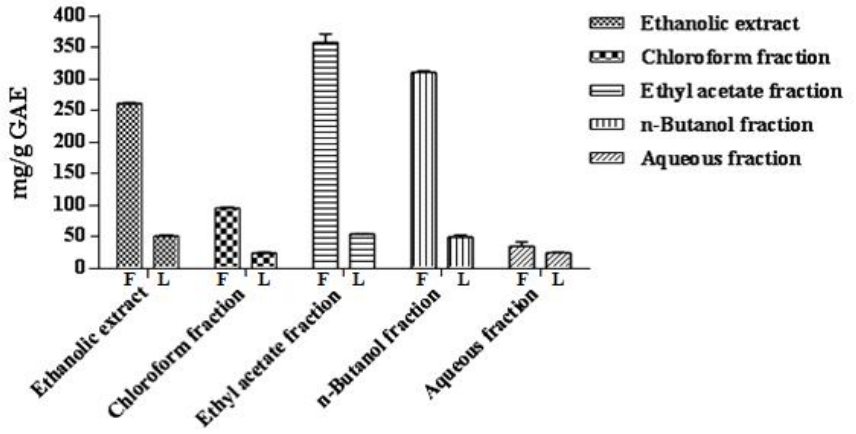

Fig. 1: Comparative phenolic content of ethanolic extract of fruits and leaves and their fractions. Total phenolic content was represented as mg/g GAE (Gallic acid equivalents). The values were expressed as mean ± standard deviation (n=3).

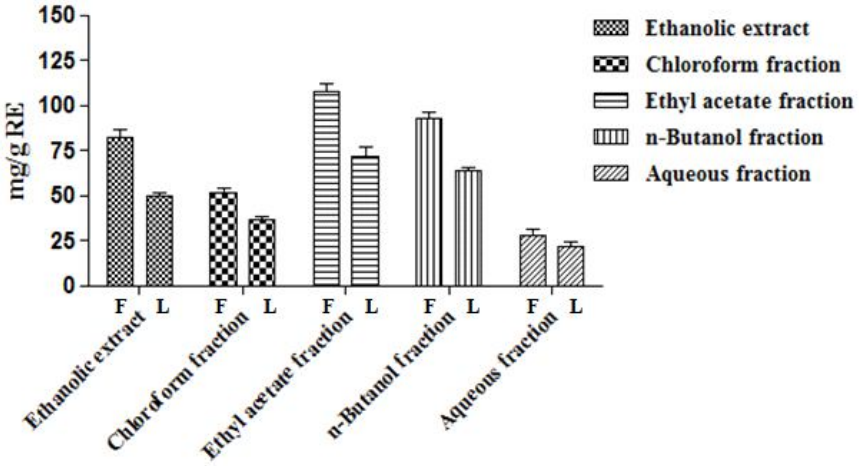

Fig. 2. Comparative flavonoid content of ethanolic extract and its fractions of fruits and leaves. Total flavonoid content was represented as mg/g RE (rutin equivalents). The values were expressed as mean ± standard deviation (n=3).

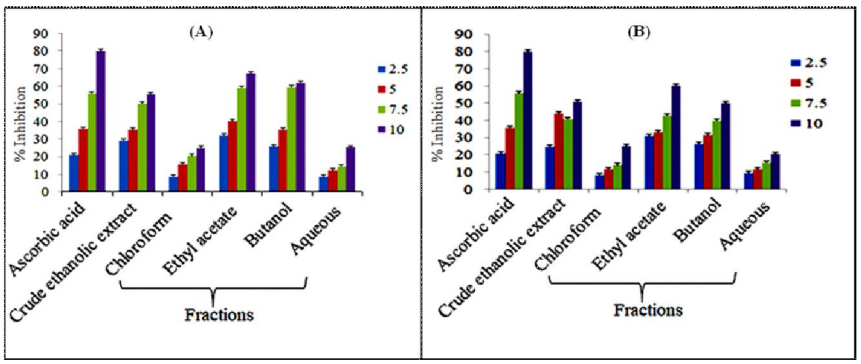

Fig. 3: DPPH radical scavenging activity of ethanolic extract and its solvent fractions. % DPPH activity was determined for fruit extract (A) and leaves extract (B) of *T. chebula* and ascorbic acid. The values were represented as mean ± S.D. of three independent experiments.

ethanolic extract (9.85±0.49 µg/ml)> n-butanol (9.88±0.75 µg/ml) > chloroform fraction (21.11±1.82 µg/ml)> aqueous fraction (31.5±0.86 µg/ml). IC_{50} value for ascorbic acid was 6.6±0.28 µg/ml (Table 2).

FRAP Assay

Similar to DPPH, FRAP activity also showed dose dependent activity in various concentration of extracts and its sub-fractions (Fig. 4 A&B). The order of FRAP activity in case of fruit was ethyl acetate fraction (8.95±0.21 µM) > crude ethanolic extract (12.95±0.08 µM)> n-butanol (12.72±0.48 µM) fraction > chloroform fraction (29.81±0.89 µM)> aqueous fraction (41.99±1.0 µM). In case of leaves, the order was ethyl acetate (10.83±0.61 µM > crude ethanolic extract (14.712±0.48 µM)> n-butanol (14.78±0.42 µM) > chloroform fraction (52.89±0.71µM)> aqueous fraction (79.03±3.90 µM) (Table 2). IC_{50} value for ascorbic acid was 11.58±0.74 µM Fe (II) equivalents.

Antibacterial potential of fruits and leaves extracts and its solvent fractions

The ethanolic extract and its fractions exhibited good antimicrobial activity against Gram positive and Gram negative bacteria, *i.e. B. subtilis*, *S. aureus*, *E. coli*, *K. pneumonia*, and *P. aeruginosa*. The results from the agar well diffusion method, followed by measurement of minimum inhibitory concentration (MIC), indicated that ethyl acetate fraction of fruits exhibited more antibacterial activity as compared to that of all other extract with diameter of zone of inhibition were 12.5±1.17mm, 12±0.70mm, 13.5±0.75mm, 12.5±0.71 mm, 13±1.12mm and MIC values were 6.25 mg/ml, 6.25 mg/ml, 0.78 mg/ml, 0.39 mg/ml, 3.12 mg/ml against *B. subtilis*, *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, respectively. Similarly, ethyl acetate fraction of leaves also showed higher antibacterial activity

Table 2: IC_{50} values of crude ethanolic extract and different solvent fractions of fruit and leaves of *T. chebula*.

Extracts/ fractions	Plant part	Half maximal inhibitory concentration (IC_{50})	
		DPPH	FRAP
Ethanolic extracts	F	8.65±0.64	12.95±0.08
	L	9.85±0.49	14.712±0.48
Chloroform fraction	F	21±0.28	29.81±0.89
	L	21.11±1.82	52.89±0.71
Ethyl acetate fraction	F	6.05±0.35	8.95±0.21
	L	8.1±0.28	10.83±0.61
n-Butanol fraction	F	6.95±0.08	12.72±0.48
	L	9.88±0.75	14.78±0.42
Aqueous fraction	F	21.51±1.97	41.99±1.0
	L	31.5±0.86	79.03±3.90
Ascorbic acid		6.6±0.28	11.58±0.74

a-µg/ml; b- µM; F-Fruits, L-Leaves. Values were expressed as mean±S.D. (n=2).

among all solvent fractions of leaves with zone of inhibition of 11±0.80mm, 10.5±0.7 mm, 9±1.25mm, 10±1.17mm, 10±0.78mm and MIC values of 3.12 mg/ml, 12.5 mg/ml, 1.56 mg/ml, 0.78 mg/ml, 6.25 mg/ml, against *B. subtilis*, *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *respectively*. This may be explained by the presence of higher amount of phenolics and flavonoids in ethyl acetate fraction as compared to that of ethanol extracts and other fractions. Positive control- Amoxyclav showed greatest inhibitory activity against all bacterial strains, while DMSO alone had no antibacterial activity (Table 3).

Discussion

In this present study, differences were observed in the antimicrobial and antioxidant activity of crude ethanolic extract and its fractions. These attributes may be due to differences in the chemical components of the plants extract such as tannins, alkaloids, phenols, flavonoids,

Table 3: Antibacterial activity of leaves and fruits extract of *T. chebula*. Agar well diffusion assays and Minimum inhibitory concentration (MIC) method were performed for different bacteria as indicated for crude extract and its fractions of fruits and leaves and represented as zone of inhibition in mm (n=3) and MIC in mg/ml.

Fractions	Plant parts	Zone of Inhibition (ZOI)				
		B. subtilis	*S. aureus*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
Crude extract	Fruit	10±0.71	9±1.25	10.5±1.12	10±0.5	9±0.58
	Leaves	9±0.75	8±0.78	8±0.70	7±1.15	8.5±1.12
Chloroform	Fruit	8±0.75	7.5±1.72	6.5±1.25	6.5±0.70	7.5±0.58
	Leaves	7±0.71	6.5±1.25	6±1.20	6±0.78	7.0±1.12
Ethyl acetate	Fruit	12.5±1.14	12±0.70	13.5±0.75	12.5±0.71	13±1.12
	Leaves	11±0.80	10.5±0.70	9±1.25	10±1.17	10±0.78
n-Butanol	Fruit	12±0.75	10±1.14	10.5±0.70	11.5±1.15	11±0.75
	Leaves	8±0.71	9.5±0.75	8±1.12	8.5±1.25	9±1.23
Aqueous	Fruit	9±1.12	7±1.72	7.5±1.25	7±0.70	8.5±0.58
	Leaves	7.5±0.71	7±1.25	7. 5±1.20	7±0.78	6.5±1.12
Amoxyclav	Fruit	14±0.71	14.5±0.75	15±0.70	14.5±0.98	13±0.78
	Leaves	14±1.25	13.5±0.75	15±0.75	13±1.0	12±1.21
MIC						
Crude extract	Fruit	6.25	12.5	3.125	6.25	12.5
	Leaves	12.5	25	6.25	3.125	12.5
Chloroform	Fruit	12.5	25	12.5	12.5	25
	Leaves	25	12.5	12.5	6.25	25
Ethyl acetate	Fruit	6.25	6.25	0.78	0.39	3.125
	Leaves	3.125	12.5	1.56	0.78	6.25
n-Butanol	Fruit	6.25	12.5	3.125	1.56	6.25
	Leaves	6.25	12.5	6.25	3.125	6.25
Aqueous	Fruit	12.5	25	6.25	12.5	25
	Leaves	12.5	25	12.5	12.5	25
Amoxyclav		0.31	0.078	0.039	0.078	0.078

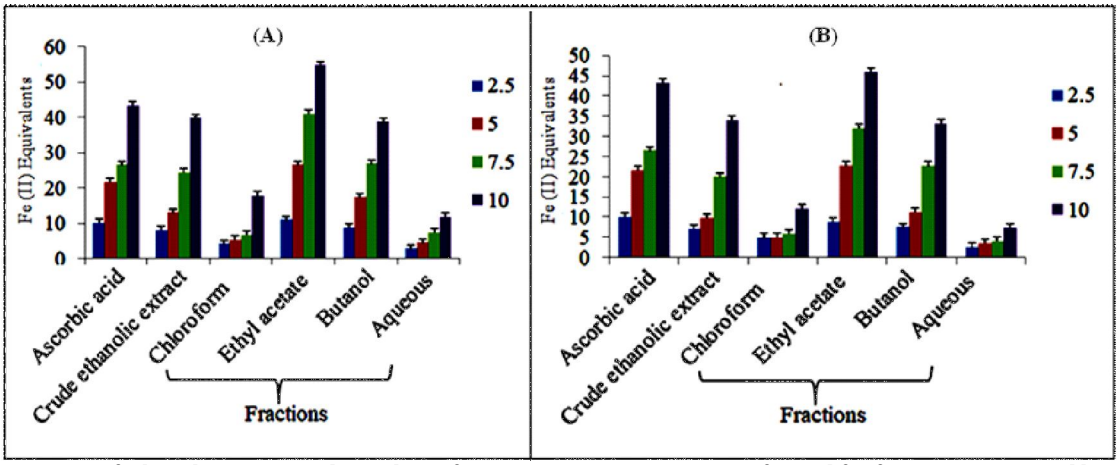

Fig. 4: FRAP assay of ethanolic extract and its solvent fractions. FRAP assay was performed for fruit extract (A) and leaves extract (B) of *T. chebula* along with standard, ascorbic acid. The values were represented as mean ± S.D. of three independent experiments.

terpenoids and saponins. Phenolics compounds are of considerable interest as they are one of the most widely occurring groups of phytochemical and have been reported to exhibit anti-microbial, antioxidant, vasodilator, anti allergenic properties (Chang and Lin, 2012). The highest values of total phenolic and flavonoid content were observed in the ethyl acetate fraction as compared to the other fractions and the ethanolic extracts. This is due to the fact that phytochemicals being polar in nature gets dissolved in the solvent that matches with their polarity. Thus, the presence of high phenolic and flavonoid content in various fractions has contributed directly to the antioxidant activity by neutralizing the free radicals. Kathirvel and Sujatha (2012) revealed that the acetone extract showed higher antibacterial activity followed by the methanol. Bag *et al.* (2012) also demonstrated that the ethanolic extract of fruit showed strong antimicrobial activity. The antibacterial activity responsible due to the presence of chemical constituents as alkaloids, glycosides, saponins, cardiac glycosides, tannins, tannic acid and simple phenol compounds. Kumar *et al.* (2018) also showed that fractions of ethanolic extract of *T. arjuna* rich in phenolic and flavonoid content showed higher anti-oxidant and antimicrobial activity. Antibacterial activity of leaves extracts of *T. chebula* was in accordance with study of Ghosh *et al.* (2008) in which methanolic leaves extracts showed good antibacterial effects against *B. subtilis* and *S. aureus*.

Results obtained in the present study strongly suggested that the polyphenols are important constituents of this plant for the antioxidant potential. Aqueous fraction and the chloroform fraction showed low activity due to fewer amounts of such compounds. The phytochemical investigations may possibly bring new natural antioxidants that might contribute to the excellent defense system against oxidative damage caused in the system. Similar to present study, ethyl acetate fraction obtained from ethanolic extract of *Ficus pseudopalma* showed higher amount of phenolic and flavonoids which in turn responsible for higher antibacterial activity (DeLas *et al.*, 2014). In line with the results of Kumar *et al.* (2018) on *T. arjuna* and Chandel *et al.* (2019) on *T. bellerica*, the present study also highlighted the importance of leaves of *T. chebula* as an alternative of fruits as source of natural antioxidants and also in therapeutic application.

Conclusion

The results indicated that ethyl acetate fractions obtained from ethanol extract exhibit higher antibacterial and antioxidant activities as compared to that of crude extract. This could be due to enrichment of specific phytocompounds in ethyl acetate fraction that are responsible for antibacterial and antioxidant activities. The ethyl acetate fraction demonstrated the highest antibacterial and antioxidant activity thus could be explored as a candidate for future search for antimicrobial and antioxidant agents for the cure and management of different ailments. Moreover, the present study also showed that leaves of *T. chebula* can also be used as substitute of fruits for therapeutic and food industries.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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