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Original Article

Antioxidant, Antibacterial Activities and Identification of Bioactive Compounds from *Terminalia chebula* Bark Extracts

Alagesan Venkatesan[1], Arumugam Kathirvel[2], Shanmugam Prakash[1], Venugopal Sujatha[1*]

[1]Phytochemistry Lab, Department of Chemistry, Periyar University, Periyar Palkalai Nagar, Salem-636 011, Tamil Nadu, INDIA.
[2]Department of Chemistry, K. S. Rangasamy College of Arts and Science (Autonomous), Tiruchengode-637 215, Tamil Nadu, INDIA.

ABSTRACT

Background: Free radical induces numerous diseases by damaging biomolecules such as lipids, proteins, RNA and DNA. Several scientists reported that numerous plants and plant extracts have potent antioxidant activities to scavenge free radicals. The present study was aimed to screen phytoconstituents, *in vitro* antioxidant and antibacterial potential in various solvent extracts of *Terminalia chebula* bark. **Methods:** Phytochemical analysis, estimation of various metabolites, *in vitro* antioxidant and antibacterial activity were done by adopting standard protocols. Selected bioactive (acetone) extract of *T. chebula* was analyzed for their phytochemical profile by GC-MS analysis. **Results:** The results of preliminary phytochemical screening analysis revealed that presence of various phytochemicals like, alkaloids, flavonoids, glycosides, terpenoids, phenolics, saponins and carbohydrates in most of the tested extracts. Acetone extract possess significant high quantity of both primary and secondary metabolites when compared with other extracts. Remarkable free radicals scavenging potential was observed in acetone extract with lowest IC_{50} values on all tested radicals namely, DPPH· (IC_{50}=144.77 µg/ml), NO· (IC_{50}=149.46 µg/ml), ·OH (IC_{50}=121.18 µg/ml), $O_2^{\cdot-}$ (IC_{50}=159.41 µg/ml), Reducing power (IC_{50}=35.85 µg/ml), Fe^{2+} ion chelating (IC_{50}=137.56 µg/ml) and TBARS (IC_{50}=201.96 µg/ml). Acetone extract expressed significant high antibacterial activity against *S. typhi* (15 mm). The result of GC-MS analysis of acetone extract shows the presence of 32 major bioactive compounds, including various phenolic, sesquiterpene, flavonoid, triazine and gibberellin compounds. **Conclusion:** The present study suggested that *T. chebula* bark extract serves as a good source of phytochemicals, natural antioxidant and antibacterial agent.

Key words: Antibacterial, Antioxidants, GC-MS analysis, Phytochemicals, *Terminalia chebula*.

Correspondence :

Dr. Venugopal Sujatha,
Assistant Professor, Phytochemistry Lab, Department of Chemistry,
Periyar University, Periyar Palkalai Nagar, Salem-636 011,
Tamil Nadu, INDIA.
Ph no: 9444886804
Tel: +91427-2345271, Fax: +91427-2345124.

E-mail: chemsujatha888@gmail.com
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INTRODUCTION

The plant kingdom is a source of drugs and nowadays there has been an increasing awareness about the significance of medicinal plants.[1] Recent years have witnessed enhanced research work reported on plants and plant materials. Drugs isolated from the plants are easily available, less expensive, safe, efficient and less side effects.[2] Plants with traditional therapeutic usage are being screened. Plants are an excellent source of natural antioxidants and antimicrobials, which may act as a potential drug in modern biomedicine.[3] Several plants have been reported to have significant free radicals scavengers such as phenols, flavonoids and terpenoids.[4] *Terminalia chebula* (*T. chebula*) (F: *Combretaceae*) is called as 'King of Medicine'. Various parts of *T. chebula* has been extensively used in Ayurveda and Siddha to treat numerous diseases such as constipation, parasites, malabsorption syndrome, hepatomegaly, vesicular, renal calculi, urinary discharges, tumors, skin disease, leprosy, intermittent fever, rheumatism, neuropathy, paralysis, memory loss, diabetes and anorexia.[5,6] *T. chebula* has been reported to possess multiple pharmacological and medicinal properties, such as antioxidant, antidiabetic, antimicrobial, anti-inflammatory, antimutagentic, antiproliferative, cardioprotective, antiarthritic, hepatoprotective, preventing, gastrointestinal motility and wound healing activity.[7] Hence, the present study was aimed to analyze phytochemical (qualitative and quantitative) profile, antioxidant and antibacterial efficacy of *T. chebula* bark extracts.

MATERIALS AND METHODS

Plant material

T. chebula bark sample were collected from Vellimalai, Tumbal, Salem District, Tamil Nadu, India. The nomenclature of collecting plant sample was identified and authenticated by Botanical Survey of India (Reference number: BSI/SRC/5/23/2011-12/Tech./32), Coimbatore, Tamil Nadu, India. The collected plant material was washed with tap water, prior to distilled water, shade dried and powdered by using electrical grinder.

Preparation of extracts

Powdered plant material (2 kg) was extracted with various solvents (hexane, chloroform, ethyl acetate, acetone, methanol and water) in increasing polarity manner in a Soxhlet apparatus until the efflux solvent became colorless. All solvent extracts were passed through Whatman (No.1) filter paper and concentrated under vacuum at 40°C to yield plant crude extracts which stored at 4°C until use.

Phytochemical analysis

Qualitative phytochemical analysis

Preliminary qualitative phytochemical analysis of all extracts were carried out according to method of Onwukeame *et al.*,.[8]

Quantitative phytochemical analysis

Determination of total phenol content

The total phenol content of *T. chebula* bark extracts were estimated by spectrophotometric assay as per the method of Barreira *et al.*,.[9] Gallic acid was used for constructing the standard curve (20-100 µg/ml, Y=0.0129x + 0.0697, R^2=0.9985) and the results were expressed as µg of gallic acid equivalents/ mg of extract (GAEs).

Determination of total flavonoid content

Flavonoid content of extracts was determined by using the method of Kathirvel and Sujatha.[10] Different concentrations (20-160 µg/ml) of

(±)-catechin was used as a reference compound to plot the standard curve (Y=0.0042x + 0.0164, R^2 =0.9991) and the results were expressed as µg of (±)-catechin equivalents (CEs) per mg of extract.

Estimation of total flavonol content

Total flavonol content was determined by adopting the protocol of Grubesic *et al.*,.[11] Rutin was used to calculate the standard curve (20-120 µg/ml, Y=0.0043x+ 0.03682, R^2=0.9652) and the results were expressed as µg of rutin equivalents per mg of extract.

Estimation of tannin content

Tannin content of all extracts was measured by Folin-Denis method.[12] Tannic acid was used to plot the standard curve (20-120 µg/ml, Y=0.0003x + 0.0091, R^2=0.9985) and the results were expressed as µg of tannic acid equivalents per mg of extract.

Estimation of protein content

Total protein content was estimated by Lowry's method.[13] Bovine serum albumin was used as reference to construct standard curve (20-160 µg/ml, Y=0.0025x+0.0074, R^2=0.9846) and the results were expressed as µg of bovine serum albumin (BSA) equivalents per mg of extract.

Estimation of ascorbic acid content

Total ascorbic acid content was estimated by the method of Omaye.[14] Ascorbic acid was server as reference to plot standard curve (20-120 µg/ml, Y=0.0043x+0.0368, R^2=0.9652) and the results were expressed as µg of ascorbic acid equivalents per mg of extract.

Estimation of carbohydrate content

Total carbohydrate content was estimated by Anthrone method.[15] Various concentrations of standard glucose was used to construct the standard curve (20-120 µg/ml, Y=0.0114x+0.0324, R^2=0.9914) and the results were expressed as µg of glucose equivalents per mg of extract.

In vitro antioxidant studies

Different concentrations (20-100 µg/ml) of *T. chebula* bark extracts were tested for various types of radicals scavenging potential. Ascorbic acid, TBHQ and BHA served as standard reference compounds for all *in vitro* antioxidant assays.

DPPH radical ($DPPH^{\cdot}$) scavenging assay

The DPPH radical scavenging activity was carried out according to the method of Arunika *et al.*,[16] with some modifications.

Hydroxyl radical ($^{\cdot}OH$) scavenging assay

The 2-deoxyribose (Fenton reaction) assay[17] was used to determine the hydroxyl radical scavenging efficacy of all extracts.

Nitric oxide radical ($NO^{\cdot}$) scavenging assay

The nitric oxide radical scavenging potential of various extracts of *T. chebula* was determined by the method of Sfahlan *et al.*,.[18]

Superoxide anion radical ($O_2^{\bullet-}$) scavenging assay

The superoxide radical scavenging activity of different extracts of *T. chebula* was tested as per the modified method of Liu *et al.*,.[19]

Ferrous ion chelating activity

Ferrous ion chelating activity was estimated according to the method of Glucin.[20]

Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acids reactive substances analysis was measured by using the method of Barreira *et al.*,.[9]

Reducing power

The reducing power activities of *T. chebula* bark extracts were determined by the method of Oyaizu.[12]

GC-MS analysis

The selected bioactive (acetone) extract was subjected to GC-MS analysis using CP3800 Saturn 2200 Gas Chromatography-Mass Spectrometer (GC-MS) system. The temperature was programmed to 80°-350°C at the rate of 3°C/min and held at 350°C at 55 min. The ion source temperature was 200°C with 20-500 amu scan range. The spectrums of unknown component were compared with Wiley and NIST libraries.

Antibacterial activity

Three grams-positive bacterial strains namely, *Enterococcus faecalis, Bacillus subtilis, Staphylococcus aureus* and three gram-negative bacterial strains *viz*., *Proteus vulgaris*, *Salmonella typhi, Shigella sonnei* were used for antibacterial study. All selected bacterial strains were obtained from clinical laboratories in and around Salem District, Tamil Nadu. The antibacterial ability of various solvents bark extracts of *T. chebula* were evaluated using agar well diffusion method, as per the modified protocol of Srinivasan *et al.*,.[21]

Statistical analysis

Statistical analyses were conducted using the SPSS software (16.0 versions). Analysis of Variance (ANOVA) in a completely randomized design and Tukey's multiple range tests was used to compare any significant differences between samples. The values were expressed as means ± standard deviations. All determinations were done at least in triplicate and all were averaged. The confidence limits used in this study were based on 95% (p<0.05).

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

The results of phytochemical analysis of *T. chebula* bark extracts revealed that presences of various classes of phytoconstituents namely, alkaloids, flavonoids, terpenoids, carbohydrates, tannins and phenolics in most of the tested extracts (Table 1). Tannins, flavonoids, phenolics, alkaloids, steroids, saponins and terpenoids were found in ethyl acetate, acetone and methanol extracts. Methanol and water extracts showed the presence of amino acids, proteins and carbohydrates.

Similar findings were reported in various extracts of different parts (leaves[22] and fruits[23]) of *T. chebula.* Kumar, (2006)[24] reported that *T. chebula* contains a number of phytoconstituents like, tannins, flavonoids, sterols, amino acids, fructose, resin and fixed oils. Fruit ethanolic extract of *T. chebula* shows presence of carbohydrates, proteins, anthraquinone, glycosides, saponins, triterpenoids, tannins, polyphenols, amino acids and flavonoids[25,26] which strengthens our outcome. Variety of phytochemicals present in the bark extracts may be responsible for its medicinal properties.

Quantitative phytochemical analysis

Different classes of phytochemicals (proteins, carbohydrates, vitamin C, tannins, flavonoids and phenolic compounds) were found in various concentrations in all tested extracts of *T. chebula* (Table 2). However, highest quantity of both primary and secondary metabolites was noticed in acetone extract such as, phenol [159.51 ± 0.86 µg/mg GAE], tannin [151.89 ± 1.92µg/mg TAE], flavonoid [117.56 ± 0.60 µg/mg CE], flavonol [73.26 ± 0.33 µg/mg RU], carbohydrate [154.67 ± 0.59 GE], ascorbic acid [134.97 ± 0.41 µg/mg AAE] and protein [76.00 ± 2.00 µg/mg BSAE] followed by methanol extract. Moderate to low quantity of tested phytochemicals were observed in remaining extracts.

Table 1: Qualitative phytochemical analysis of various solvent crude extracts of *T. chebula* bark

Phytoconstituents	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol	Water
Alkaloids	-	++	++	++	++	+
Flavonoids	-	+++	+++	+++	+++	+
Glycosides	-	+	++	++	++	+
Cardiac glycosides	-	+	++	+	++	-
Tannins	+	-	+++	+++	+++	+++
Phenolics	-	-	+++	+++	+++	+++
Lignins	-	-	-	-	-	-
Aminoacids	-	-	-	-	++	++
Steroids	-	-	+	++	++	+
Proteins	-	-	-	-	+	++
Carbohydrates	-	-	-	+	++	+
Saponins	-	-	+++	+++	+++	++
Terpenoids	-	-	+	++	++	++
Fats/Oils	+	+	+	+	+++	+++
Anthocyanins	-	-	+	-	-	+
Leucoanthocyannins	-	+	+	-	+	+

+++ = Copiously present, ++ = Moderately present, + = Slightly present, - = Absent.

Table 2: Quantitative phytochemical analsysis of various solvent crude extracts of *T. chebula* bark

Content (µg/mg extract)	Extracts*					
	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol	Water
Total phenol content	28.68 ± 0.59^{a}	62.68 ± 0.25^{b}	137.98 ± 0.17^{d}	159.51 ± 0.86^{f}	143.41 ± 0.52^{e}	80.39 ± 0.70^{c}
Total flavonoid content	22.48 ± 0.63^{a}	71.29 ± 0.48^{b}	$87.16 \pm 0.96^{b,c}$	117.56 ± 0.60^{d}	$111.21 \pm 1.53^{c,d}$	$81.05 \pm 0.41^{b,c}$
Total flavonol content	30.31 ± 1.09^{a}	38.52 ± 0.14^{b}	51.67 ± 0.14^{d}	73.26 ± 0.33^{f}	63.78 ± 1.18^{e}	46.16 ± 0.91^{c}
Total tannin content	14.11 ± 1.92^{a}	54.11 ± 3.85^{b}	121.89 ± 1.92^{d}	151.89 ± 1.92^{f}	139.67 ± 0.00^{e}	95.22 ± 1.92^{c}
Total protein content	17.33 ± 1.15^{a}	30.67 ± 1.15^{b}	38.67 ± 1.15^{c}	76.00 ± 2.00^{e}	50.67 ± 2.31^{d}	28.67 ± 1.15^{b}
Total ascorbic acid content	12.93 ± 1.22^{a}	63.20 ± 1.18^{b}	114.86 ± 0.21^{d}	134.97 ± 0.41^{f}	119.67 ± 0.88^{e}	108.60 ± 0.77^{c}
Total carbohydrate content	10.91 ± 0.91^{a}	69.83 ± 0.85^{b}	111.52 ± 0.92^{d}	154.67 ± 0.59^{f}	117.48 ± 0.41^{e}	96.31 ± 0.88^{c}

*- The values are mean of triplicates with (±) standard deviation (mean ± S.D, n=3). The superscript letters (a-f) present in rows represents the effectiveness of extracts (f>e>d>c>b>a) which significantly differs when subject to Tukey's multiple comparison test (at $p<0.05$).

Table 3: IC_{50} values (µg/ml) of different solvent crude extracts of *T. chebula* bark

Extracts	Scavenging activities and reducing power IC_{50} (µg/ml)*						
	DPPH	Nitric oxide	Hydroxide	Superoxide	Reducing power	Ferrous ion chelation	lipid peroxidation
Haxane	687.06 ± 0.052	682.22 ± 0.285	346.48 ± 0.046	848.65 ± 0.229	367.52 ± 0.511	689.25 ± 0.472	791.01 ± 0.616
Chloroform	463.47 ± 1.562	549.43 ± 0.271	145.44 ± 0.514	621.19 ± 0.350	175.84 ± 0.251	498.22 ± 0.178	556.32 ± 0.236
Ethyl acetate	167.29 ± 0.150	256.48 ± 0.289	128.62 ± 0.567	248.11 ± 0.086	68.03 ± 0.727	161.51 ± 0.416	327.89 ± 0.220
Acetone	144.77 ± 0.208	149.46 ± 0.303	121.18 ± 0.049	159.41 ± 0.035	35.85 ± 0.159	137.56 ± 0.484	201.96 ± 0.761
Methanol	157.48 ± 0.269	178.33 ± 0.468	122.21 ± 0.025	166.29 ± 0.520	42.58 ± 0.433	149.15 ± 0.822	271.76 ± 1.380
Water	196.57 ± 0.058	288.82 ± 0.699	131.19 ± 0.301	509. 49 ± 1.300	94.87 ± 0.440	277.47 ± 1.947	480.68 ± 0.442
Ascorbic acid	121.90 ± 0.017	156.22 ± 0.106	120. 60 ± 0.165	148.55 ± 0.733	39.18 ± 0.058	297.50 ± 0.520	160.97 ± 0.712
BHA	138.28 ± 0.237	146.40 ± 0.000	122.36 ± 0.265	146.66 ± 0.605	75.09 ± 0.216	226.39 ± 0.765	137.73 ± 0.503
TBHQ	170.32 ± 0.333	140.52 ± 0.396	125.26 ± 0.353	169.57 ± 0.503	89.04 ± 0.816	271.23 ± 0.420	140.81 ± 0.512

*- Data represents mean ± S.D, (n=3).

Table 4: Antibacterial activity of different solvent crude extracts of *T. chebula* bark

Organisms	Zone of inhibition (Diameter in mm)*						
	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol	Water	Control#
E. faecalis	10.00 ± 1.00^{c}	07.33 ± 0.58^{a}	13.33 ± 0.58^{c}	14.67 ± 0.58^{c}	12.33 ± 0.58^{c}	00.00 ± 0.00^{a}	34.00 ± 1.00^{c}
S. aureus	07.33 ± 0.58^{a}	08.67 ± 0.58^{a}	11.67 ± 0.58^{a}	12.00 ± 1.73^{b}	10.33 ± 0.58^{b}	06.33 ± 0.58^{b}	28.00 ± 0.00^{a}
B. subtilis	05.67 ± 1.15^{b}	08.67 ± 0.58^{a}	12.67 ± 0.58^{d}	14.00 ± 1.00^{e}	13.33 ± 0.58^{d}	00.00 ± 0.00^{a}	28.33 ± 0.58^{a}
V. vulgaris	08.67 ± 0.58^{b}	11.00 ± 1.00^{b}	11.00 ± 1.00^{a}	12.33 ± 0.58^{b}	00.00 ± 0.00^{a}	00.00 ± 0.00^{a}	32.33 ± 0.58^{b}
S. typhi	09.33 ± 1.53^{b}	12.33 ± 1.53^{c}	11.67 ± 1.15^{a}	15.67 ± 1.15^{d}	14.33 ± 0.58^{c}	07.00 ± 1.00^{b}	32.67 ± 1.15^{b}
S. sonnei	07.67 ± 1.15^{a}	10.33 ± 1.15^{b}	12.33 ± 0.58^{b}	09.67 ± 0.58^{a}	12.33 ± 0.58^{c}	00.00 ± 0.00^{a}	31.67 ± 0.58^{b}

*- The values are mean of triplicates with (±) standard deviation (mean ± S.D, n=3). Different superscript letters (a-d) in the column indicate significant differences (at $p < 0.05$) when subject to Tukey's multiple comparison test.

High amount of total phenols, flavonols, flavonoids, tannins, ascorbic acid, carbohydrate and protein has been reported in leaf acetone extract of *T. chebula*[22] which supports the present findings. Similar results were noticed in various extracts of different parts of *T. chebula* like, seed, leaves, stem and fruit.[27,28] Previous study reported that successive solvent extraction systems may leads to the differences in extraction of phytoconstituents in plant extracts.[29] Moreover, reports on medicinal plant extracts state that concentration of phytochemicals harbor a positive correlation with antioxidant activity of extracts.[3] Present results clearly indicates the presences of high quantity phytochemicals namely, phenols, flavonols, flavonoids, tannins, ascorbic acid, carbohydrate and protein are play important role in antioxidant potential of these extracts.

In vitro antioxidant activity

All tested extracts of *T. chebula* show different degrees of antioxidant ability in a dose dependent manner in all tested methods (Figure 1 and Table 3). Significant high antioxidant potential was found in acetone extract on all tested radicals scavenging assays followed by methanol extract. Acetone extract shows excellent ferrous reducing potential with lowest IC_{50} value (35.85 µg/ml) followed by hydroxyl radicals scavenging activity (121.18 µg/ml). Ethyl acetate and water extracts expressed sustainable antiradical potential on all tested radicals with moderate IC_{50} value. Minimal free radical scavenging ability was observed in chloroform and hexane extracts with high IC_{50} value.

Our results suggested that various concentrations have different activities and maximum activity was observed at 1000 µg/ml concentration. DPPH is a stable free radicals and it gives more accurate and concurred results for determination of antioxidant potential.[30] The radical scavenging activity of *T. chebula* plant extract possessed significant activity in acetone followed by other extracts, which were capable of reducing DPPH radical into hydrogen ion, thus the concentration of the color change in the reaction depends upon the number of electrons transferred during the reaction.[31] Hence, it could be assumed that the higher antioxidant potential of acetone extract was due to the electron sharing by the phenolic compounds present in them.

Reactive oxygen species formed during their reaction or with superoxide such as NO_2, N_2O_4, and NO_2 - are very reactive. The reactivates of $NO^{\bullet}$ and $O^{2\bullet-}$were found to be relatively lower, but their metabolite $ONOO^-$ (peroxynitrite) is enormously reactive and directly induces toxic reactions, as well as SH-group oxidation, lipid peroxidation, protein tyrosine nitration and DNA modifications.[32] In our studies, it was found that the acetone higher scavenging activity (149.46 µg) followed by the other extract (Figure 1). Similar kind of observations was observed in previous studies.[22] The current results were correlated with the earlier findings[33] has shown noticeable activity against nitric oxide radicals.

Hydroxyl radical scavenging activity exhibited lowest IC_{50} of acetone extract was 121.18 µg/ml. The possible scavenging ability of phenolic substances might be due to the active hydrogen's donor's ability of hydroxy replacement. This activity was further compared to other related medicinal plants, i.e. *Ananascosmosus*[34], *Allium sativum*[35] are reported potential antioxidant activity. Hence forth, the test *T. chebula* possesses higher amount of antioxidant potential in acetone extract.

The superoxide scavenging activity of *T. chebula* bark was increased markedly with the increase of concentrations. The inhibition concentration (IC_{50}) of the bark acetone extract was 159.41 µg/ml. It's very low compared with other extract. It plays a vital role in the formation of hydroxyl radical or singlet oxygen in living organisms.[36] The results of our present study were comparable with the results of other reports on superoxide radical scavenging activity on *Clitoria ternatea*,[37] *Pothos scandens* are similar findings.

Reducing capacity of the bark in various extracts of the *T. chebula* was measured by its ability to transform Fe^{3+} to Fe^{2+} at various concentrations (200, 400, 600, 800 and 1000 µg/ml).

The results revealed that the reducing activity considerably increased as the concentration of the extract was increased with a maximum increase at 1000 µg/ml (Figure 2). The acetone extract showed significantly higher activities than the control exhibited greater reducing power, indicating that *T. chebula* bark acetone extract consists of hydrophilic polyphenolic compounds that cause the greater reducing power.[38] *In vitro* antioxidant previously reported fruit methanolic extract of *T. chebula* as a reducing agent and effectiveness as scavengers of free radicals.[39]

The main approach to avoid ROS generation that is related with redox active metal catalysis involves chelating of the metal ions. *T. chebula* bark acetone extract the most active extract interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine. IC_{50} values of the acetone extract for chelating activity were 137.56 µg/ml is lower than the other extract. The IC_{50} of chelating effect of other extracts on Fe^{2+} and ferrozine complex formation is shown in Table 3.

The results of the investigations revealed that *T. chebula* bark had potent lipid peroxidation inhibition activity because lower IC_{50} value 201.96 µg/ml. So, it conclude that the acetone extract of *T. chebula* bark exhibited significant in lipid peroxide inhibition activity on compared with the standard. The activity may be related to the presence of phenols and flavonoids in the plant extract. *T. chebula* possess anti-lipid peroxidation, anti-superoxide radical formation and free radical scavenging activity.[40] Bark and fruit acetone extract shows potential anti carcinogenic activity[41].

Antibacterial activity

All extracts of *T. chebula* show a broad spectrum of antibacterial activity (5-15 mm) against most of the tested pathogens (Table 4). The results of antibacterial activity revealed that acetone extract harbor significant

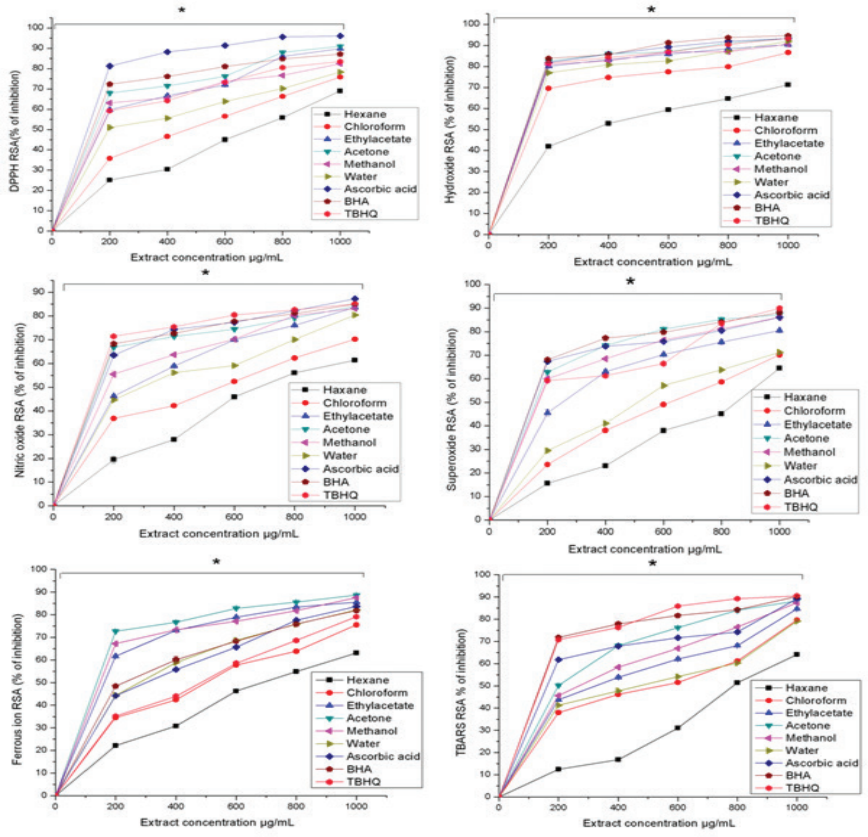

Figure 1: Radical scavenging activity of different solvent crude extracts of *T. chebula* bark.

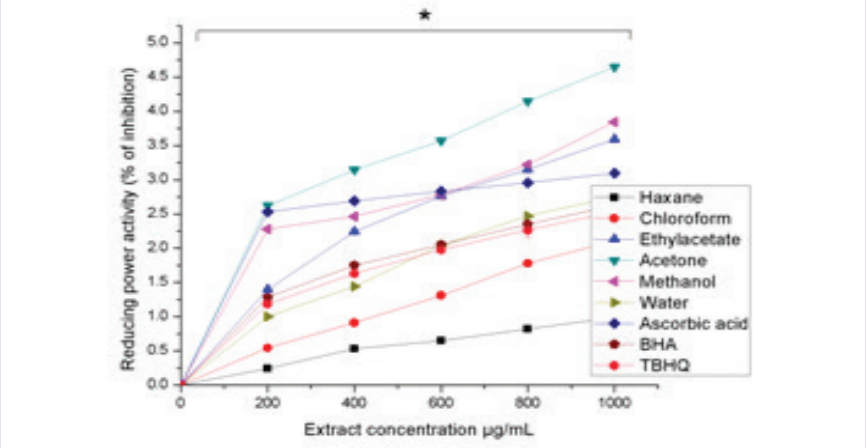

Figure 2: Reducing power of different solvent crude extracts of *T. chebula* bark.

antibacterial activity against all tested pathogen and maximum growth inhibition was observed against *S. typhi* (15 mm) followed by *E. faecalis* (14 mm). The moderate antibacterial potential was noticed in methanol and ethyl acetate extracts against most of the tested microbes. Hexane, chloroform and water extracts show only minimal/nil activity against most of the tested pathogens.

Medicinal plants are important source for discover a new antimicrobial agents with significant activity against infective microbes.[42] Several investigators have been reported the antimicrobial potential of various parts of *T. chebula*.[43] Leaf acetone extract of *T. chebula* exhibited high antibacterial activity, especially against negative strain.[22] Similar findings were noticed in the present study. Acetone was reported as effective solvent for extracting alkaloids, phenolic, flavonoids, and tannin compounds

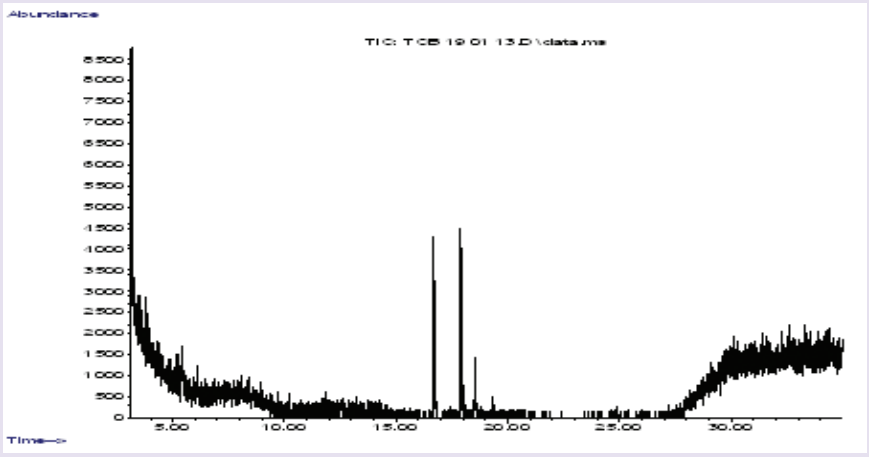

Figure 3: GC-MS Chromatogram of acetone extract of *T. chebula* bark.

which exhibited antibacterial activity[21] which are quite comparable with our results. The present results were good agreement with previous findings on antibacterial activities of *T. chebula* fruit extracts (Figure 3).[23,44]

GC-MS analysis

The result of acetone extract GC-MS analysis indicates the presence of 32 compounds (Figure 3). Ferulic acid [Phenolic compound, RT-16.68, Peak area-6.38], 3,4,5-trihydroxy benzoic acid (Gallic acid) [Phenolic compound, RT-17.89, Peak area-7.24], alpha.-Santalol [Sesquiterpene, RT-18.54, Peak area-2.02] were identified as predominant components of acetone extract. 2,3,7,8-tetrahydroxychromeno[5,4,3-cde]chromene-5,10-dione (Ellagic acid) RT-29.45, Peak area-3.67, Gibberellic acid RT-29.62, Peak area-1.43 compounds were detected in significant quantities.

Previous study has been reported that 35 and 64 different compounds were identified in *T. chebula* ethanolic and ethyl acetate fraction of fruit extract respectively[45,46] which strengthen the present outcome. Ellagic acid and Gallic acid are polyphenolic antioxidant compounds that occur in many plants[47] and are found to have antioxidant, antimutagenic, anti-inflammatory and cardioprotective activity.[48] Ferulic acid also serves as antioxidant agent.[49] α-santalol which has shown outstanding chemopreventive effects against skin cancer under both *in vivo* and *in vitro* conditions.[50] The presence of high quantity of these components in acetone extract of *T. chebula* may be the responsible for its bioactivity.

CONCLUSION

The findings of present investigation clearly indicates that acetone extract of *Terminalia chebula* bark possess significant antioxidant and antibacterial capacity and a good source of various phytoconstituents which recommends further research needed for isolation of bioactive principles.

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CONFLICT OF INTEREST

The author declare no conflict of interest.

ABBREVIATIONS USED

DPPH•: 2,2-diphenyl-1-picrylhydrazyl radical, **NO•:** Nitric oxide radical, **•OH:** Hydroxyl radical, **$O_2^{\bullet-}$:** Superoxide anion radical, **Fe^{2+}:** Ferrous ion, **IC_{50}:** Inhibitory concentration 50, **GCMS:** Gas Chromatography-Mass spectrometer, **BHA:** Butylated hydroxyanisole, **TBHQ:** Tertbutyl hydoquinone, **TBARS:** Thiobarbituric acid reactive substances.

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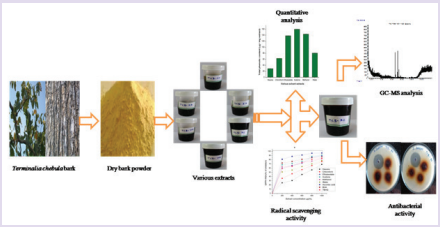
PICTORIAL ABSTRACT



SUMMARY

- *Terminalia chebula* bark serves as a good source of phytochemicals and antioxidant and antibacterial agents.
- Acetone extract possesses significant antioxidant capacity.
- Acetone extract possesses significant ferrous ion, reducing and hydroxyl radical scavenging potential.
- Acetone extract has wide spectrum antibacterial activity.
- 32 compounds were identified in GC-MS analysis of acetone extract of *Terminalia chebula* bark, inferring its pharmacological property.

ABOUT AUTHORS

Mr. Alagesan Venkatesan: Ph.D research scholar, Phytochemistry Lab, Department of Chemistry, Periyar University, Periyar Palkalai Nagar, Salem - 636 011, Tamil Nadu, India. His area of research interests is Phytochemistry and Nanochemistry.

Dr. Arumugam Kathirvel: Is researcher and recently got Ph.D. degree. Currently, he is working as Assistant Professor, Dept. of Chemistry, KSR College of Arts and Science, Tiruchengode, Tamilnadu. His area of research interests is plant biomolecules and its biological potentials.

Dr. Venugopal Sujatha: Is working as Assistant Professor, Phytochemistry Lab, Department of Chemistry, Periyar University, Periyar Palkalai Nagar, Salem Tamil Nadu, India, since October 2008. Her research fields mainly focused on the Medicinal chemistry, Phytochemistry and Nano chemistry. She has published over 35 research articles in various reputed and peer reviewed journals.

Mr. Shanmugam Prakash: Ph.D research scholar, Phytochemistry Lab, Department of chemistry, Periyar University, Periyar Palkalai Nagar, Salem - 636 011, Tamil Nadu, India. His area of research interests is phytochemistry and Nanochemistry.