

Original Article

COMPARATIVE STUDY ON THE ANTIOXIDANT ACTIVITIES OF METHANOLIC AND AQUEOUS EXTRACTS OF *TERMINALIA BELLERICA*

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Received: 10 Aug 2019, Revised and Accepted: 12 Oct 2019

ABSTRACT

Objective: The present study was undertaken to investigate the antioxidative activity of fruit pulp, seed and bark of *T. bellerica*.

Methods: Methanol (70%) and water were used for the extraction, and the respective extracts were analyzed for total phenolic contents along with the antioxidant activities through different assays.

Results: Amongst the tested extracts, methanolic extracts were found to be better than aqueous with the highest total phenolic contents (TPC). Methanolic fruit pulp showed lower IC₅₀ for free radical (118.7 µg/ml), superoxide anion radical (77.65 µg/ml) and hydroxyl radical (73.76 µg/ml) scavenging activities. Methanolic fruit pulp also showed lower IC₅₀ for lipid peroxidation (115.6 µg/ml) and ferric thiocyanate (184.98 µg/ml) assay than aqueous extracts in comparison to standard quercetin. Further, correlation between TPC and antioxidant studies revealed that phenolics are mainly responsible for antioxidant activity of *T. bellerica*.

Conclusion: The current study suggests that the methanolic extract of *T. bellerica* could be a potential source of natural antioxidants for food and pharmaceutical companies.

Keywords: Reactive oxygen species, Total phenolic content, Antioxidant, *Terminalia. Bellerica*

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DOI: <http://dx.doi.org/10.22159/ijcpr.2019v11i6.36354>

INTRODUCTION

Reactive oxygen species (ROS) can cause severe damage to the normal cells of the body. This damage can be to the DNA, proteins and other macromolecules. Oxidative stress, caused by an imbalance between antioxidant systems and the production of oxidants (ROS), seems to be associated with many multifactorial diseases especially cancers, cardiovascular diseases and inflammatory disorders [1, 2]. The increase in ROS generation or decreased antioxidant availability can result in a net increase in intracellular oxidative damage. The mechanism of action of many synthetic antioxidants involves free radical (FR) scavenging property, which protects against oxidative damage but has adverse side effects [3]. The synthetic antioxidant may cause cellular toxicity; however, there is an alternative is the consumption of natural antioxidants from various food supplements and traditional medicines [4]. There is increased interest among phytotherapy researchers to use medicinal plants with antioxidant activity for protection against oxidative stress.

Terminalia bellerica (TB) Roxb (Combretaceae), is a large deciduous tree found throughout India that reaches height up to 30 meters. Trunk of the tree is straight with brownish-grey color. Leaves are long, alternate, oval and are clustered towards the end of branches. These are 7-14 cm in breadth and 10-12 cm in length. Simple and solitary flowers are white or yellow in color and appear in the month of May. Fruits are ovoid in shape and appear grey in color. TB has been valued in Ayurvedic and traditional systems of medicine for treatment of wide range of diseases having many pharmacological properties such as anti-inflammatory, immunomodulatory, anticancer, hepatoprotective and antimicrobial [5-9]. It is an integral part of ancient formulation, *Triphala* which is used for a variety of ailments in Ayurveda. GC-MS analysis revealed that TB contains various polyphenolic and other bioactive compounds such as gallic acid, quinic acid (chlorogenic acid), ethyl galate, 9,12 octadecadienoic acid, glucopyranose, sitosterol, protein, tannins, galactose, glucose, mannitol, fructose, ramnose etc [10]. Antioxidant activities increase proportionally with the polyphenol content, primarily because of their redox properties. Among the

diverse roles of polyphenols, they protect cell constituents against destructive oxidative damage, thus limiting the risk of various degenerative diseases associated with oxidative stress by acting as potent FR scavengers [11]. Recent studies focused on the comparison of antioxidant activities of methanolic and aqueous extracts of TB and their correlation with total phenolic content.

MATERIALS AND METHODS

Plant materials

Plant materials were collected from herbal garden of Narendra Dev University of Agriculture and Technology Kumarganj, Faizabad, U. P., India and identified with the help of Dr. MN Srivastava, Senior Scientist, Botany Division, CSIR-Central Drug Research Institute, Lucknow, India and the voucher specimens (2322 CSIR-CDRI) were submitted in CDRI herbarium.

Chemicals and reagents

Quercetin, gallic acid, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and thiobarbituric acid (TBA) were purchased from Sigma-Aldrich, St. Louis, USA. Ascorbic acid, Folin Ciocalteu's phenol reagents were the product of E. Merk, Mumbai, India. Nitro blue tetrazolium (NBT), phenazine methosulphate (PMS), reduced nicotinamide adenine dinucleotide (NADH), potassium ferricyanide, trichloroacetic acid (TCA), ferric chloride (FeCl₃), ferrous sulphate (FeSO₄) and sodium dodecyl sulphate (SDS) were purchased from SRL India. All other reagents and chemicals used were of analytical grade.

Extraction procedure

Twenty grams of the dried and powdered plant sample of TB fruit pulp, seed and bark was extracted with 70% methanolic solvent (in distilled water) and aqueous system for overnight at room temperature in an orbital shaker. The methanolic fruit pulp (MEFP), seed (MES), bark (MEB) and aqueous fruit pulp (AQFP), seed (AQS) bark (AQB) extracts were separated from the residues by filtering through Whatman No. 1 filter paper. The residues were extracted until decoloration with the same fresh solvent and extracts

combined. The combined extracts were concentrated and freed of solvent under reduced pressure at 40 °C by using a rotary evaporator and lyophilized till dryness. The dried crude concentrated extracts were stored at -4 °C and used for the antioxidant activity determination.

Antioxidant studies

Total phenolic content (TPC)

TPC of powdered plant material was extracted with 50% methanol+1% HCl, filtered and made up to 10 ml each with water. TPC was measured with the method of Ragazzi and Veronese [12]. To 0.1 ml plant extract, 0.5 ml of Folin's reagent (1 N) and 1.0 ml of Na₂CO₃ were added subsequently. The test mixture was mixed properly and kept at room temperature for 30 min and volume was made up to 12.5 ml with distilled water. The absorbance of this solution was measured at 720 nm. The TPC was reported as mg of gallic acid equivalent (GAE)/g of dry weight.

Free radical scavenging activity (FRSA)

FRSA of the extracts was measured by using DPPH stable radical according to the method of Yen and Duh [13]. Each extract (0.1 ml) was added to a freshly prepared DPPH solution (6 × 10⁻⁵ M in HPLC grade 2.9 ml methanol) and mixed vigorously. The reduction of the DPPH radical (DPPH[•]) was measured by continuous monitoring of the decrease in absorbance at 515 nm until a stable value was obtained.

$$\text{Inhibition (\%)} = \frac{[(\text{blank absorbance} - \text{sample absorbance}) / \text{blank absorbance}] \times 100}{100}$$

The inhibitory concentration (IC₅₀), which represents the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, representing a parameter widely used to measure the antioxidant activity, was calculated from a calibration curve by linear regression. EC₅₀ was calculated as IC₅₀ (µg/ml)/concentration of DPPH/ml and expressed as µg/mg DPPH. For rational reasons of clarity, the ARP was determined as the reciprocal value of the EC₅₀, representing a comparable term for the effectiveness of antioxidant and radical scavenging capacity:

$$\text{ARP} = 1 / \text{EC}_{50} \times 100$$

The larger the ARP, the more efficient the antioxidant

Superoxide anion radical scavenging activity (SARSA)

This assay was based on the capacity of the extract to inhibit the reduction of nitro blue tetrazolium (NBT) by the method of Nishikimi *et al.* [14]. Three milliliters reaction mixture containing different aliquot of plant extracts (50, 100, 150 and 200 µl) with 0.1 M phosphate buffer (pH 7.8), 60 µM PMS, 468 µM nicotinamide adenine dinucleotide reduced (NADH) and 150 µM NBT was incubated for 5 min at ambient temperature. Absorbance was read after 6 min at 560 nm using a UV-Vis spectrophotometer. The percentage inhibition (PI) of superoxide (O₂^{•-}) generation was measured by comparing the absorbance of the control and those of the reaction mixture containing the test sample.

Reducing power (RP)

RP of the extracts was determined by using a slightly modified method of ferric reducing-antioxidant power assay [15]. Each extract (1.0 ml) was mixed with 2.5 ml of phosphate buffer (0.1 M, pH 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide and was incubated at 50 °C for 20 min. After completion of the incubation period, 2.5 ml of 10% (w/v) trichloroacetic acid (TCA) was added to terminate the reaction. The upper layer (2.5 ml) was diluted with an equal volume of deionized water. Finally, 0.5 ml of 0.1% (w/v) FeCl₃ was added and after 10 min the absorbance was measured at 700 nm against a blank. RP was expressed as ascorbic acid equivalents (1 ASE = 1 mmol ascorbic acid). ASE value is inversely proportional to RP.

Lipid peroxidation (LPO)

A modified thiobarbituric acid-reactive species (TBARS) assay method of Ohkawa *et al.* [16], was applied to measure the LPO formation, using egg homogenate as lipid-rich media. Egg homogenate (10% in 0.2 M PBS, 0.5 ml), test extract (0.1 ml) and DW

(0.85 ml) were mixed in a test tube. Finally, FeSO₄ (0.07 M, 0.05 ml) was added to the reaction mixture and incubated at 37 °C temperature for 30 min to induce LPO. Thereafter, acetic acid (20%, 1.5 ml), TBA (0.8% prepared in 1.1% SDS, 1.5 ml) and TCA (20%, 0.05 ml) was added, vortexed and then heated in a boiling water bath for 60 min. After cooling, butanol (5 ml) was added to each tube and centrifuged for 10 min at 3000 rpm. The absorbance of the upper organic layer was measured at 532 nm by UV-Vis spectrophotometer (Labtronics, model LT-2910).

Hydroxyl radical scavenging activity (HRSA)

OH[•] were generated by a mixture of Fe³⁺-EDTA, H₂O₂ and ascorbic acid and assessed by monitoring the degraded fragments of deoxyribose, through malondialdehyde (MDA) formation [17]. The reaction mixtures contained ascorbic acid (50 µM), FeCl₃ (20 µM), EDTA (2 mmol), H₂O₂ (1.42 mmol), deoxyribose (2.8 mmol) with different concentrations of the plant extracts in a final volume of 1 ml, was incubated at 37 °C for 1 hour and then 1 ml of 2.8% TCA (w/v in water) and 1 ml of 1% TBA (w/v) were added. The mixture was heated in a boiling water bath for 30 min. It was cooled and absorbance was taken at 532 nm.

Ferric thiocyanate assay (FTC)

The reaction mixture containing 400 µl of different concentration of ethanolic plant extracts, 200 µl of diluted linoleic acid (25 mg/ml in 99% ethanol) and 400 µl of 50 mmol phosphate buffer (pH 7.4) was incubated for 15 min at 40 °C. A 100 µl aliquot of this was then mixed with a reaction mixture containing 3 ml of 70% ethanol, 100 µl of ammonium thiocyanate (300 mg/ml in DW) and 100 µl of ferrous sulphate. Red color developed was measured at 535 nm [18].

Statistical analysis

All analytical data are subjected to an analysis of variance (ANOVA). Each value is a mean ± standard deviation (SD) of three replications (n=3). Statistical analysis was conducted by using prism software (graph pad prism software version 3.0, USA). The results obtained were considered statistically significant if p < 0.05. The R² value and the regression equation were calculated by plotting a graph showing the TPC on the x-axis and the antioxidant deciding parameters on the y-axis, using MS office excel 2007.

RESULTS

Total phenolic content (TPC)

The TPC in methanolic and aqueous extracts of TB ranged from 98.68 to 127.60 mg/g GAE and 56.97 to 80.11 mg/g GAE (fig. 1). The highest value of TPC was present in MEFP (127.60 mg/g GAE) followed by MES (108.58 mg/g GAE) and MEB (98.68 mg/g GAE) which signifies its high antioxidant activity than aqueous extracts.

Free radical scavenging activity (FRSA)

Methanolic and aqueous extracts of TB were examined for their potential to scavenge FRs and measured as PI. Methanolic extracts were considered to be the most potent significant FR scavenger than aqueous extracts and its value of inhibition ranged from 62.95 to 68.34% (fig. 2). FRSA values of methanolic extracts were in following order MES (68.34%) > MEFP (68.34%) > MEB (62.95%) while in aqueous extracts the order was AQS (53.82%) > AQB (53.50%) > AQFP (51.58%). Thus methanolic extracts showed high FRSA than aqueous in comparison to standard quercetin (90.25%). The IC₅₀ value of MEFP was 118.7 µg/ml, whereas, in the case of MES and MEB, it was 130 and 146.7 µg/ml, respectively.

Superoxide anion radical scavenging activity (SARSA)

The antioxidant potential of methanolic extracts against O₂^{•-} was considered to be significant in comparison to the standard quercetin. The result presented in fig. 3 shows that methanolic extracts inhibits NBT reduction significantly high than aqueous extracts. The order of SARSA of methanolic and aqueous extracts were MEFP (75.86%) > MEB (73.86%) > MES (72.99%) and AQFP (62.91%) > AQS (56.82%) > AQB (56.19) in comparison to standard quercetin (91.77%) at 200 µg/ml concentration. The IC₅₀ value at which methanolic extracts showed significant SARSA was 77.65 (MEFP), 155.53 (MEB) and 191.75 (MES) µg/ml.

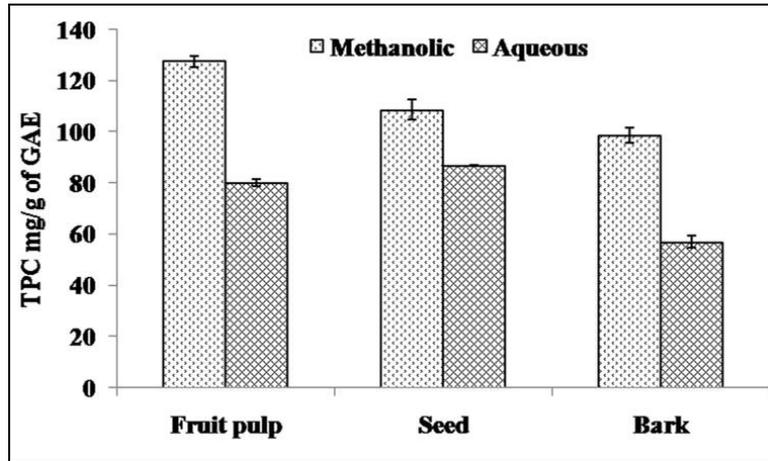


Fig. 1: Total phenolic content of methanolic and aqueous extracts of *T. bellerica*. Values are mean±SD of three replications (n=3). MEFP: methanolic fruit pulp; MES: methanolic seed; MEB: methanolic bark; AQFP: aqueous fruit pulp; AQS: aqueous seed; AQB: aqueous bark

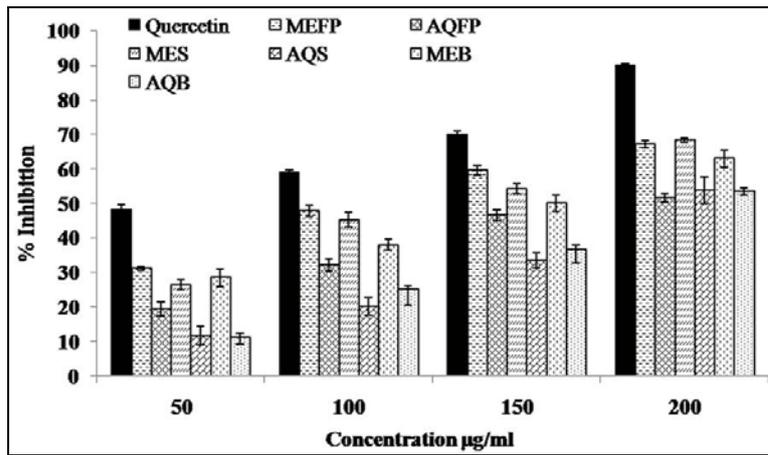


Fig. 2: Free radical scavenging activity of methanolic and aqueous extracts of *T. bellerica* and standard quercetin against DPPH radicals at varying concentrations. Values are mean±SD of three replications (n=3). MEFP: methanolic fruit pulp; MES: methanolic seed; MEB: methanolic bark; AQFP: aqueous fruit pulp; AQS: aqueous seed; AQB: aqueous bark

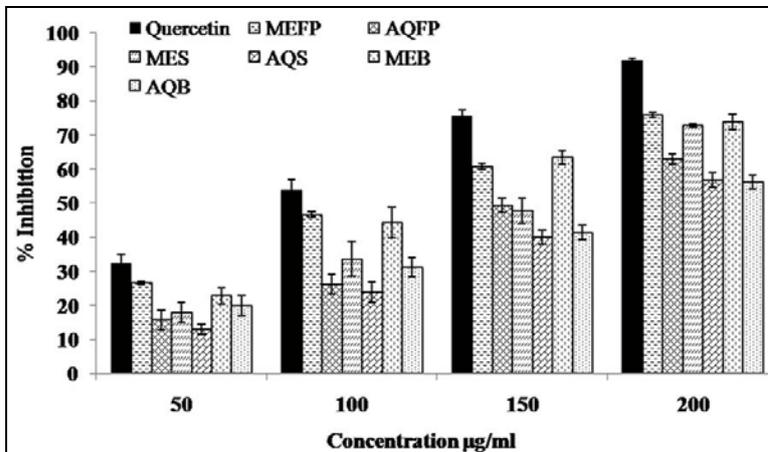


Fig. 3: Inhibitory effects of methanolic and aqueous extracts of *T. bellerica* and standard quercetin on superoxide anion radical at varying concentrations. Values are mean±SD of three replications (n=3). MEFP: methanolic fruit pulp; MES: methanolic seed; MEB: methanolic bark; AQFP: aqueous fruit pulp; AQS: aqueous seed; AQB: aqueous bark

Lipid peroxidation (LPO)

Studies on the inhibition of LPO in the presence of extracts were carried out and expressed as PI. The methanolic and aqueous

extracts prevent LPO induced by FeSO₄ and PI varied from 63.02 to 71.51% and 54.40 to 60.75%, respectively. Maximum LPO inhibition was shown by MEFP (71.51%) than MES (64.70%) and MEB (63.02%) in a concentration-dependent manner at 100-400 µg/ml in

comparison to standard (95.39%). Methanolic extracts showed anti-LPO activity at lower IC_{50} value 115.6 (MEFP), 130.3 (MES) and

144.5 (MEB) $\mu\text{g/ml}$ (fig. 4) in comparison to aqueous extracts 290 (AQFP), 300 (AQS) and 331 (AQB) $\mu\text{g/ml}$.

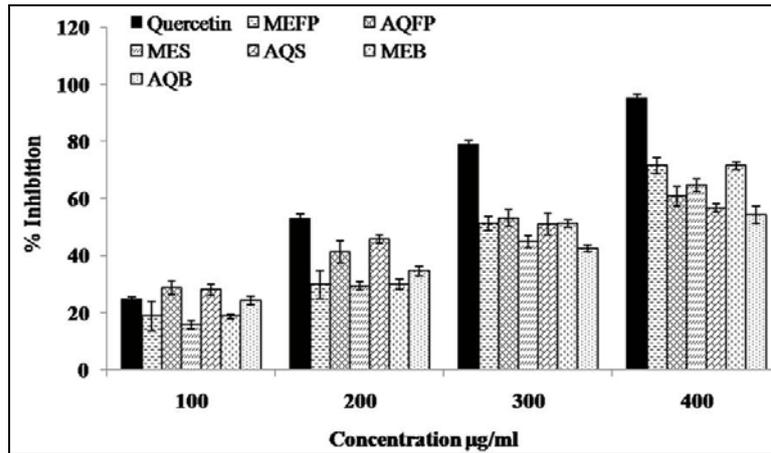


Fig. 4: Inhibitory effects of methanolic and aqueous extracts of *T. bellerica* and standard quercetin on LPO using egg homogenate as a lipid-rich source at varying concentrations. Values are mean \pm SD of three replications (n=3). MEFP: methanolic fruit pulp; MES: methanolic seed; MEB: methanolic bark; AQFP: aqueous fruit pulp; AQS: aqueous seed; AQB: aqueous bark

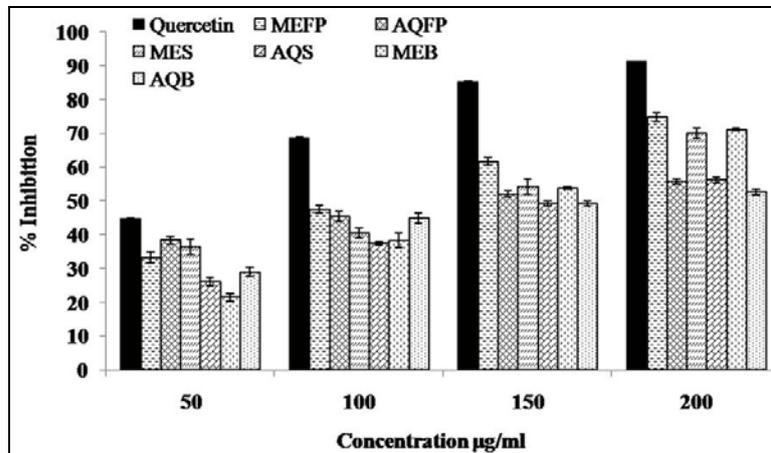


Fig. 5: Inhibitory effects of methanolic and aqueous extracts of *T. bellerica* and standard quercetin on hydroxyl radical-mediated deoxyribose degradation at varying concentrations. Values are mean \pm SD of three replications (n=3). MEFP: methanolic fruit pulp; MES: methanolic seed; MEB: methanolic bark; AQFP: aqueous fruit pulp; AQS: aqueous seed; AQB: aqueous bark

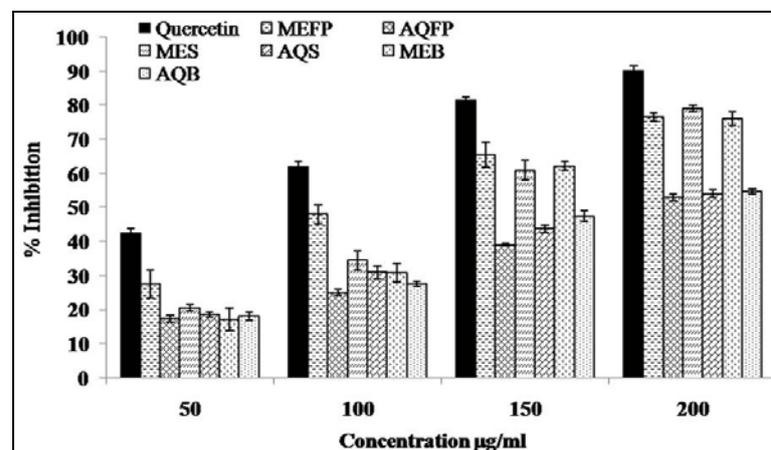


Fig. 6: Inhibitory effects of methanolic and aqueous extract of *T. bellerica* and standard quercetin on ferric ion chelation by ferric thiocyanate assay method at varying concentrations. Values are mean \pm SD of three replications (n=3). MEFP: methanolic fruit pulp; MES: methanolic seed; MEB: methanolic bark; AQFP: aqueous fruit pulp; AQS: aqueous seed; AQB: aqueous bark

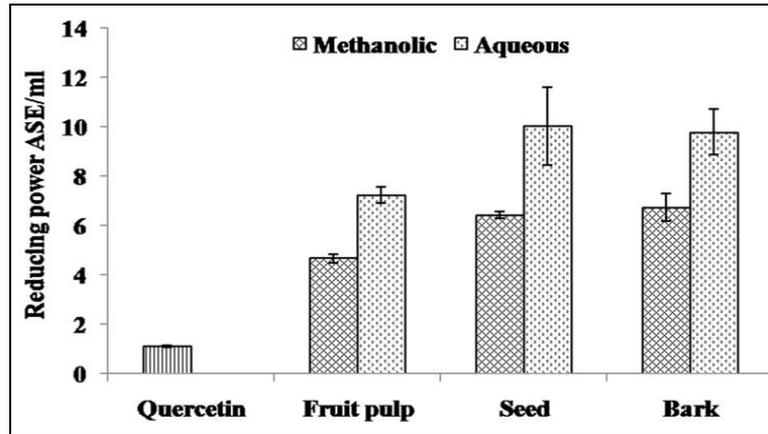


Fig. 7: Reducing power (ASE/ml) of methanolic and aqueous extracts of *T. bellerica* and standard quercetin. Values are mean±SD of three replications (n=3). MEFP: methanolic fruit pulp; MES: methanolic seed; MEB: methanolic bark; AQFP: aqueous fruit pulp; AQS: aqueous seed; AQB: aqueous bark

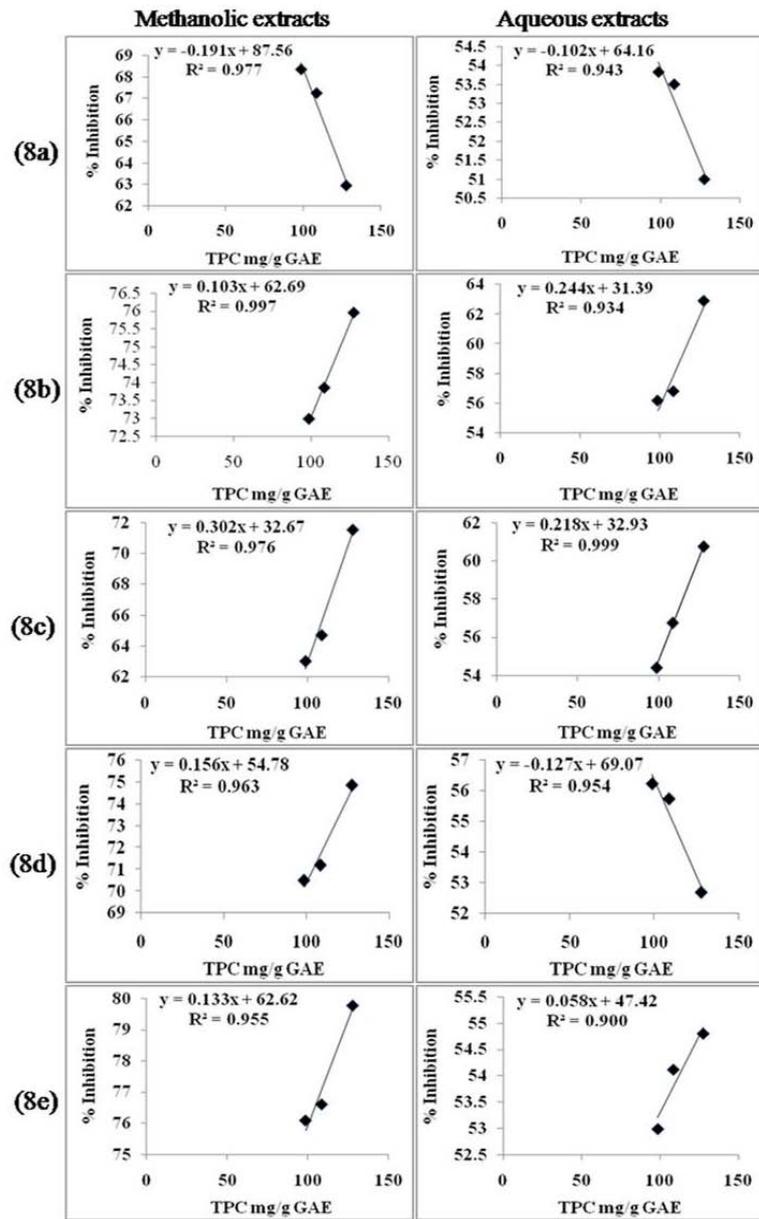


Fig. 8: Linear correlation between TPC (x axis) in the plant extracts in relation to their antioxidant activity (y axis). (8a) TPC versus FRSA (8b) TPC versus SARSA (8c) TPC versus LPO (8d) TPC versus HRSA (8e) TPC versus FTC assay

Hydroxyl radical scavenging activity (HRSA)

Methanolic and aqueous extracts were further studied for their ability to chelate iron and/or to scavenge OH[•] by using deoxyribose degradation assay. The methanolic extracts were found to be most potent OH[•] scavenger with inhibition of 70.51 to 74.86% than aqueous extracts of 52.70% to 56.23% in comparison with quercetin (91.12%) (fig. 5). The biochemical studies revealed that methanolic extracts caused a concentration-dependent (50-200 µg/ml) inhibition of deoxyribose oxidation. The IC₅₀ at which methanolic and aqueous extracts showed HRSA was 73.76 (MEFP), 81.99 (MES), 90.76 (MEB) µg/ml and 425.9 (AQFP), 488.0 (AQS), 548.0 (AQB) µg/ml, respectively.

Ferric thiocyanate (FTC)

In tested extracts, methanolic extracts showed maximum inhibition (76.08% to 79.78%) than aqueous extracts (53.00% to 54.80%) to inhibit production of FRs which initiates oxidation of lipids and proteins. Quercetin showed 92.12% inhibition to inhibit the production of peroxides. The inhibition is increased with increasing concentration of the extracts from 50 to 200 µg/ml in the reaction mixture and the maximum inhibition was shown by MES (79.78%) (fig. 6). The IC₅₀ of methanolic extracts was in the range 184.98 (MEFP) > 212.5 (MES) > 221.4 (MEB).

Reducing power (RP)

The RP of a compound may act as a significant indicator of its potential antioxidant activity [19]. With regards to RP, higher reducing capacity might be attributed to the higher amount of phenolic compounds. Methanolic extracts had significantly high Fe³⁺ to Fe²⁺ transformation capacity (6.73 to 4.68 ASE/ml) than aqueous extracts (10.03 to 7.25 ASE/ml) in comparison to standard quercetin (1.12 ASE/ml) (fig. 7), showing high phenolic content in methanolic extracts.

Correlation between antioxidant activities and TPC

Phenolics are the major contributors to the antioxidant activity. The correlation between TPC and FRSA of methanolic and aqueous extracts had a correlation coefficient of R² = 0.977 (y = -0.191x + 87.56) and R² = 0.943 (y = -0.102x + 64.16). A good correlation also exists between TPC and other antioxidant activities (fig. 8).

DISCUSSION

Phenolic compounds and polyphenols are the most abundant structures in plants. Antioxidant compounds are usually in the phenolic form. The antioxidant properties of phenolic compounds originate from their properties of proton loss, chelate formation and dismutation of radicals. In fact, in some studies, theoretical methods have been proposed to estimate the antioxidant activities of phenolic substances. Their structure-activity relationships are examined for this purpose. Phenols are compounds that have the ability to destroy radicals because they contain hydroxyl groups. These important plant components give up hydrogen atoms from their hydroxyl groups to radicals and form stable phenoxyl radicals; hence, they play an important role in antioxidant activity. Therefore, determination of the quantity of phenolic compounds is very important in order to determine the antioxidant capacity of plant extracts [20, 21]. In fig. 1, the quantity of TPC in methanolic and aqueous extracts of TB is shown. According to Shahriar *et al.* [22] and Venkatesan *et al.* [23] hexane and chloroform extract of *Terminalia arjuna* fruit (73.00 and 61.72 mg/g of GAE) and *Terminalia chebula* bark (28.68 and 62.68 mg/g of GAE) showed lower TPC in comparison to our reported values of methanolic extracts.

The DPPH method is a preferred method to determine FRSA because it is fast, easy and reliable and does not require a special reaction and device. DPPH is a stable, synthetic radical that does not disintegrate in water, methanol or ethanol. The FRSA of extracts depends on the ability of antioxidant compounds to lose hydrogen and the structural conformation of these components [24, 25]. The DPPH[•], which is at its maximum wavelength at 517 nm, can easily receive an electron or hydrogen from antioxidant molecules to become a stable diamagnetic molecule as DPPH-H. [26] Owing to the

DPPH[•] ability to bind H, it is considered to have a radical scavenging property. Discoloration occurs due to the decreasing quantity of DPPH[•] into reaction mixture which reflects the FRSA of the tested extract [27]. In fig. 2, FRSA of different concentrations of methanolic and aqueous extracts of *T. bellerica* are demonstrated. The antioxidant activities of the extracts were compared with quercetin, which is a known natural antioxidant. The radical scavenging effect of the methanolic extract was significant high in comparison to the aqueous extract.

O₂^{•-} are a precursor to active FRs that have the potential of reacting with biological macromolecules and thereby inducing tissue damage [28]. It has been implicated in several pathophysiological processes due to its transformation into more ROS such as OH[•] that initiates LPO. Also, O₂^{•-} has been observed to directly initiate LPO [29]. O₂^{•-} is normally formed first, and its effects can be magnified because it produces other kinds of FRs (H₂O₂ and ¹O₂) and oxidizing agents [30] which induce oxidative damage in lipids, proteins and DNA [31]. O₂^{•-} derived from dissolved oxygen by PMS-NADH coupling reaction and reduces NBT in this system. In this method, O₂^{•-} reduces the yellow dye (NBT²⁺) to produce the blue formazan which is measured spectrophotometrically at 560 nm. Plant extracts that contain antioxidants are able to inhibit the blue NBT formation [32, 33]. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of O₂^{•-} in the reaction mixture. Fig. 3 clearly indicates that TB is a potent scavenger of O₂^{•-}. According to Venkatesan *et al.* [23] methanolic extracts showed O₂^{•-} inhibition at IC₅₀ 166.29 µg/ml which is almost equal to our reported value 155.53 µg/ml.

In LPO assay, antioxidant potential is measured by evaluating the capability of test sample to hamper the oxidation of polyunsaturated fatty acids (PUFA) into TBARS. Peroxidation generates peroxy radicals which decompose to MDA. It forms a stable product with TBA, which serve as a mean to quantify the level of peroxidation [34]. This assay is very useful mean to assess LPO *in vitro* due to its simplicity and reproducibility. According to Sherin *et al.* [35] hexane, chloroform and ethyl acetate extracts of TB leaf showed 50% inhibition at concentration 0.350, 0.280, 0.520 mg/ml which was much higher than our reported values of methanolic extracts.

The OH[•] can induce oxidative damage to DNA, lipids and proteins and are involved in various neurodegenerative and cardiovascular diseases [36]. The HRSA of the extracts was determined by its ability to compete with deoxyribose for OH[•]. In this assay, 2-deoxy-2-ribose was oxidized when exposed to OH[•] generated by the fenton-type reaction. The oxidative degradation can be detected by heating the products with TBA under acidic conditions to develop a pink chromogen with a maximum absorbance at 532 nm [37].

Inhibition of linoleic acid oxidation was also used to assess the antioxidant activity of the tested extracts. Antioxidant activity of different extracts was determined by inhibition of peroxidation in linoleic acid system using thiocyanate method [38]. Linoleic acid is a PUFA, upon oxidation peroxides are formed which oxidize Fe²⁺ to Fe³⁺, the later forms complex with thiocyanate ion (SCN⁻), concentration of which is determined spectrophotometrically by measuring absorbance at 535 nm. Higher the absorbance higher will be the concentration of peroxides formed during the reaction, consequently lower will the antioxidant activity. According to Sultana *et al.* [39] methanolic extracts of *T. arjuna* showed 44.4% inhibition at 200 µg/ml concentration which was much lower than our tested extracts.

The RP of a compound acts as an indicator of its potential antioxidant activity [40]. In the RP assay, the presence of antioxidants in the samples would result in the reduction of Fe³⁺ to Fe²⁺ by donating an electron. The amount of Fe³⁺ reduction can be then monitored by measuring the formation of [Fe³⁺]₄[Fe²⁺(CN)₆]₃ complex (pearl's Prussian blue) at 700 nm indicates an increase in reductive ability [41]. Fe³⁺ reduction is often used as a significant indicator of electron-donating activity which is an important mechanism of phenolic antioxidant action [42]. The methanolic extracts showed highest activity than aqueous extracts in a dose-

dependant manner, which is due to the presence of these phenolic compounds.

It was observed that the FRSA of the methanolic and aqueous extracts was positively correlated with their total amount of phenolic compounds ($R^2 = 0.977$ and $R^2 = 0.943$). Many studies in the literature present positive correlations between the quantity of phenolic compounds and the DPPH[•] scavenging effect [43, 44]. In our study as well, a positive correlation was observed between the antioxidant activities and the composition of total phenolics.

CONCLUSION

The results of the present study revealed that TB possessed potent free radical scavenging ability in methanolic and aqueous extracts. In all the antioxidant assays performed, the methanolic extract was found to be a more potent radical scavenger compared to that of aqueous extracts. The activity observed may be attributed to the presence of more phenolic contents in the methanolic extract and further we conclude that methanolic extracts of TB are potential candidates for natural antioxidants in food and pharmaceutical industries.

ACKNOWLEDGMENT

Rasna Gupta is grateful to Head, Department of Biochemistry, RML Avadh University for providing laboratory facilities.

AUTHORS CONTRIBUTIONS

All the author have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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