ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Technol. Aliment. 14(2) 2015, 97–105
www.food.actapol.net pISSN 1644-0730 eISSN 1889-9594 DOI: 10.17306/J.AFS.2015.2.11

ANTIOXIDANT ACTIVITIES OF LEAF GALLS EXTRACTS OF *TERMINALIA CHEBULA* (GAERTN.) RETZ. (COMBRETACEAE)

Ravi Shankara Birur Eshwarappa[1,2,3]✉, Y. L. Ramachandra[3], Sundara Rajan Subaramaihha[2], Sujan Ganapathy Pasura Subbaiah[2], Richard Surendranath Austin[4], Bhadrapura Lakkappa Dhananjaya[5]✉

[1]Department of Chemistry, School of Graduate Studies, Jain University
Bangalore – 560002, **India**
[2]Research Unit in Vrukshayurveda, A Division of Centre for Advanced Studies in Biosciences, Jain University
Bangalore – 560019, Chamrajpete, **India**
[3]PG Department of Studies and Research in Biotechnology, Kuvempu University
Shankarghatta – 577451, Karnataka, **India**
[4]Department of Biochemistry, University of Mysore
Mysore – 570006, Karnataka, **India**
[5]Toxinology/Toxicology and Drug Discovery Unit, Centre for Emerging Technologies (CET), Jain University
Ramanagara – 562112, Kanakpura Taluk, **India**

ABSTRACT

Background. Free radicals are implicated in several metabolic diseases and the antioxidant therapy has gained an utmost importance in the treatment. The medicinal properties of plants have been investigated and explored for their potent antioxidant activities to counteract metabolic disorders. In this study, the chemical composition and free radical scavenging potential of leaf gall extracts (ethanol, petroleum ether, chloroform and aqueous) of *Terminelia chebula* is evaluated, which is extensively used in the preparation of traditional medications to treat various metabolic diseases.

Material and methods. The presences of phenolics, flavonoids, triterpens, saponins, glycosides, phytosterols, reducing sugars were identified in the extracts according to standard procedures. The free radical scavenging activities of the extract were also analysed by standard procedures.

Results. The methanol extract had the highest total phenolic and flavonoid content. The antioxidant activities of leaf gall extracts were examined using diphenylpicrylhydrazyl (DPPH), Super oxide radical scavenging, Hydroxyl scavenging and ferric reducing power (FRAP) methods. In all the methods, the ethanolic extract showed higher free radical scavenging potential than all the other extracts.

Conclusion. As the higher content of both total phenolics and flavonoids were found in the ethanolic extract, so the significantly high antioxidant activity can be positively correlated to the high content of total polyphenols/flavonoids of the ethanol extract. The results of this study confirm the folklore use of *T. chebula* leaves gall extracts as a natural antioxidant and justify its ethnobotanical use. Further, the results of antioxidant properties encourage the use of *T. chebula* leave gall extracts for medicinal health, functional food and nutraceutical applications.

Key words: *Terminelia chebula*, plants, galls, antioxidant, gallic acid, metabolic diseases, drug

✉beravish@gmail.com, ✉chandu_greeshma@rediffmail.com, phone +91 80 2757 7200, fax +91 80 2757 7199

INTRODUCTION

Reactive oxygen species (ROS) are highly active molecules generated in cells under normal metabolic activities. ROS in excess production causes oxidative damage to proteins, lipids, enzymes, and DNA molecules (Halliwell, 1997). Living cells possess powerful scavenging mechanisms to avoid excess ROS--induced cellular injury, but with ageing and under influence of external stresses, these mechanisms become inefficient leading to metabolic distress. Therefore, free radicals are implicated in several metabolic disorders that include heart diseases, acquired immunodeficiency syndrome, diabetes mellitus, arthritis, cancer, ageing, liver disorder etc., In the treatment of these metabolic diseases, the antioxidant therapy has gained an utmost importance. Plants and plant based herbal preparations have been used to treat ailments since prehistoric times, and the treatment of various diseases with plant-based medicines has remained an integral part of many cultures across the globe. The World Health Organization estimates that 4 billion people (i.e. 80% of the world's population) use herbal medicines in some aspects of primary healthcare and there is a growing tendency to "Go Natural" (Gossell-Williams et al., 2006). In these aspects, all round the world, the medicinal properties of plants have been investigated and explored for their potent antioxidant activities to counteract metabolic disorders, that have no side effects but high economic viability (Auudy et al., 2003; Shrestha et al., 2013; Shrestha et al., 2014).

T. chebula (Gaertn.) Retz. (Combretaceae) commonly known as black myrobalan and haritaki, is an important medicinal plant native to tropical regions of southern Asia viz., India, Nepal, China, Sri Lanka, Malaysia, Vietnam. It is amply referred to as 'King of medicines' as it has been the component of many formulation for treatment of various diseases in all the streams of Indian system of medicines like Ayurveda, Siddha, Unani and Homeopathy (The Wealth..., 1969; The Ayurvedic..., 1978). It consists of gall-like excrescences formed by insects on the leaves, petioles and branches of the plant insect *Dixothrips onerosus* (Thysanoptera). The galls are vasiform, lobed, greenish yellow, fleshy, truncated gall, 25 to 33 mm long, smooth when immature, longitudinally striated or ridged when old (Santha et al., 1991). These galls are commonly known as Karkatshringi and is an important ayurvedic drug used in preparations like the Dasamularista, Cyavanaprasa and Shringyadi curna which are used in the treatment of diseases like swasa (asthma), yakshma (tuberculosis), ajeerna (indigestion), hydroga (heart diseases), jwara (fevers) and yakrt roga (liver disorders) to mention a few (The Wealth..., 1969; The Ayurvedic..., 1978). Karkatshringi also finds usage in the treatment of children's ear infections, suppress haemorrhage from gums and also used to suppress bleeding from nose (Sukh, 1997; Shrestha et al., 2014). Hakims consider galls useful in pulmonary infections, diarrhoea and vomiting (Nadkarni, 1976). The accepted source of Karkatasringi is the galls of *Rhus Succedanea* L., but *P. integerrima* and *T. chebula* are also generally used in preparations (The Ayurvedic..., 1978; The Siddha..., 1978). Gall extracts of *T. chebula* have been found to posses anti-inflammatory activity, antibacterial, anti-tyrosinase activity, anti-cancer activity, and anti-ageing activity (Vonshak et al., 2003; Manosroi et al., 2010; 2011; 2013; Upadhye and Rajopadhye, 2010; Shankara et al., 2012). The Galls are used in some of the ayurvedic formulations like 'Chvyanprash avaleha', 'KumariAsava', 'KumariKalp' etc. and prescribed for weakness as rejuvenating agent and tonic (Gunakari Ayurvediy Aushadhe). The use of leaf galls as a rejuvenator may be attributed to antioxidant property. As the ethanomedical uses of galls of *T. chebula* suggest it might possess antioxidant activities. Therefore, in the present study, we evaluate the photochemical constituents and the antioxidant potential of leaf galls of *T. chebula* to exemplify their potential development as drug used against metabolic diseases.

MATERIAL AND METHODS

Materials

Folin-Ciocalteu reagent and quercetin were obtained from Qualigens, Mumbai, India. Ascorbic acid, gallic acid, quercetin, L-ascorbic acid, potassium thiocyanate, ethylene diamine tetra acetic acid (EDTA), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-deoxyribose, thiobarbituric acid (TBA), sodium nitroprusside, N-(1-Naphthyl) ethylenediamine dihydrochloride, potassium hexacyanoferrate ($K_3Fe(CN)_6$), trichloroacetic acid (TCA), ferric chloride were procured from SRL

Chemicals, India. All the other reagents and solvents were of analytical grade.

Plant material

The gall induced leaves of *T. chebula* were purchased from local market of Bangalore, India. The plant materials were authenticated by Dr. S. Sundara Rajan and the voucher specimen (JU-RUV-52) were deposited at Research centre of vrkshayurveda, Jain University, Bangalore. The galls were cleaned with distilled water, dried to dryness and crushed into fine powder by using electric grinder.

Preparation of extract

The coarsely powdered gall materials were extracted with ethanol, petroleum ether, chloroform and aqueous solvents seperaly in a soxhlet apparatus for 24 h. The extracts were evaporated to dryness under reduced pressure using a Rotavapor (BuchiFlawil, Switzerland) and a portion of the residue was used for the phytochemical and antioxidant assays.

Phytochemical analysis

Qualitative analysis

The preliminary qualitative phytochemical analyses of carbohydrates, saponins, alkaloids, flavonoids, tixed oils and fats, phenolic and tannins, glycosides, phytosterols and triterpenoids in the extracts were carried out using the standard methods as described by Harborne (1984), Trease and Evans (1989), Kokate et al. (1998), Khandelwal (2005), Shrestha et al. (2013).

Quantitative analysis

Determination of total phenolic content. The total phenolics were determined in the *T. chebula* leaf gall extracts (ethanol, petroleum ether, chloroform and aqueous) using Folin-Ciocalteau reagent method (Kaur and Kapoor, 2002), employing Gallic acid as standard. Briefly, 200 ml of all the extracts (2 mg/ml) were made up to 3 ml with distilled water, then mixed thoroughly with 0.5 ml of Folin-Ciocalteau reagent. After mixing for 3 min, 2 ml of 20% (w/v) sodium carbonate was added and allowed to stand for a further 60 min in the dark. The absorbance of the reaction mixtures was measured at 650 nm, and the results were expressed as mg of gallic acid equivalent (GAE)/g of dry weight.

Determination of total flavonoid content. Total flavonoid content of the extracts (ethanol, petroleum ether, chloroform and aqueous) was determined using the aluminium chloride colorimetric method as described by Chang et al. (2002). In brief, 50 µl of methanol and aqueous extracts (2 mg/ml) were made up to 1 ml with methanol then mixed with 4 ml of distilled water and subsequently with 0.3 ml of 5% $NaNO_2$ solution. After 5 min of incubation, 0.3 ml of 10% $AlCl_3$ solution was added and then allowed to stand for 6 min, followed by adding 2 ml of 1 M NaOH solution to the mixture. Then water was added to the mixture to bring the final volume to 10 ml and the mixture was allowed to stand for 15 min. The absorbance was measured at 510 nm. Total flavonoid content was calculated as quercetin from a calibration curve. The calibration curve was worked out by preparing quercetin solutions at concentrations 12.5 to 100 $mg \cdot ml^{-1}$ in methanol. The result was expressed as mg quercetin equivalent (QUE)/g of dry weight.

Evaluation of antioxidant potential of *T. chebula* leaf gall extract

Antioxidant and free radical scavenging potential of *T. chebula* leaf gall extracts (ethanol, petroleum ether, chloroform and aqueous) is evaluated by using DPPH, FRAP, Super Oxide and hydroxyl radical assays.

DPPH free radical scavenging activity (DPPH). Quantitative measurement of radical scavenging properties of *T. chebula* leaf gall extracts (ethanol, petroleum ether, chloroform and aqueous) was carried out according to the method of Bloi (1958). Briefly, a 0.1 mM solution of 2,2-diphenyl-1-picryl-hydrazyl (DPPH*) in methanol was prepared and 1 ml of this solution was added to 3 ml of the both methanol and aqueous extracts at different concentration (1–250 µg/ml). Ascorbic acid was used as a positive control. After incubation for 30 min in the dark, the discoloration was measured at 517 nm. Measurements were taken in triplicate. The capacity to scavenge the DPPH* radical was calculated and expressed as percentage inhibition using the following equation:

$$I\% = (\text{absorbance of control} - \text{absorbance of test} / \text{absorbance of control}) \times 100$$

The IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation prepared from the different concentrations of all the extracts.

Ferric reducing/antioxidant power activity (FRAP). Ferric reducing/antioxidant power (FRAP) was determined following the method reported by Zhao et al. (2008). *T. chebula* leaf gall extracts (ethanol, petroleum ether, chloroform and aqueous) at various concentrations (1–250 µg/ml) was mixed with 2.5 ml of 200 mM phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. Mixtures were incubated at 50°C for 20 min, 2.5 ml of 10% trichloroacetic acid was added and the tubes were centrifuged at 10,000 rpm for 10 min. Five milliliters of the upper layer of the solution was mixed with 5.0 ml distilled water and 1 ml of 0.1% ferric chloride. The absorbance of the reaction mixtures was measured at 700 nm. Ascorbic acid was taken as standard and the final results were expressed as mg ascorbic acid equivalent/g of dry weight.

Hydroxyl Radical Scavenging activity. Quantitative measurement of hydroxyl radical scavenging properties of *T. chebula* leaf gall extracts (ethanol, petroleum ether, chloroform and aqueous) was carried out by measuring the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+}/Ascorbate/EDTA/H_2O_2 system (Halliwell et al., 1988). *T. chebula* leaf gall extracts (methanol and aqueous) at various concentrations (1–500 µg/ml) was mixed with the reaction mixture containing 0.1 ml of 3.0 mM deoxyribose, 0.5 ml of $FeCl_3$ (0.1 mM), 0.5 ml of EDTA (0.1 mM), 0.5 ml of ascorbic acid (0.1 mM), 0.5 ml of H_2O_2 (1 mM) and 0.8 ml of phosphate buffer (20 mM, pH 7.4) and made up to final volume of 3.0 ml. The reaction mixture was incubated at 37°C for 1 h. A 1 ml portion of the incubated mixture was mixed with 1 ml of 10% trichloroacetic acid and 1.0 ml of 0.5% thiobarbituric acid to develop pink chromogen that is measured at 532 nm. The hydroxyl radical scavenging capacity was calculated and expressed as % inhibition of deoxyribose degradation using following equation:

$$I\% = (\text{absorbance of control} - \text{absorbance of test} / \text{absorbance of control}) \times 100$$

The IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation prepared from the different concentrations of both the methanol and aqueous extracts.

Superoxide anion scavenging activity. Superoxide anion scavenging capacity of *T. chebula* leaf gall extracts (ethanol, petroleum ether, chloroform and aqueous) was assessed by the method of Nishikimi et al. (1972). About 1 mL of reaction mixture contained 156 µM nitroblue tetrazolium in 100 mM phosphate buffer (pH 7.4), 468 µM NADH in 100 mM phosphate buffer (pH 7.4) with 0.1 mL of extract (200–1000 µg/ml) were mixed. The reaction started by adding 100 µL of 60 µM phenazine methosulphate solution in 100 mM phosphate buffer (pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 min, correspondingly blank contained all the reagents except gall extract of *T. chebula* and the absorbance of oxidized product formazan was recorded at 560 nm. Decreased absorbance of the reaction mixture indicated increased superoxide anion scavenging activity. Superoxide anion radical is a reactive molecule and causes severe cytotoxicity in living cells, thus removing nitric oxide is of prime importance in antioxidant therapy. The superoxide anion derived from dissolved oxygen by phenazine methosulphate/NADH coupling reaction reduces nitro blue tetrazolium. The decrease in the absorbance at 560 nm with the gall extract thus indicates the consumption of superoxide anion in the reaction mixture. The capacity to scavenge the superoxide anion was calculated and expressed as percent inhibition using the following equation:

$$I\% = (\text{absorbance of control} - \text{absorbance of test} / \text{absorbance of control}) \times 100$$

The IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation prepared from the different concentrations of both the methanol and aqueous extracts.

Statistical analysis

The experiments were carried out in triplicate and results are given as the mean ±standard deviation. The data in all the experiments were analysed (Microsoft

Excel 2007) for statistical significance using Students t-test and differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

As antioxidant therapy is gaining importance in the treatment of several metabolic diseases (diabetes mellitus, arthritis, cancer, ageing, liver disorder etc.,), where free radicals are implicated. All over the world, scientific developmental programs have aimed at investigating the medicinal properties of plants for their potent antioxidant properties (Auudy et al., 2003; Shrestha et al., 2014). In these lines, the antioxidant potential of extracts (ethanol, petroleum ether, chloroform and aqueous) of leaf galls of *T. chebula* is evaluated and its phytochemical constituents determined. Firstly, in the phytochemical screening, the qualitative presence of phenolics, flavonoids, triterpens, saponins, glycosides, phytosterols, reducing sugars were identified in the extracts (Table 1). The antioxidant activities of plant/herb extracts are often explained by their total phenolic and flavonoid contents. The total amount of phenolic and flavonoid content of extracts of leaf galls of *T. chebula* is presented in Table 2. The results obtained indicate that in comparison with all the exact, the ethanol extract had the highest total phenolic and flavonoid of 538 ±1.4 mg of GAE/g d.w. and 478 ±2.2 mg of QUE/g d.w., respectively. These results show that the ethanol extract possessed significant activity in releasing most of the secondary metabolites from leave galls of *T. chebula.* This may be due to the fact that phenolic and flavonoid compounds are often extracted in higher amounts by using polar solvents such as aqueous methanol/ethanol (Sultana et al., 2007). Differences in the polarity of the extracting solvents could result in a wide variation in the polyphenolic and flavonoid contents of the extract (Choi et al., 2007; Shrestha et al., 2013). Phenolic antioxidants are products of secondary metabolism in plants, and their antioxidant activity is mainly due to their redox properties and chemical structure, which can play an important role in chelating transitional metals and scavenging free radicals (Mohamed et al., 2010). Similarly, the mechanisms of action of flavonoids are also through scavenging or chelating processes (Kessler et al., 2003). In addition, compounds such as flavonoids, which contain hydroxyl functional groups, are responsible for the antioxidant effects of plants (Das and Pereira, 1990).

Table 1. Preliminary phytochemical analysis of leaf gall extracts of *T. chebula*

Chemical constituents	Petroleum ether	Chloroform	Aqueous	Ethanol
Flavonoids	–	–	+	+
Steroids	+	+	–	+
Triterpenes	+	+	+	+
Tannins	–	–	+	+
Saponins	–	–	+	+
Alkaloids	–	+	–	+
Glycosides	–	–	–	+
Carbohydrates	–	–	–	–

+ indicates present, – indicates absent.

Table 2. Total phenolic and total flavonoid content in different extracts of *T. chebula* leaf gall

Gall extracts	Total phenolic mg of GAE/g d.w.	Total flavonoids mg of QUE/g d.w.
Petroleum ether	107 ±1.4	96 ±2.2
Chloroform	208 ±3.2	162 ±2.6
Aqueous extract	410 ±1.5	331 ±1.2
Ethanolic extract	538 ±1.4	478 ±2.2

The higher amount of total phenolic and flavonoid content of leaf galls extracts of *T. chebula* suggested that it possesses high antioxidant activity. DPPH is a stable free radical, which has been widely accepted as a tool for estimating free radical-scavenging activities of antioxidants (Naik et al., 2003). The percentage inhibition of DPPH in the presence of *T. chebula* gall extracts are shown in Figure 1. The ethanol extract showed highest scavenging activity of 97.24% at 250 µg/mL concentration, whereas aqueous, chloroform and petroleum ether extracts showed 80.67%, 56.32% and 28.32% scavenging activity at the same concentration respectively. The EC_{50} values

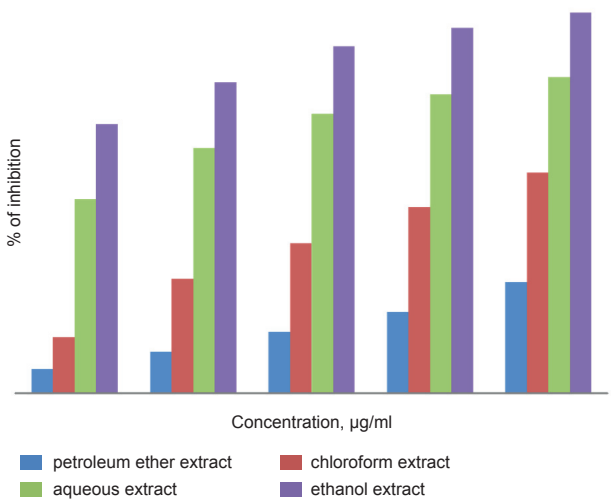

Fig. 1. Free radical scavenging activity of leaf gall extracts of *T. chebula*. Activity was measured by the scavenging of DPPH radicals and expressed as % inhibition. Each value is expressed as the mean ±standard deviation

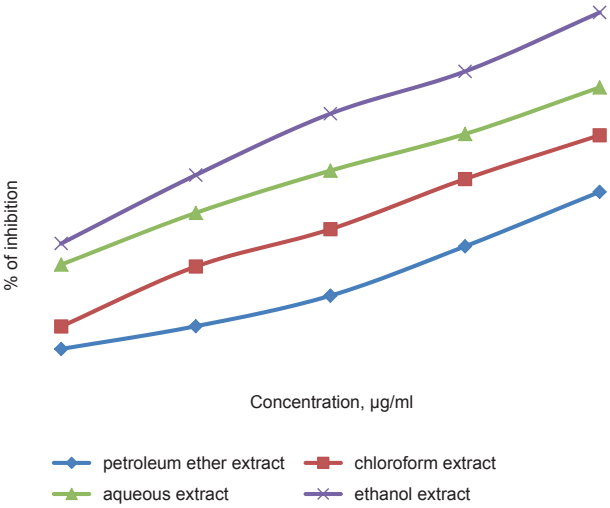

Fig. 2. Super oxide anion scavenging activity of leaf gall extracts of *T. chebula*. Activity was measured and expressed as % inhibition. Each value is expressed as the mean ±standard deviation

of ethanolic extract were found to be 96 ±02 µg/ml (Table 3). Therefore, in the present study the methanol extract exhibited higher DPPH scavenging activity, when compared to all the other extracts. The observed noticeable effect on scavenging free radicals of ethanol extract can be related to the high phenolic constituents present (Table 2). Phenolic antioxidants are products of secondary metabolism in plants, and the antioxidant activity is mainly due to their redox properties and chemical structure, which can play an important role in chelating transitional metals, inhibiting lipoxygenase and scavenging free radicals (Decker, 1997). Phenolic compounds are also effective hydrogen donors, which make them good antioxidants (Rice-Evans et al., 1995). The percentage inhibition of superoxide radical scavenging potential extracts of *T. chebula* is as shown in Figure 2. The ethanolic extract of *T. chebula* is found to be effective in scavenging super oxide radicals than all the other extracts. The scavenging activity in terms of IC_{50} value of ethanolic extract of *T. chebula* gall are calculated as 370 ±02 µg/ml (Table 3). Therefore, in the present study the ethanol extract exhibited higher superoxide scavenging activity, when compared to the aqueous leaf gall extract of *T. chebula*. These observed noticeable effects on scavenging free radicals of the ethanol extract can be related to the high phenolic constituents present (Table 2).

The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins. The hydroxyl radical scavenging activity of leaf galls of *T. chebula* is as shown in Figure 3. The percentage inhibition of hydroxyl radical scavenging potential extracts of *T. chebula* is as shown in Figure 3.

Table 3. IC_{50} values of *T. chebula* gall extracts with standard ascorbic acid

T. chebula gall extract	IC_{50} values, µg/ml		
	DPPH assay	superoxide radical scavenging assay	hydroxyl radical scavenging assay
Petroleum ether	274 ±02	810 ±03	543 ±02
Chloroform	201 ±03	760 ±08	400 ±02
Aqueous extract	143 ±06	500 ±04	270 ±02
Ethanolic extract	96 ±02	370 ±02	190 ±04
Ascorbic acid	12.93 ±03	40.17 ±06	24.28 ±06

Each value is expressed as mean ±SD.

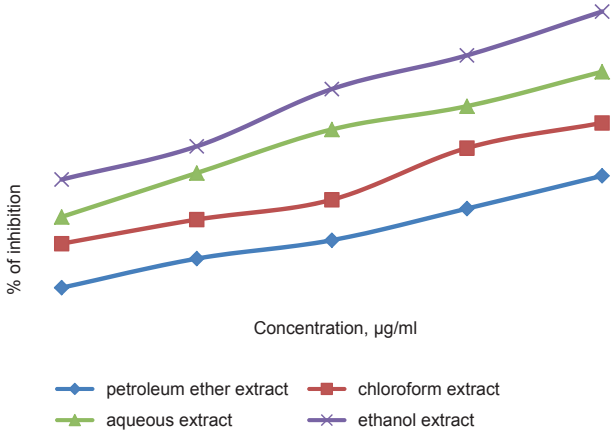

Fig. 3. Hydroxyl radical scavenging activity of leaf gall extracts of *T. chebula*. Activity was measured and expressed as % inhibition. Each value is expressed as the mean ± standard deviation

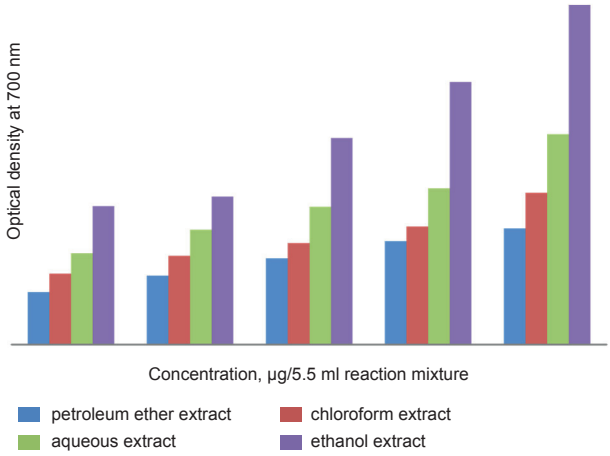

Fig. 4. Ferric reducing power of leaf gall extracts of *T. chebula*. Activity is expressed at absorbance at 700 nm. Each value is expressed as the mean ±standard deviation

The ethanolic extract of *T. chebula* is found to be effective in scavenging hydroxyl radicals than all the other extracts. The scavenging activity in terms of IC_{50} value of ethanolic extract of *T. chebula* gall is of 190 ±04 µg/ml (Table 3), indicating that the methanol extract processes potent hydroxyl radical scavenging activities which can be related to the high phenolic constituents present (Table 2).

The reducing capacity of compounds or extracts may serve as a significant indicator of its potential antioxidant activity. The presence of a reductant, such as the antioxidant substances in plant extracts, causes the reduction of Fe^{3+} ferricyanide complex to the ferrous form, Fe^{2+}. The reduction capabilities of *T. chebula* leaf gall extracts is as indicated in Figure 4. In comparison with all the extracts, the ethanol extract had better reducing power at a concentration of 250 µg/ml (Fig. 4). The ferric reducing power of ethanolic extract of *T. chebula* leaf galls may be attributed to the high phenolic and flavonoid contents of the extracts (Table 2). The ability to reduce Fe (III) may be attributed to the hydrogen donation from phenolic compound (Shimada et al., 1992), which is related to the presence of a reducing agent (Duh, 1998). In addition, the number and position of hydroxyl group of phenolic compounds also govern their antioxidant activity (Rice-Evans et al., 1995).

As exemplified earlier, the antioxidant activities of plant/herb extracts are often explained by their total phenolic and flavonoid contents. In this study, it was observed that there was a strong correlation of antioxidant activities with that of total phenolic and flavonoid content in the leaf gall extracts of *T. chebula*. The leaf galls of *T. chebula* are reported to be very rich in tannins, triterpenoids, flavonoids, essential oils, and others phenolic constituents (Mansori et al., 2013; Santha et al., 1991). The results given in this investigation showed that the phenolic and flavonoid content was higher in polar extracts (ethanol) and subsequently its higher antioxidant potential. Therefore, it seems clear that the presence of polar phenolics is fundamental in the evaluation of free radical-scavenging activity (Al-Reza et al., 2009). The activity of antioxidant has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Yildirim et al., 2000). These observations confirm the folklore use of *T. chebula* leaves gall extracts as a natural antioxidant and justify the ethnobotanical, approach in the search for novel bioactive compounds.

CONCLUSION

The findings of this study support the view that the extracts obtained using a high polarity solvent (methanol) were considerably more effective radical scavengers. Further, it was observed that there was a strong correlation of higher antioxidant activities with that of high total phenolic and flavonoid content in the methanolic leaf gall extracts of *T. chebula*. The results of this study confirm the folklore use of *T. chebula* leaves gall extracts as a natural antioxidant and justify the ethnobotanical approach in the search for novel bioactive compounds. Further, the results support the use of gall extracts as promising sources of potential antioxidants that may be effective as preventive agents in the pathogenesis of some metabolic diseases. Therefore, the results encourage the use of *T. chebula* leave gall extracts for medicinal health, functional food and nutraceuticals applications, due to their antioxidant properties. Future work will be interesting to know the chemical composition and better understand the mechanism of action of the antioxidants present in the extract for development as drug for therapeutic application.

ACKNOWLEDGEMENTS

We acknowledge Dr. R. Chenraj Jain, President, Jain University Trust., Dr. N. Sundararajan, Vice Chancellor, Jain University and Prof. K. S. Shantamani, Chief Mentor, Jain University, Bangalore for their kind support and encouragement and for providing facilities for carrying out this work. DBL thank Jain University for the constant support and encouragement for carrying out research aspects.

REFERENCES

Al-Reza, S. M., Rahman, A., Kang, S. C. (2009). Chemical composition and inhibitory effect of essential oil and organic extracts of *Cestrum nocturnum* L. on food-borne pathogens. Int. J. Food Sci. Technol., 44, 1176–1182.

Auudy, B., Ferreira, F., Blasina, L., Lafon, F., Arredondo, F., Dajas, R., Tripathi, P. C. (2003). Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. J. Ethnopharm., 84, 131–138.

Blois, M. S. (1958). Antioxidants determination by the use of a stable free radical. Nature, 181, 1199–1200.

Chang, C., Yang, M., Wen, H., Chern, J. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 10(3), 178–182.

Choi, Y., Jeong, H. S., Lee, J. (2007). Antioxidant activity of methanolic extracts from some grains consumed in Korea. Food Chem., 103, 130–138.

Das, N. P., Pereira, T. A. (1990). Effects of flavanoids on thermal autooxidation of palm oil: structure activity relationship. J. Am. Oil Chem. Soc., 67, 255–258.

Decker, E. A. (1997). Phenolics: prooxidants or antioxidants? Nutr. Rev., 55, 396–407.

Duh, P. D. (1998). Antioxidant activity of Budrock (*Arctium laooa* Linn.) its scavenging effect on free radical and active oxygen. J. Am. Oil Chem. Soc., 75, 455–461.

Gossell-Williams, M., Simon, O. R., Westm, M. E. (2006). The past and present use of plants for medicines. West. Indian Med. J., 55, 217–218.

Gunakari Ayurvediy Aushadhe. Ayurveda Rasashala, Pune.

Halliwell, B. (1997). Antioxidants and human disease: a general introduction. Nut. Rev., 55, 44–52.

Halliwell, B., Grootveld, M., Gutteridge, J. M. C. (1988). Methods for the measurement of hydroxyl radicals in biochemical systems: deoxyribose degradation and aromatic hydroxylation. Meth. Biochem. Anal. 33, 59–90.

Harborne, J. J. (1984). Phytochemical methods: A guide to modern techniques of plant analysis. New York: Chapman and Hall.

Kaur, C., Kapoor, H. C. (2002). Anti-oxidant activity and total phenolic content of some Asian vegetables. Int. J. Food Sci. Technol., 37, 153–161.

Kessler, M., Ubeaud, G., Jung, L. (2003). Anti- and pro-oxidant activity of rutin and quercetin derivatives. J. Pharm. Pharmacol., 55, 131–142.

Khandelwal, K. R. (2005). Practical pharmacognosy: Techniques and experiments (pp. 149–156). Pune: Nirali Prakashan.

Kokate, C. K., Purohit, A. P., Gokhale, S. B. (1998). Pharmacognosy (pp. 106–114). Pune: Nirali Prakashan.

Manosroi, A., Jantrawut, P., Akazawa, H., Akihisa, T., Manosroi, J. (2010). Biological activities of phenolic compounds isolated from galls of *Terminalia chebula* Retz. (Combretaceae). Nat. Prod. Res., 24(20), 1915–1926.

Manosroi, A., Jantrawut, P., Akihisa, T., Manosroi, W., Manosroi, J. (2011). In vitro and in vivo skin anti-aging evaluation of gel containing noisomes loaded with a semi-purified fraction containing gallic acid from *Terminalia chebula* galls. Pharm. Biol., 49(11), 1190–1203.

Manosroi, A., Jantrawut, P., Ogihara, E., Yamamoto, A., Fukatsu, M., Yasukawa, K., Tokuda, H., Suzuki, N., Manosroi, J., Akihisa, T. (2013). Biological activities of phenolic compounds and triterpenoids from the galls of *Terminalia chebula.* Chem. Biodiv., 10(8), 1448–1463.

Mohamed, A. A., Khalil, A. A., El-Beltagi, H. E. S. (2010). Antioxidant and antimicrobial properties of kaff maryam (*Anastatica hierochuntica*) and doum palm (*Hyphaene thebaica*). Grasas Aceites, 61(1), 67–75.

Nadkarni, K. M. (1976). Indian materia medica (pp. 1062––1063). India: Popular Prakashan.

Naik, G. H., Priyadarsini, K. I., Satav, J. G., Banavalikar, M. M., Sohoni, D. P., ..., Biyani, M. K. (2003). Comparative antioxidant activity of individual herbal components used in ayurvedic medicine. Phytochem., 63, 97–104.

Nishikimi, M., Appaji, N., Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun., 46(2), 849–854.

Rice-Evans, C. A., Miller, N. J., Bolwell, P. G., Bramley, P. M., Pridham, J. B. (1995). The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Rad. Res., 23, 375–383.

Santha, T. R., Shetty, J. K., Yoganarasimhan, S. N., Sudha, R. (1991). Farmacognostical staudies on the South Indian market sample of karkatasringi (kudukkaipoo) – terminalia chebul (Gaertn. Leaf gall). Anc. Sci. Life, 11(1–2), 16–22.

Shankara, B. E. R., Ramachandra, Y. L., Sundara Rajan, S., Preetham, J., Sujan Ganapathy, P. S. (2012). *In vitro* antibacterial activity of *Terminalia chebula* leaf gall extracts against some human pathogenic strains. Intl. Curr. Pharm. J., 1(8), 217–220.

Shimada, K., Fujikawa, K., Yahara, K., Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soyabean oil in cyclodextrin emulsion. J. Agric. Food Chem., 40, 945–948.

Shrestha, S., Kaushik, V. S., Eshwarappa, R. S., Subaramaihha, S. R., Ramanna, L. M., Lakkappa, D. B. (2014). Pharmacognostic studies of insect gall of *Quercus infectoria Olivier* (Fagaceae). Asian Pac. J. Trop. Biomed., 4(1), 35–39.

Shrestha, S., Subaramaihha, S. R., Subbaiah, S. G., Eshwarappa, R. S., Lakkappa, D. B. (2013). Evaluating the antimicrobial activity of methanolic extract of *rhus succedanea* leaf gall. Bioimpacts, 3(4), 195–198.

Sukh, D. (1997). Ethanotherapeutics and modern drug development. The potential of Ayurveda. Curr. Sci., 73, 909–928.

Sultana, B., Anwar, F., Przybylski, R. (2007). Antioxidant activity of phenolic components present in barks of barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam trees. Food Chem., 104, 1106–1114.

The Ayurvedic Formulary of India (Pt. I). 1978. Delhi, India: Goverment of India.

The Siddha Formulary of India. 1978. New Delhi: Goverment of India.

The Wealth of India: Raw Materials. Vol. 8. 1969. New Delhi, India: C.S.I.R.

Trease, G. E., Evans, W. C. (1989). Pharmacognosy. Delhi: ELBS Publ.

Upadhye, A. S., Rajopadhye, A. A. (2010). Pharmacognostic and phytochemical evaluation of leaf galls of Kakadshringi used in Indian system of medicine. J. Sci. Ind. Res., 69, 700–704.

Vonshak, A., Barazani, O., Sathiyamoorthy, P., Shalev, R., Vardy, D., Golan-Goldhirsh, A. (2003). Screening South Indian medicinal plants for antifungal activity against cutaneous pathogens. Phytother. Res., 17(9), 1123–1125.

Yildirim, A., Mavi, A., Oktay, M., Kara, A. A., Algur, O. F., Bilaloglu, V. (2000). Comparison of antioxidant and antimicrobial activities of Tilia (*Tilia argentea* Desf Ex DC), Sage (*Salvia triloba* L.), and Black Tea (*Camellia sinensis*) extracts. J. Agric. Food Chem., 48, 5030–5034.

Zhao, H., Fan, W., Dong, J., Lu, J., Chen, J., ..., Shan, L. (2008). Evaluation of antioxidant activities and total phenolic contents of typical malting barley varieties. Food Chem., 107, 296–304.

Received – Przyjęto: 27.09.2014

Accepted for print – Zaakceptowano do druku: 26.02.2015

For citation – Do cytowania

Eshwarappa, R. S. B., Ramachandra, Y. L., Subaramaihha, S. R., Subbaiah, S. G. B., Austin, R. S., Dhananjaya, B. L. (2015). Antioxidant activities of leaf galls extracts of *Terminalia chebula* (Gaertn.) Retz. (Combretaceae). Acta Sci. Pol. Technol. Aliment., 14(2), 97–105. DOI: 10.17306/J.AFS.2015.2.11