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Research Article

Evaluation of Phytochemical and *In vitro* antioxidant potentials of *Terminalia bellirica* (Gaerth) Roxb

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ABSTRACT

Free radicals are toxic by products of natural cell metabolism and are responsible for causing a wide number of health problems. Free radicals or ROS formed in the body as a result of biological oxidation. Antioxidants are micronutrients that have the ability to neutralize free radicals or their actions. The aim of the present study was investigate the phytochemical and *In vitro* antioxidant activity of fruit of ***Terminalia bellirica* (Gaerth.)Roxb.** The preliminary phytochemical screening was done to find out the presence of various secondary metabolites**.** *In vitro* antioxidant activity of aqueous extract ***Terminalia bellirica* (Gaerth) Roxb**. was performed by DPPH scavenging assay, FRAP, Superoxide radical scavenging assay, $ABTS^{+}$ assay and nitric oxide assay. All the antioxidant activities were compared with standard ascorbic acid. Qualitative Phytochemical screening of ***Terminalia bellirica* (Gaerth) Roxb**. showed the presence of alkaloids, flavonoids, saponin, steroids, terpenoids, tannin, glycosides, sugar and quinines in the fruit pericarp, mesocarp and seed of ***Terminalia bellirica* (Gaerth) Roxb**. In *in vitro* antioxidant study , the IC_{50} value of fruit pericarp , mesocarp and seed were found to be 27µg , 18 µg and 180 µg in DPPH scavenging assay , 444.04 µg ,540.05 µg and 111.01 µg in FRAP, 964.32 µg ,794.15 µg and 900 µg in superoxide radical scavenging assay and 300 µg , 454.54 µg and 300 µg in nitric oxide assay , 22.62 µg ,41.20 µg and 6.18 µg in $ABTS^{+}$ assay. From the results of the above findings concluded that the aqueous extracts of ***Terminalia bellirica* (Gaerth) Roxb** possess highest free radical scavenging activity which is mainly attributed to the presence of flavonoids and terpenoids**.**

Key words: DPPH, In vitro antioxidant activity, IC_{50}, Terminalia bellirica (Gaerth) Roxb

INTRODUCTION

Oxygen is vital for aerobic life process. Mostly 5% or more of the inhaled oxygen is converted into reactive oxygen species. Reactive oxygen species (ROS) are present in biological system from a variety of sources. When generation of ROS overtaking antioxidant defense of the cell , the free radicals start to inhibits the cell proteins , lipids and carbohydrates , which causes a number of physiological disorders.

Antioxidant is commonly referes to the activity of numerous vitamins, minerals and other phytochemicals to protect the damage caused by ROS [(1).] Antioxidant defense system scavenges and minimizes free radicals formation. The actions of free radicals are counteracted by antioxidants, either endogenous or exogenous [(2).] The curative effects of numerous herbs are commonly ascribed to their free radical scavenging activity. It has been suggested that there is an inverse relationship between dietary intake of antioxidant rich food and incidence of human disease [(3)].

Herbal plants are the treasury of antioxidant drugs that are potent free-radical scavenging molecules. The natural antioxidants from herbal origin such as plant polyphenols and flavonoids which scavenge the free radicals. Mostly medicinal plants play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [(4)]. Plant and plant products are being used as a source of medicine since long. The medicinal properties of plants have been recently investigated in the developing countries, due to their potent antioxidant activities, no side effects and economic viability [(5)].

The plant selected under study was *Terminalia bellirica* (Gaerth) Roxb , belonging to the family Combretaceae. It has various curative property which include rejuvenative and laxative , and used to dress wounds to arrest the bleeding , lowering cholesterol and blood pressure a lowering cholesterol and blood pressure and prevents ageing, imparts longevity, boosts immunity, improves mental faculties and enhances the body resistance against diseases. Hence the present study was an attempt to assess the phytochemical and *in vitro* antioxidant potentials of ***Terminalia bellirica* (Gaerth) Roxb.**

MATERIALS AND METHODS

Collection and authentication of the plant materials

Plant source selected for the present study is ***Terminalia bellirica* (Gaerth) Roxb**. The fruit of the selected plant was collected from authenticated dealer in Trichy, identified with the help of Flora of Presidency of Madras [(6)] **,** and authenticated with the specimen deposited at RAPINAT Herbarium, Department of Botany, St. Joseph's college, Trichy.

Preparation of Test Sample

The fruit of the ***Terminalia bellirica*** **(Gaerth) Roxb** was divided into three parts such as Fruit Pericarp, Fruit Mesocarp and Fruit Seed. The selected test samples were coarsely powdered and used for phytochemical and *in vitro* antioxidant studies.

Preparation of aqueous extract

The dried fruit of the ***Terminalia bellirica*** **(Gaerth) Roxb** was powdered coarsely using electric blender. 200gm of each part of fruit of ***Terminalia bellirica*** **(Gaerth) Roxb** was taken and extracted with water. To one part of the plant powder six parts of water was added, boiled and reduced to one third and filtrated. Then the filterate was evaporated to dryness. Paste form of the extract obtained was subjected to *in-vitro* assays.

Preliminary Phytochemical Screening

The aqueous extract of ***Terminalia bellirica*** **(Gaerth).** was tested for different phytoconsituents like alkaloids, glycosides, saponins, tannins, terpenoids, phenolic compounds, protein, carbohydrates using standard procedures[(7)].

In-vitro **antioxidant assays**

The free radical scavenging of aqueous extract of *Terminalia bellirica* (Gaerth) was performed by using DPPH radical scavenging assay[(8)]. , FRAP[(9)] , superoxide radical [(10)] and nitric oxide radical scavenging[(11)] and $ABTS^{+}$ [(12)] assay.

RESULTS AND DISCUSSION

Table I: Preliminary phytochemical Screening Different parts of *Terminalia bellirica* (Gaerth) Roxb

Test for	FPTB	FMTB	FSTB
	Powder	**Powder**	**Powder**
Terpenoids	Absent	Absent	Absent
Flavones	**Present**	**Present**	**Present**
Steroids	**Present**	**Present**	**Present**
Glycosides	**Present**	**Present**	**Present**
Sugar	**Present**	**Present**	**Present**
Alkaloids	**Present**	**Present**	**Present**
Quinines	Absent	Absent	Absent
Phenols	Absent	**Present**	**Present**
Tannins	**Present**	**Present**	**Present**
Saponins	**Present**	**Present**	**Present**
Coumarin	**Present**	**Present**	**Present**

FPTB – Fruit Pericarp of ***Terminalia bellirica*** **(Gaerth) Roxb**

FPTB – Fruit Mesocarp of ***Terminalia bellirica*** **(Gaerth) Roxb**

FPTB – Fruit Seed of ***Terminalia bellirica*** **(Gaerth) Roxb**

Phytochemicals, as plant bioactive molecules with distinct bio-activities towards animal biochemistry and metabolism are being widely examined for their ability to provide health benefits. The results of phytochemical analysis showed the presence of alkaloids, flavonoids, saponin, steroids, terpenoids, tannin, glycosides, sugar and quinines in the fruit pericarp, mesocarp and seed of *Terminalia bellirica* (Gaerth) Roxb. Thus, the reported pharmacological activities of fruit of *Terminalia bellirica* (Gaerth) Roxb could be attributed to the presence of saponins and flavonoids.

In vitro **antioxidant activity**

Table II: DPPH radical scavenging activity of aqueous fruit extract of *Terminalia bellirica (*Gaerth) Roxb

AQUEOUS EXTRACT OF *Terminalia bellirica (*Gaerth) Roxb					
Fruit Pericarp		**Fruit Mesocarp**		**Fruit Seed**	
Concentration (µg)	% **of Scavenging Activity**	**Concentration (µg)**	% **of Scavenging Activity**	**Concentration (µg)**	% **of Scavenging Activity**
10	37.03	**5**	7.40	**50**	18.51
20	40.74	**10**	37.03	**100**	29.62
30	55.55	**15**	44.44	**150**	40.74
40	66.66	**20**	55.55	**200**	55.55
50	74.07	**25**	74.07	**250**	62.96
IC_{50} =27µg		**IC_{50} =18µg**		**IC_{50} =180µg**	
Ascorbic acid – 58.2% / 50 µg					

The representation of scavenging the stable DPPH radical is a extensively used method to estimate the free radical scavenging ability of various samples. DPPH is a stable nitrogen-centered free radical. The color changes from violet to yellow upon reduction by either the process of hydrogen- or electron- donation is the basis of determination of free radical scavenging activity of samples. Substances which can perform this reaction can be measured as antioxidants and therefore radical scavengers [(13)].

In the present study, the percentage of scavenging effect on the $DPPH^{\bullet}$ radical was concomitantly increased with the increase in the concentration of both aqueous (10 to 50 µg - FPTB, 5 to 25 µg – FMTB and 50 to 250 µg FSTB) extracts of different parts of of the fruit of ***Terminalia bellirica (*Gaerth) Roxb.** IC_{50} for DPPH radical-scavenging activity of aqueous extract of fruit mesocarp , percarp and fruit seed of ***Terminalia bellirica*** **(Gaerth) Roxb** was calculated as 27 µg ,18 µg and 180 µg respectively. From the results it is known that of ***Terminalia bellirica (*Gaerth) Roxb** possess hydrogen donating capabilities for aqueous fruit extracts and does

scavenging free radicals. Furthermore, it was noticed that the aqueous extract of fruit mesocarp has more pronounced scavenging activity than that of the standard, Ascorbic acid and fruit pericarp and fruit seed.

Table III: Ferric reducing/antioxidant power (FRAP) assay of aqueous fruit extract of *Terminalia bellirica* (Gaerth) Roxb

AQUEOUS EXTRACT OF *Terminalia bellirica* (Gaerth.) Roxb.					
Fruit Pericarp		**Fruit Mesocarp**		**Fruit Seed**	
Concentration (µg)	**% of Reductive Activity**	**Concentration (µg)**	**% of Reductive Activity**	**Concentration (µg)**	**% of Reductive Activity**
200	7.6	**200**	25	**50**	7.6
400	45.45	**400**	29.41	**100**	29.41
600	67.56	**600**	55.55	**150**	67.56
800	84.41	**800**	75.51	**200**	70
1000	90	**1000**	86.04	**250**	75
IC_{50} =444.04µg		**IC_{50} =540.05µg**		**IC_{50} =111.01µg**	
Ascorbic acid – 80.52% / 100 µg					

FRAP assay is based on the ability of antioxidants to reduce Fe 3+ to Fe 2+ in the presence of 2,4,6-tri(2-pyridyl)- s-triazine (TPTZ), forming an intense blue Fe^{2+} -TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH 3.6). The absorbance increase is proportional to the antioxidant content. The reducing power of a compound is connected to its electron transferability and may serve as a significant indicator of its potential antioxidant activity. In this assay, the yellow color of the test solution changes to emerald green depending on the reducing power of test specimen. Greater absorbance at 700 nm indicated greater reducing power .

Table III indicates the reductive capabilities of the aqueous extracts of fruit pericarp, mesocarp and seed of ***Terminalia bellirica* (Gaerth) Roxb.** Total reducing power of fruit of ***Terminalia bellirica* (Gaerth) Roxb** was found to be higher in the aqueous extract than standard. The reducing power of extracts increased with increasing concentration. Among the different parts tested, the aqueous extract of fruit seed of ***Terminalia bellirica* (Gaerth) Roxb** showed higher

reducing power (**IC_{50} =111.01µg**) followed by fruit pericarp (**IC_{50} =444.04µg**) and fruit mesocarp (**IC_{50} =540.05µg**). This result indicates that the extracts may consist of polyphenol compounds that usually show great reducing power.

Table IV: Superoxide radical scavenging activity of aqueous fruit extract of *Terminalia bellirica* (Gaerth) Roxb

AQUEOUS EXTRACT OF *Terminalia bellirica* (Gaerth.) Roxb.					
Fruit Pericarp		**Fruit Mesocarp**		**Fruit Seed**	
Concentration (µg)	**% of Scavenging Activity**	**Concentration µg**	**% of Scavenging Activity**	**Concentration µg**	**% of Scavenging Activity**
200	**11.11**	**200**	**14.81**	**200**	**0**
400	**18.51**	**400**	**18.51**	**400**	**3**
600	**25.92**	**600**	**25.92**	**600**	**18.51**
800	**33.33**	**800**	**37.03**	**800**	**33.33**
1000	**51.85**	**1000**	**62.96**	**1000**	**55.55**
IC_{50} =964.32µg		**IC_{50} =794.15µg**		**IC_{50} =900µg**	
Ascorbic acid – 53.2 % / 100 µg					

Superoxide anion plays an important role in the formation of more reactive species such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which encourage oxidative damage in lipids, proteins, and DNA. Therefore, analysis of SO radical is very important method of verifying the mechanism of antioxidant potential of medicinal plants. [(14)].

The aqueous and ethanol extracts of different fruit parts of ***Terminalia bellirica* (Gaerth) Roxb** were found to possess concentration dependent scavenging activity on superoxide generated by photo-reduction of riboflavin and the results are given in **Table IV.** The IC_{50} values for scavenging superoxide radical of aqueous extracts of different fruit parts of ***Terminalia bellirica* (Gaerth) Roxb** were found to be **964.32µg, 794.15µg** and **900µg** respectively.

Table V: Nitrous oxide scavenging activity of aqueous fruit extract of *Terminalia bellirica* (Gaerth) Roxb

AQUEOUS EXTRACT OF *Terminalia bellirica* (Gaerth) Roxb					
Fruit Pericarp		**Fruit Mesocarp**		**Fruit Seed**	
Concentration (µg)	% **of Scavenging Activity**	**Concentration (µg)**	% **of Scavenging Activity**	**Concentration (µg)**	% **of Scavenging Activity**
100	**40**	**100**	**5**	**100**	**30**
200	**45**	**200**	**20**	**200**	**45**
300	**50**	**300**	**30**	**300**	**50**
400	**50**	**400**	**40**	**400**	**55**
500	**55**	**500**	**55**	**500**	**65**
IC_{50} = 300µg		**IC_{50} = 454.54µg**		**IC_{50} = 300µg**	
Ascorbic acid – 58.5 % / 50 µg					

Nitric oxide (NO) is a significant chemical agents produced by macrophages, endothelial cells, neurons, etc. which is involved in the guideline of a variety of physiological processes. Excess concentration of NO is related with numerous diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals.36 Hence, plant acquires protection from free radical generation due to the presence of antioxidant molecules and the secondary metabolites such as phenolics, flavonoids and polypropanoids have the capacity to scavenge free radicals by donating protons [(15)].

In the present study, Nitric oxide was useful information on the reactivity of the compounds generated from sodium nitroprusside and measured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by ***Terminalia bellirica* (Gaerth) Roxb** fruit extracts. **Table V** illustrates the percentage inhibition of nitric oxide generation by extract.

The aqueous extracts of different fruit parts of ***Terminalia bellirica* (Gaerth) Roxb** were found to possess concentration dependent scavenging activity on nitricoxide generated by oxidation of sodium nitro prusside and the results are given in **Table V .** The IC_{50} values for scavenging superoxide radical of aqueous extracts of different fruit parts of ***Terminalia bellirica* (Gaerth) Roxb** were found to be **300µg** (Fruit Pericarp & Fruit seed) **454µg** (Fruit Mesocarp) respectively.

Table VI: ABTS$^+$ radical Inhibitory activity of aqueous fruit extract of *Terminalia bellirica* (Gaerth) Roxb

AQUEOUS EXTRACT OF *Terminalia bellirica* (Gaerth) Roxb					
Fruit Pericarp		**Fruit Mesocarp**		**Fruit Seed**	
Concentration (µg)	**% of Inhibition**	**Concentration (µg)**	**% of Inhibition**	**Concentration (µg)**	**% of Inhibition**
10	**22.47**	**10**	**3.37**	**10**	**80.89**
20	**37.07**	**20**	**13.48**	**20**	**82.02**
30	**66.29**	**30**	**23.48**	**30**	**83.14**
40	**83.14**	**40**	**37.07**	**40**	**85.39**
50	**88.76**	**50**	**60.67**	**50**	**88.76**
IC $_{50}$ =22.62µg		**IC $_{50}$ =41.20µg**		**IC $_{50}$ =6.18µg**	
Ascorbic acid = 65.8					

ABTS assay is an excellent tool to determine the antioxidant activity of hydrogen donating and chain breaking antioxidants. The decolorization of the ABTS••, through measuring the reduction of the radical cation as the percentage inhibition of absorbance at 734nm. ABTS•+ was generated by incubating ABTS•• chromophore through the reaction.

In the present study, the ability of fruit extracts of ***Terminalia bellirica* (Gaerth) Roxb** to scavenge ABTS$^+$ was determined and the maximum ABTS$^+$ scavenging activity was found to be higher in aqueous fruit seed extract of **(IC_{50} = 6.18µg)** followed by fruit pericarp **(IC_{50} = 22.62µg)** and fruit mesocarp **(IC_{50} = 41.20µg)**.

CONCLUSION

From the results, it can be cocluded that the aqueous extracts of fruit pericarp, mesocarp and seed of ***Terminalia bellirica* (Gaerth) Roxb** showed strong antioxidant activity against various free radicals. The fruit pericarp of ***Terminalia bellirica* (Gaerth) Roxb** has significant antioxidant activity. This antioxidant potentials are mainly due to the presence of secondary metabolites which include terpenoids , flavonoids etc. Therefore, further investigation is needed to isolate and identify the antioxidant compounds present in the aqueous extract. Furthermore, the in vivo antioxidant activity of this extract needs to be assessed prior to clinical use.

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