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Antioxidant, Antimicrobial and Phytochemical Study of Different Solvent Extracts of Fruits of *Terminalia bellerica* (Gaertn.) Roxb., from Dibrugarh, Assam

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Terminalia bellerica (Gaertn.) Roxb. is widely cultivated plant due to its significance use as traditional medicine. The fruits of the plant were collected from Dibrugarh district of Assam, India. The present study aimed to evaluate the phytochemical, antioxidant and antimicrobial activity of different solvent extracts of the fruits of *Terminalia bellerica*. Antioxidant and phytochemical analysis were carried out using standard methods and the results revealed the presence of tannins, flavonoids, phenols and glycosides in the fruit sample. Among the solvent used for the extraction process, Ethyl acetate extract recorded the highest phenol content (6.56±0.004mgCE/gm dried body weight) and antioxidant activity (67.00± 0.12%) against DPPH. Similarly, ethyl acetate extract recorded the highest (22±1mm) inhibition against *B. subtilis* compared to Chloramphenicol (30mcg) and Clotrimazole (10mcg). It is concluded that the ethyl acetate extract of the fruits *Terminelia bellerica* yielded the best results and more efficacious in terms of antimicrobial activity which makes it more useful in new drug development.

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1. INTRODUCTION

Terminalia bellerica (Gaertn.) Roxb. is a medicinal plant from the family Combretaceae having various pharmaceutical and nutraceutical uses. It is commonly known as Bahera or Belleric or Bastard. In Assam it is known as Bhomora. The plant is found in greater part of India, in Gangetic plains, Chota Nagpur, Bihar, Orissa, West Bengal, Konkan, Deccan and most of South India [1,2]. The plant is also a secondary host of tasar silkworm [3]. The fruit is used in Triphala which is a popular herbal rasayana treatment in India having antibacterial effects against various pathogenic bacteria. Powder of the fruits is also used for cough and cold.

The local Monpa community of Arunachal Pradesh uses it as a part of their dietary component, in making pickles and also used by the herbalist in the treatment of various diseases like conjunctivitis, kidney diseases, and constipation [4]. The people of Coimbatore district also used the plant in their traditional medicinal practices [5]. The fruit of the plant is used in polyherbal formulation in Ayurvedic and Thai folk medicine having various medicinal properties [6]. The ethyl acetate fraction of fruits possess antioxidant activity [7,8] also recorded essential oils, phenolics, flavonoids in fruits of the plant.

The plant *Teminalia bellerica* (Gaertn.) Roxb. Is also a commonly used medicinal plant by the local people of Assam. Inspite of the tremendous medicinal uses, the plant parts are not examined in laboratory for their antioxidant and antimicrobial activity from this study area. The present study aimed to evaluate the total phenolic content and total flavonoid content, antioxidant and antimicrobial activity of different solvent extracts of fruits of the plant.

2. MATERIALS AND METHODS

2.1 Collection and Processing of Samples

Samples were collected from Dibrugarh, Assam and cleaned properly and washed under running water to remove dust and other debris. The materials were air dried at 28^0 C. The materials were grounded to fine powder using electric grinder. The fine powder was kept in air tight bottles for further analysis.

2.2 Preparation of Extracts

Extracts were prepared in four solvents viz-ethyl acetate, methanol, chloroform and hexane by cold maceration methods. The solvents were selected on the basis of polarity level and their extraction ability. The extracts were kept in air tight glass bottles at 5° C for further analysis. Hot petroleum ether extract was also prepared using soxhlet extractor.

The dried extracts were dissolved in DMSO (Dimethyl Sulfoxide) to obtain sample solution at 1mg/ml of concentration. Aqueous extracts were dissolved in distilled water at 1mg/ml of concentration.

2.3 Quantitative Phytochemical Analysis

Quantitative estimation for total phenol content (TPC) and total flavonoid content (TFC) were performed following standard methods noted below:

Determination of total Phenol Content (TPC): Total phenol content (TPC) of the sample extract was estimated following the method described by Malik and Singh [9]. For determination of Total phenol content a extract solution was prepared by mixing the extracts with DMSO at a concentration of 1mg/ml. 0.2 ml of the extract solution was taken in 10 ml test tube and made up to a volume of 3ml by adding distilled water. Then sequential addition of 0.5 ml Folin-ciocalteau reagent (1:1 with water) and 2 ml Na_2Co_3 (20%) was done. The solution were warmed for 1 min. and then cooled. Development of blue colour indicates the presence of phenol. Absorbancy of the solution was measured at 760 nm and phenol content was determined using the standard curve of Catechol. The total phenol content in extracts was expressed in terms of Catechol Equivalent (mg CE/g extract).

Determination of total Flavonoid Content (TFC): The Aluminium chloride method was used for determination of total flavonoid content of the sample extracts as described by Mervat and Hanan [10]. The extracts were mixed with DMSO to form a solution having concentration of 1mg/ml. 0.2 ml of extract solutions were taken in test tubes in triplicate form and volume was made to 3 ml by adding methanol. 0.1 ml $AlCl_3$ (10%), 0.1 ml sodium potassium tartarate and

2.8 ml distilled water were added sequentially to the solution and shaken vigorously and carefully. After 30 mins of incubation absorbancy of the solutions were taken at 415 nm using spectrophotometer and flavonoid content was determined using the standard curve of Quercetin. The total flavonoid content in extracts was expressed in terms of Quercetin Equivalent (mg QE/g extract).

Antioxidant activity assay of the sample extracts: DPPH radical scavenging activity and ABTS radical scavenging activity tests were performed for determination of antioxidant activity of the crude extracts of different parts of the plants.

Determination of antioxidant activity assay of the sample extract by DPPH method: DPPH radical scavenging activity was determined by the method described by Anti-Stanojevic et al. [11]. In this method, 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical was converted to 1,1-diphenyl-2-picryl hydrazine by the reaction of the radicals present in the sample. The scavenging capacity of the sample was determined through the degree of change in colour from purple to yellow of the sample solution. 0.5ml of extract solutions (1mg/ml) were taken in test tubes in triplicate form and the volume of the solution were made to 3ml with methanol. Test tubes with 3ml of methanol in triplicate from were used as blank. 0.15ml of freshly prepared DPPH solution was added to each of the test tubes. The solutions were then shaken and left to stand at room temperature for 30 minutes in dark. A control solution was prepared by mixing DPPH solution in methanol. Absorbancy was recorded at 517 nm using UV-Vis spectrophotometer. The capacity of scavenging free radicals by the sample extracts was calculated using the following formula

DPPH radical scavenging activity (%) = $[(Abs_{control}-Abs_{sample}/Abs_{control}.] \times 100$

Where,

$Abs_{control}$ is the absorbance of DPPH radical + methanol

Abs_{sample} is the absorbance of DPPH radical + sample extract

Determination of antioxidant activity assay of the sample extracts by ABTS method: The ABTS assay was carried out following the method of Re et al. [12]. A stock solution was prepared by mixing equal proportion of 7 mM ABTS solution and 2.4 mM potassium persulfate solution and kept for 12 hrs at room temperature in dark. 1 ml of the solution was mixed with 60 ml methanol to obtain an absorbancy of 0.706 + 0.001 units at 734 nm using the UV-Vis spectrophotometer. Freshly prepared ABTS solution was used for each assay. 1 ml extract solution (1mg/ml) was allowed to react with 1 ml of the ABTS solution and the absorbance was taken at 734 nm after 7 min using the UV-Vis spectrophotometer. The ABTS scavenging capacity of the extract was compared with standard ascorbic acid and calculated the percentage of inhibition.

ABTS radical scavenging activity (%) = $[(Abs_{control}-Abs_{sample})/Abs_{control}] \times 100$

Where,

$Abs_{control}$ is the absorbance of ABTS radical + methanol;

Abs_{sample} is the absorbance of ABTS radical + sample extract/standard.

Antimicrobial activity assay of the sample extracts: Antimicrobial activity of the bacterial strains was carried out by agar well diffusion method described by Nair et al.[13]

2.4 Antimicrobial Activity Study

The antimicrobial test was carried by agar well diffusion method described by Nair et al. [13] using 6 mm borer in triplicate. The activity was determined by measuring the diameter of zone of inhibition (ZOI) exhibited by the extract.

2.5 Selected Strains for Antimicrobial Study

Five Gram-Positive bacterial strains viz, Bacillus subtilis (MTCC 441), Bacillus cereus (MTCC 8750), Staphylococcus aureus (MTCC 3160), Staphylococcus epidermis (MTCC 3615) and Proteus vulgaris (MTCC 443), Enterococcus faecalis (MTCC 3017) and Penecillium chrysogenum (MTCC 947) were used in the study. Strains were obtained from the Microbial Type Culture collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacterial strains were maintained on nutrient agar slants and fungal strains on PDA slants and stored in freeze. Strains were regularly sub-cultured using nutrient broth for bacterial strains and PDB for fungal strains.

2.6 Standard Antibiotics

Standard antibiotics viz, Chloramphenicol (C) 30 mcg, Clotrimazole (CC) 10 mcg were taken for bacterial and fungal strains for comparison of ZOI with the solvent extracts [14].

3. RESULTS AND DISCUSSION

The results of qualitative phytochemical analysis is presented in Table 1. Alkaloids, flavonoids, phenol, carotenoid, reducing sugar, glycosides, tannins are present in the test samples. On the other hand; steroids, terpenoids, phlobatannin, saponin, anthraquinone and cardiac glycosides are absent in the sample. Devi et al. [15], Hazra [8] and Kumar & Khurana, [16] also recorded phytochemicals in extracts of the fruits.

Total phenols and flavonoids content of different solvent extracts are presented in Table 2. Ethyl acetate extract recorded highest total phenol content of 6.56±0.004mgCE/gm (dried weight) and methanol extract recorded highest (4.45±0.002 mg QE/ gm dried weight) flavonoid content. Gupta et al. [17] recorded that more phenol content in methanol extract than the aqueous extract used for the study. The polar solvent can extract more phytoconstituents from the plants.

The antioxidant activity of different solvent extracts is presented in Table 3. The ethyl acetate extract showed highest (67.00±0.12%) antioxidant activity against DPPH and methanol extract recorded highest (88.00±1.00%) antioxidant activity against ABTS at 500µl of sample at a concentration of 1mg/ml. Antioxidant activity of all the sample extracts recorded more inhibition against ABTS than DPPH. Chen et al. [7] proved that the ethyl acetate fraction of the fruits possess antioxidant activity. Singh and Asha [4] also evaluated the antioxidant percentage of methanol extract of fruits and compared it with standard ascorbic acid using DPPH as free radical. Gupta et al. [17] recorded that the methanol extract have more antioxidant activity than the aqueous extract. Elizabeth et al. [18] studied the antioxidant activity and phytochemicals present in methanol, ethyl acetate, chloroform and aqueous extracts of seed of the plant. In our study the antioxidant inhibition against ABTS is comparatively more than inhibition against DPPH. The study recorded that the ethyl acetate extracts have relatively high antioxidant activity. It has higher antioxidant activity against ABTS than DPPH. Highly polar ethyl acetate and methanol solvent extract are more potent than the low polar solvents.

The antimicrobial activity of different solvent extracts of the plant are presented in Table 4. Different solvent extracts recorded inhibition against *B. subtilis, B. cereus, S. aureus and P. chrysogenum*. The inhibition is compared with the standard antibiotics Chloramphenicol (30mcg) and Clotrimazole (10 mcg). Highest inhibition (22±1mm) was recorded by ethyl

Table 1. Qualitative phytochemical analysis of the fruits of *Terminalia bellerica* (Gaertn.) Roxb

Sample	Phytochemicals														
	Tannins	Flavonoids	Alkaloids	Phenol	Glycosides	Steroids	Terpenoids	Phlobatannin	saponin	Cardiac glycosides	anthraquinone	Free anthraquinone	carotenoid	Reducing sugar
Fruits	+	+	+	+	+	-	-	-	-	-	-	-	+	+

'+' indicate presence, '-' indicate absence of the constituents

Table 2. TPC and TFC of different solvent extract of fruit of *Terminalia bellerica* (Gaertn) Roxb

Solvents	Total phenol content (mg catechol equivalent/gm dry extract)	Total flavonoid content (mg quercetin equivalent/gm dry extract)
Ethyl acetate	6.56±0.004	1.44±0.112
Methanol	2.63±0.000	4.45±0.002
Chloroform	3.20±0.000	4.15±1.002
Hexane	1.77±0.001	2.50±0.001

**Values tabulated are average of triplicate*

Table 3. Antioxidant activity of different solvent extract of *Terminalia bellerica* (Gaertn.) Roxb

Solvent extract (500µl)	**DPPH radical scavenging activity (% inhibition in mg/ml)**	ABTS **radical scavenging activity (% inhibition in mg/ml)**
Ethyl acetate	67.00±0.12	85.80±1.01
Methanol	72.60±0.00	88.00±1.00
Chloroform	64.40±0.20	80.56±0.02
Hexane	55.00±0.01	76.34±0.12
Ascorbic acid	96.32±1.02	98.32±0.02

**Values tabulated are average of triplicate*

Table 4. Antimicrobial activity of different solvent extracts of *Terminalia bellerica* (Gaertn.) Roxb

Solvent extracts	**Diameter of Zone of Inhibition (mm)**								
	B. subtilis	***B. cereus***	***S. aureus***	***S. epidermis***	***P. vulgaris***	***E. faecalis***	***E. coli***	***P. chrysogenum***	***C. albicans***
Ethyl acetate	22±1	16±2	12±0	-	-	-	-	10±1	-
Methanol	12±2	8±0	-	-	-	-	-	-	-
Chloroform	-	8±0	-	-	-	-	8±0	-	-
Hexane	-	-	-	-	-	-	-	10±0	-
Hot petroleum ether extract	12.1±1.02	-	8±0	-	-	-	-	-	-
Chloramphenicol (30mcg)	15±0	-	-	30±0	-	8±0	-	-	-
Clotrimazole (10mcg)	20±0	10±0	11±1	20±0	8±0	-	26±2	11±0	32±0

Zone of inhibition including 5mm well diameter

acetate extract against *B. subtilis* which is followed by B. cereus (16 ±2 mm). Extracts showed no inhibition against *P. vulgaris, S. epidermis and E. faecalis.* Devi et al. [15] recorded antimicrobial activity of aqueous extract of the fruits against some human pathogenic bacteria. Dharmaratne et al. [19] also recorded anti-microbial activity of aqueous and methanol extracts of fruits of the plants against microorganisms; they also showed that the extraction of the fruits in boiling water is more potent in showing antimicrobial activity. Gupta et al*.* [17] also recorded that methanol extract was more potent in extracting phytochemicals from the plant which were responsible for their antimicrobial activity. The present study did not recorded inhibition against fungal strains *C. albicans*. According to Bais et al*.* [20] and Hassan et al. [21] the difference in antimicrobial action against the bacteria and fungi may be due to the inhibition of cell wall formation in the cell resulting in a leakage of cytoplasmic constituents by the active components of the extract. Madani and Jain [22]; Yadav, [23]; Shaikh et al. [24] also recorded antimicrobial activity of the plant against various micro-organism. Antibacterial activity obtained were found to be encouraging as compared to that of standard antibiotics though the standard antibiotics showed larger inhibitory effect than the different solvent extract of *T. bellerica.*

4. CONCLUSION

The present study revealed that *T. bellerica* is a good source of natural antioxidant which might be helpful in preventing the progress of various oxidative stresses and may be a good antimicrobial agent against certain diseases caused by B.subtilis, B. cereus and S.aureus. It is observed from our study that TPC, TFC, antioxidant and antimicrobial activity of the plant varies among different solvent extract. The variation in phytochemicals present antioxidanat and antimicrobial activity in the present study and earlier study may be due to habitat differences of the plant which plays an important role in production of secondary metabolites. Moreover; method of extraction, concentration of the extract may also influence the result.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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