Original Research Article

Antioxidant, antimicrobial and phytochemical study of different solvent extracts of fruits of Terminalia bellerica (Gaertn.) Roxb., from Dibrugarh, Assam

Abstract : Terminalia bellerica (Gaertn.) Roxb. is widely used traditional medicinal plant cultivated for its medicinal importance. The fruits of the plant were collected from Dibrugarh district of Assam, India. The present study aims to determine the phytochemical, antioxidant and antimicrobial activity of different solvent extracts of the fruits. Tannins, flavonoids, phenol, glycosides are present in fruit sample. Ethyl acetate extract recorded highest phenol content (6.56±0.004mgCE/gm dry wt.) and antioxidant activity (67.00± 0.12%) against DPPH. Similarly, ethyl acetate extract recorded highest (22±1mm) inhibition against B. subtilis which is compared to Chloramphenicol(30mcg) and Clotrimazole (10mcg). The study can be concluded that the fruits of the plant can be used in near future for development of new drug and ethyl acetate extract is more potent than the other solvent extracts tested.

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 (Sharma et al. 2005; Deb et al. 2016). The plant is also a secondary host of tasar silkworm (Anonymous, 1976). The fruit is used in Triphala which is a popular herbal rasayana treatment in India having antibacterial effects against various pathogenic bacteria. Powder of fruits also used for cough and cold.

In Tawang, Arunachal Pradesh, the local Monpa community uses it as food and as medicine in various diseases (Singh and Asha, 2017). The people of Coimbatore district also used the plant in their traditional medicinal practices (Kirtikar and Basu, 1999). The fruit of the plant is used in polyherbal formulation in Ayurvedic and Thai folk medicine having various medicinal properties (Intharuksa et al. 2016). The ethyl acetate fraction of fruits possess

antioxidant activity (Chen et al. 2019). Hazra (2019) 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 aims to determine the total phenolic content and total flavonoid content, antioxidant and antimicrobial activity of different solvent extracts of fruits of the plant.

MATERIAL AND METHODS

Collection and processing of samples

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

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.

Quantitative phytochemical analysis

Following methods were used for the phytochemical analysis; antioxidant and antimicrobial activity of the plant- Phytochemical analysis, Total phenol content (TPC) and Total flavonoid content (TFC), antioxidant activity.

The quantitative phytochemical analysis was performed following the standard laboratory methods described by Edeoga et al (2005); Aja et al (2010) and Ajayi et al (2011). Quantitative

estimation of TPC was done by the method described by Malik and Singh (1980) and TFC by the method described by Mervat and Hanan (2009). Antioxidant activity study performed using DPPH and ABTS radical scavenging method as described by Anti-Stanojevic et al (2009) and Re et al (1999) respectively.

Antimicrobial activity study

The antimicrobial test was carried by agar well diffusion method described by Nair et al (2005) using 6 mm borer in triplicate. The activity was determined by measuring the diameter of zone of inhibition (ZOI) exhibited by the extract.

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.

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.

RESULT AND DISCUSSION

The results of qualitative phytochemical analysis is presented in Table 1. Fruits of the plant recorded phytochemicals like- tannins, flavonoids, alkaloids, phenol, glycosides, reducing sugar, carotenoids. Hazra (2019) investigated the phytochemicals present in different parts of the fruits (epicarp, mesocarp and seed) and whole fruit. Phytochemicals like- alkaloids, flavonoids, glycosides, cardiac glycosides, terpenoids, tannins, phlobatannins are present in the test samples.

In seed phytochemicals are less than the other parts of the fruits. Devi et al. (2014) and Kumar and Khurana, (2018) also recorded phytochemicals in extracts of the fruits.

Total phenol and flavonoid content of different solvent extracts are presented in Table 2. Ethyl acetate extract recorded highest Total phenol content (6.56±0.004mgCE/gm dry wt.) and methanol extract recorded highest (4.45±0.002 mgQE/ gm dry wt.) flavonoid content. Gupta et al. (2019) 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. (2019) proved that the ethyl acetate fraction of the fruits possess antioxidant activity. Singh and Asha (2017) 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. (2019) recorded that the methanol extract have more antioxidant activity than the aqueous extract. Elizabeth et al. (2019) 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 are presented in Table 4. Antimicrobial activity of the solvent extracts recorded inhibition against *B. subtilis, B. cereus, S. aureus and P. chrysogenum*. The inhibition is compared with the standard antibiotics Chloramphenicol (30mcg). Highest inhibition (22±1mm) was recorded by ethyl acetate extract against *B. subtilis*. All the extracts showed no inhibition against *P. vulgaris, S. epidermis, E. faecalis* and *C. albicans.* Devi et al. (2014) recorded antimicrobial activity of aqueous extract the fruits against tested human pathogenic bacteria. Dharmaratne et al. (2018) also recorded anti-microbial activity of aqueous and methanol extracts of fruits of the plants against tested micro-organisms. They also showed that the extraction of the fruits in boiling water is more potent in

antimicrobial activity. Gupta et al. (2019) also recorded that methanol extract is more potent in extracting phytochemicals from the plant which are responsible for their antimicrobial activity. All the extracts from our study did not recorded any inhibition against fungal strains *C. albicans*. According to Bais et al (2002) and Hassan et al. (2007) 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. The phytochemicals present in the sample extracts, may be the another reason of inactiveness of the samples against fungi. Madani and Jain, (2008); Yadav, (2012); Shaikh et al. (2014) also recorded antimicrobial activity of the plant against various micro-organism.

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 present, '-' indicate absent

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

Solvents	Total phenol content	Total flavonoid content (mg

	(mg catechol equivalent/gm dry extract)	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

Table 3: Antioxidant activity study 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

Table 4: Antimicrobial activity study 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

Diameter of the cork borer=6mm, '-' indicates no inhibition

CONCLUSION

The study proved that the ethyl acetate extract of fruits of *Terminalia bellerica* is more potent than the other extracts used for test. The more polar solvent can extract more phytochemicals which are responsible for the antioxidant and antimicrobial activity of the fruits.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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

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