Phytochemical Investigation, Antioxidant and Antimicrobial Activities of Terminalia Bellerica Fruit Extract

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Abstract*— The purpose of the current research was to assess the phytochemical, antioxidant, and antibacterial effects of various fruit solvent extracts of Terminalia bellerica (TB). The fruit sample included tannins, flavonoids, phenols, and glycosides, according to the phytochemical studies. In comparison to 2,2-diphenylpicrylhydrazyl (DPPH), the methanolic extract exhibited greater antioxidant activity, with values of 46.54% at 5 mg/ml, 78.54% at 10 mg/ml, and 85.48 at 15 mg/dl. Chloroform and petroleum ether had the inhibitor's efficacy against the microorganisms in the antibacterial activity that was found. The maximal zone of inhibition (ZOI) for S. aureus and P. aeruginosa was shown by chloroform, while the highest ZOI for E. coli and B. subtilis was shown by petroleum ether. To assess the toxicity of the crude extract and its fractions in comparison to Lemna min, 0.015µg/mL of paraquat was utilized as the reference drug. The aqueous fraction showed the highest growth inhibition at medium and minimal dosage (100 µg/ml).

Index Terms- Antioxidant, Antimicrobial, Terminallia Bellerica & Phytochemical investigation.

I. INTRODUCTION

Terminalia bellerica (TB) is a medicinal plant from the family Combretaceae. It is commonly known as Bahera and is found widely throughout India and has enormous medicinal properties (1). The fruit can be used to treat cough, cold, asthma, hair growth, conjunctivitis, astringent and anti diarrheal properties. It is a large deciduous tree about 20-50 m tall, found throughout the Indian forests and plains (2). The plant helps in loss of appetite, and piles, lowering cholesterol, and blood pressure, boosts immunity and prevents ageing. It also enhances the body's resistance against diseases (3). Based on these facts, it is expected that the photochemical investigation and evaluation of the fruits of TB for antioxidant and antimicrobial activities.

II. MATERIALS AND METHODS

2.1. Collection and identification of plant

The fresh specimens of plants *T. bellerica* were collected from upper Dir and Chitral (Bonni). Each plant sample was washed, separated and dried in air for about 2 weeks at room temperature and was used for the following tests and analyses. These plants were ground to 60 mesh size and were preserved in airtight bottles. Some fresh specimens were used to study morphological characters and some of it was utilized for section cutting (4).

2.2. Determination of swelling index

About 1.0 g fine powder accurately weighed was taken into 25 ml of glass stoppered measuring cylinder. The internal diameter of the cylinder was about 16 mm, and the length of the graduate portion was about 125 mm, marked in 0.2 ml in the division from 0 to 25 ml in an upward direction. 25 ml, of water was taken and the mixture was thoroughly shaken every 10 min for 1 hrs. kept for 3 hrs at room temperature and the volume in ml occupied by the plant material, including any sticky mucilage was measured. The mean value of the individual determination, related to 1.0g of plant material was calculated (5).

2.3. Determination of foaming index

About 1.0 g of a coarse powder of drug was placed into a 500 ml conical flask containing 100 ml of boiling water and it was maintained for 30 min. Cooled and filtered into a 100 ml volumetric flask and the volume was made up with distilled water. The decoction was poured into 10 stoppered test tubes in successive portions of 1, 2, 3ml, etc. Up to 10ml, and adjusted the volume of the liquid in each tube with water to 10ml. The tubes were stopper and shaken in lengthwise motion for 15 sec, two shakes per second. After 15 min and height of the foam was measured (6).

2.4. Preliminary screening of phytochemicals

The preliminary phytochemical studies were performed to test the different chemical groups present in the drugs 10% (w/v) solution of extract was taken unless otherwise mentioned in the respective individual test (7).

2.5. Determination of antioxidant activity of *T.bellerica*

- Extract preparation

The dried powder of the leaves sample (10 g) of *T. bellerica* was defatted with petroleum ether using a cold maceration process. The defatted fruit and leaves sample (10 g) were mixed with 100 ml ethanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 7 days to ensure complete extraction. The extracts were filtered through Whatman No. 1 filter paper and then centrifuged at 4000g for 5 min. The solvent phase was collected and evaporated at 40oC. The dried crude extracts were stored at 4 °C in air-tight bottles until use.

- Scavenging assay (DPPH)

The DPPH assay method is based on the reduction of DPPH a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 330 nm (purple colour). The antioxidant activity of different *T. bellerica* extracts was measured in terms of hydrogen donating or radical scavenging ability using the DPPH. The ability of extracts to scavenge DPPH radicals is determined according to the method of Blois. 1 ml of 0.1m DPPH solution was mixed with 3 ml of extract (10, 100, 500) in methanol. The mixture was shaken vigorously and incubated for 35 min in the dark at room temperature. The absorbance was measured at 330 nm methanol and distilled water was used to get the absorbance zero. A blank sample containing the distilled water and DPPH was also prepared. The radical scavenging activities of the tasted samples expressed as a percentage of inhibition were calculated (8).

2.6. Determination of antimicrobial activity

The agar well diffusion method was modified. Soyabean casein digest agar was used for bacterial cultures. The culture medium was inoculated with the microorganisms suspended in soyabean casein digest broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks (distilled water, hexane and alcohol). A standard antibiotic (chloramphenicol, concentration 100 mg/ml) was simultaneously used as a positive control. The plates were then incubated at 37°C for 18 h. The antibacterial activity was evaluated by measuring the inhibition-zone diameter observed (9).

2.7. Phototoxic assay

The Lemna bioassay is a quick measurement of the phytotoxicity of the materials. The medium was prepared by mixing various constituents in 1L distilled water (stock solution), and pH was adjusted to 5.5 to 7.0 by the addition of potassium hydroxide beds and finally placed in an autoclave at 121°C for 15 min. The working medium is prepared by mixing 100 ml of stock solution and 900 ml of distilled water. 30 mg of sample was mixed in 1.5 ml of solvent (methanol/ethanol) used as stored solution. Three sterilized flasks were inoculated with 10, 100, and 1000 µl of stock solution to have 10, 100 and 1000 µg/ml of concentration respectively. The solvent evaporated for the whole night under sterile conditions. Every flask, 20 ml of working medium and then ten plants of Lemna minor, each containing a rosette of 2-3 fronds, to every flask, was added. Flasks containing medium and plants were used as a negative control, while flasks containing solvents, standard (paraquat) plant growth promotes and inhibitors served as a positive control. All flasks were caped with cotton and incubated in a growth cabinet (Fisons Fi Totron 600H) at 30 °C, 56 ± 10 % RH and 9000 lux light intensity for 12 hrs daily for 7 days. The no of fronds for each flask was calculated and documented on the 7th day (10).

III. RESULTS AND DISCUSSION

3.1. Macroscopical features of plant *T. bellerica*

The plant sample with an appropriate quantity was obtained after the preliminary preparations such as drying under shadow for 3 weeks then comminuted into fine particles and pulverized into a fine powder. The grinded plant (20 kg) was dipped in MeOH (80% v/v) with rare stirring at room temperature. After 2 weeks, the materials dissolved in MeOH were separated through the process of filtration. The procedure was repeated (n=3) and the clear material obtained was converted into syrupy liquid in vaco at

40oC to bestow dark brown paste. The crude MeOH extort (463.5g) was suspended in distilled water and consecutively extracted with n-hexane (20.71 % w/w), chloroform (15.96 % w/w), ethyl acetate (12.94 %), n-butanol (19.41 % w/w), and finally the aqueous (30.96 % w/w) fraction was obtained. Each organic extract was then evaporated to dryness and stored at ambient temperature for further studies (11).

3.2. Physico-chemical evaluation of *T. Bellerica*

The evaluation of crude powder was performed and results are summarized in Table 1. It can be observed that plants have a very low value of swelling index with a moderate value of foaming index which implies the presence of tannins with little amount of mucilage, and pectin. The high value of total ash may be the presence of a high amount of minerals (12).

Table 1: Physico-chemical parameters of *T. bellerica*

Parameters	Observations
	Physicochemical
	Ash values (% w/w)
Total ash value	38.00 ± 0.02
Acid insoluble ash	12.43 ± 0.02
Water soluble ash	3.53 ± 0.03
Pharmacological	
Foaming index	136.32
Swelling index	0.89 ± 0.15

3.3. Antioxidant activity of *T. bllerica* fruit

T. bellerica showed good antioxidant activity in water, ethanol and methanol extracts. Measuring the decay in absorbance at 330 nm due to the DPPH radical reduction, indicating the antioxidant activity of the *T.bellerica* in a short time. The antioxidant activity of different concentrations (5, 10, 15 mg/ml) of ethanol and methanol of *T. bellerica* was determined by the DPPH method. In the present study, the methanolic extract of *T. bellerica* has higher antioxidant activity. The scavenging effects on the ethanolic extract from *T. bellerica* were 54.22 in 5 mg/ml, 67.44 in 10 mg/ml and 63.56 in 15 mg/ml respectively and results are presented in Fig 1 and Fig 2. The scavenging effects on the methanolic extract from *T. bellerica* were 46.54% in 5mg/ml, 78.54% in 10mg/ml and 85.48% in 15 mg/ml respectively (13).

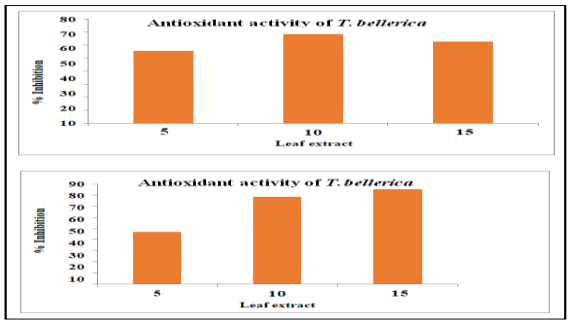

Fig 1: Antioxidant activity of methanolic and ethanolic extract of *T. bellerica*

3.4. Antimicrobial activity of *T. bellerica*

The result of the antibacterial activity of *T. bellerica* observed that chloroform extracts of fruits, showed the inhibitory activity against the pathogens. It showed maximum zone of inhibition (ZOI) against S.aureus then E.coli, B.subtilis and P.aeruginosa. Whereas, ethanol, methanol 80% & petroleum ether extracts showed an inhibitory effect against E.coli, S.aureus and B.subtilis. Acetone extract showed ZOI against B.subtilis and E.coli. Ethanol 80% extract showed ZOI against E.coli and P.aeruginosa (Fig 2). In the case of aqueous extract, it did not show non-significant activity (14).

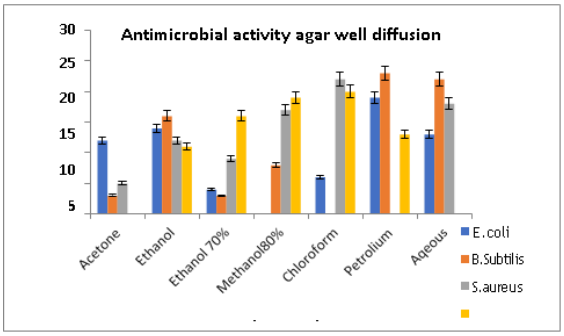

Fig 2: Antimicrobial Activity of the *T. bellerica*

3.5. Phytotoxicity assay of plant *T. bellerica*

The test was carried out to check the toxicity of the crude extract and fractions against the Lemna min towards the vegetative part of a plant phytotoxic assay also called Lemna bioassay for inhibitors and promoters of plant growth. Paraquat was used as a standard drug at a dose of 0.015µg/mL. The experiments were performed under control conditions. The detail of the experimental procedure is already described in the experimental part. Crude extract inhibited 4 % growth at 1000 µg/ml, 10 % at 100 µg/ml

and 9 % at 10 µg/ml. The n-hexane fraction 100 % growth inhibition was observed at the highest dose. While the inhibition pattern at doses of 10 µg/ml and 100 µg/ml was 8 % and 46 % inhibition at 10 µg/ml and 100 µg/ml correspondingly. It means that percent inhibition by n-hexane is dose-dependent. In the case of n-hexane, chloroform, ethylactate, butanol and aqueous fractions, growth inhibition was 100 % at the dose of 1000 ug/ml. At the dose of 100 ug/ml growth inhibition in the order of 42 %, 45 %, 41 %, 37 % and 28% for n-hexane, chloroform, ethyl acetate, butanol and aqueous fraction was observed respectively. Similarly at a dose of 10 µg/ml, n-hexane, chloroform, ethyl acetate, butanol and aqueous fractions showed the rate of inhibition, results are presented in Fig 3. Maximum growth inhibition at the highest dose (1000 µg/ml) was observed in all fractions except crude extract. Maximum growth inhibition at medium and minimum dose (100 µg/ml) was exhibited by aqueous fraction (15).

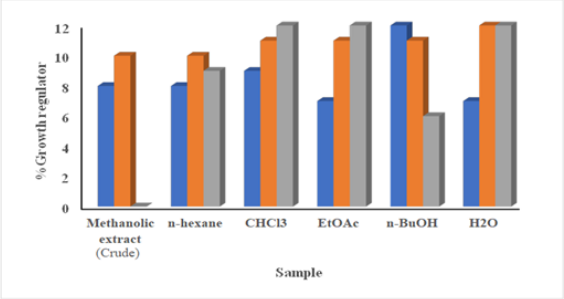

Fig 3: Growth regulator (%) in all fractions at different concentrations

CONCLUSION

The results of this investigation, the traditional application of fruit extracts which possess compounds with antimicrobial and antioxidant properties that can be used as phytomedicine for the treatment of microbial diseases and to reduce oxidative stress. In conclusion, *T. bellerica* produced effective and satisfactory results in research on phytotoxicity, antioxidants, and antimicrobials. Further pharmacological evaluations, toxicological studies and possible isolation of the therapeutic activities from this fruit are the future challenges.

REFERENCES

[1] Deb A, Sikha B, Biswajit D. Pharmacological activities of baheda (Terminalia bellerica): a review. J Pharmacogn Phytochem 2016;5:194-7.

[2] Rashed K, Potocnjak I, Giacometti J. Terminalia bellerica aerial parts ethyl acetate extract exhibits antioxidant, anti-inflammatory and anti-fibrotic activity in carbon tetrachloride intoxicated mice. J Funct Foods 2014;8:319-30.

[3] Shah CP, Santani DD. In vitro susceptibilities of human bacterial pathogens associated with dacryocystitis to Terminalia bellerica. Int J Curr Pharm Res 2011;3:32-3.

[4] Chavan LV, Anita P, Naresh C. Antioxidant availability of Terminalia bellerica (Roxb.) in relation to its medicinal uses. Pharmacogn J 2010;2:338-44.

[5] Taso R, Deng Z. Separation procedures for naturally occurring antioxidant phytochemicals. J Chromatogr B: Anal Technol Biomed Life Sci 2004;812:85-99.

[6] Prakash, D., Suri, S., Upadhyay, G and Singh, B. N. Total phenol, antioxidant and free radical scavenging activities of some medicinal plants. International Journal of Food Science and Nutrition 2007; 58, 18 - 28.

[7] Oke OM, Hamburger PS. Screening of some Nigerian medicinal plants for antioxidant activity using 22, diphenyl picryl – hydrazyl radical. African Journal of Biomedical Research 2002; 5: 77- 79.

[8] Kavitha D, Padma PR. A study of the antimicrobial effect of Albizia amara leaf extracts. Advances in Plant Sciences 2011; 24 Suppl 1: 49-52.

[9] Prabuseenivasan S, Jayakumar M, Ignacimuthu S. In vitro antibacterial activity of some plant essential oils. BMC complementary and alternative medicine 2006; 6(3)9;1-9.

[10] Bauer AW, Kirby WMM, Sherris JC, Truc M. Performance standards for antimicrobial disc susceptibility. Am.J.Clin Pathol. 1996; 45: 493-496.

[11] Marnett LJ. Lipid peroxidation-DNA damage by malondialdehyde. Mutat Res 1999;424:83-95.

[12] Gupta R, Singh RL, Singh P. Quantification of phytochemicals and evaluation of the antioxidant potential of ethanolic leaf extract of Terminalia bellerica, Terminalia chebula and Emblica officinalis vis-Ã -vis Triphala. Int J Pharm Sci Rev Res 2015;32:14-22.

[13] Tsuda T, Watanabe M, Ohshima K, Yamamoto A, Kawakishi S, Osawa T. Antioxidative components isolated from the seed of tamarind. J Agric Food Chem 1994;42:2671-4.

[14] Gulcin I, Koksal K, Elmastas M, Aboul-Enein HY. Determination of in vitro antioxidant and radical scavenging activity of Verbascum oreophilum C. Koch Var Joannis (Fam. Scrophulariaceae). Res J Biol Sci 2007;2:372-82.

[15] Mahesh R, Nagulendran KR, Velavan S, Ramesh T, Begum VH. Studies on the antioxidative and free radical scavenging activities of myrobalan (Terminalia chebula retz) through various in vitro models. Pharmacologyonline 2007;2:1-11.