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RESEARCH ARTICLE

Antimicrobial activity and antioxidant properties of *Brucea mollis* Wall. ex Kurz: a medicinal plant of Northeast India

Prapty Das[1] · Sneha Hasnu[1] · Lipika Lahkar[1] · Saurov Mahanta[1] · Sashin Kumar Borthakur[1] · Bhaben Tanti[1]

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Abstract

Brucea mollis Wall. ex Kurz is a potent medicinal plant occurring on hill slopes of Karbi Anglong district of Assam. The species is popularly known to have antimalarial activity in traditional medicine by a few ethnic communities of Northeastern regions. In the present study, an attempt was made to study the antimicrobial and antioxidant activity of *B. mollis*. The antibacterial and antifungal activity of plant extracts was screened by the disc diffusion method of Kirby-Bauer sensitivity test against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Aspergillus fumigatus* and *Aspergillus niger*. Among all the extracts (leaf ethanol, leaf methanol, callus ethanol, and callus methanol), the callus ethanol extracts was found to exhibit better antimicrobial activity against all the bacterial strains at 20 mg/ml concentration. Callus ethanol extracts exhibited maximum inhibition 17.33 ± 0.33 mm against *B. subtilis*. In case of antifungal activity, leaf methanol extracts exhibited 17.66 ± 0.33 mm against *A. fumigatus* and leaf ethanol extracts exhibited 18.33 ± 0.33 mm inhibition against *A. niger* at 20 mg/ml. The minimum inhibitory concentration (MIC) of the both leaves and callus against the bacterial and fungal strains exhibited lowest 0.156 mg/ml MIC against (*A*) *niger* from leaf methanol extract; against (*B*) *subtilis*, *S. aureus* and *A. fumigatus* from callus ethanol extract and against *S. marcescens* from callus methanol extract. Moreover, DPPH, Hydrogen peroxide (H_2O_2) radical scavenging activity, reducing power assay, total phenolic, and flavonoid content were investigated. Both the extracts showed antioxidant activity in a dose-dependent manner and ethanolic extract exhibited better antioxidant properties than methanolic extract.

Keywords Antimicrobial activity · Aantioxidant properties · Medicinal plant · *Brucea mollis* · MIC

Introduction

Brucea mollis belonging to the family Simaroubaceae is a bitter deciduous shrub well known for its potent antimalarial properties in Northeast India. It is a potent medicinal plant used traditionally by several ethnic communities of Northeast India. Its local name is known as 'koinine' in Assam (India) and distributed in Nepal, China, Bhutan, Cambodia, Laos, Malaysia, Myanmar, Philippine, Thailand, and Vietnam (Borthakur et al. 2018; Das et al. 2018; Pullaiah 2006). In India, the species is found in parts of West Bengal, Sikkim, Arunachal Pradesh, Assam, Nagaland, Manipur, and Meghalaya (Hajra et al. 1997). The species has several medicinal properties like anticancer, antitumor, antileukemic, antiplasmodial, antibacterial and pesticidal effect (Bharati and Singh 2012). There are reports on phytochemical constituents of *B. mollis* from leaf, stem, roots, etc. and several compounds have been isolated and identified. Alves et al. (2014) reported the presence of quassinoids, alkaloids, triterpenes, steroids, coumarins, anthraquinones, flavonoids and metabolites which have been isolated from the members of the family Simaroubaceae and of these quassinoids are the most dominant and abundant group in the family.

Medicinal plants which constitute a major source of natural organic compounds are widely used in different healthcare practices. The medicinal effects of plants are basically due to the presence of alkaloids, steroids, tannins and phenolic compounds, which are secondary metabolites synthesized in specific regions or parts of the plant (Joseph and Raj 2010). Nowadays, there is a growing demand for pharmaceutical-based products. Though there are numerous medicinal plants that have been explored and utilized

✉ Bhaben Tanti
btanti@gauhati.ac.in

[1] Plant Molecular Biology Laboratory, Department of Botany, Gauhati University, Guwahati, Assam 781014, India

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in the expanding pharmaceutical industries yet many of them still remain unexplored (Baruah et al. 2017; Sarma and Tanti 2017). There has been a high demand for the study of traditional medicinal plants with their medicinal properties which have been increasingly used in pharmaceutical activities due to low toxicity and economic viability (Rai et al. 2008). There are several reports on antimicrobial and antioxidant activity from various edible and medicinal plants and attention has been paid to the utilization of antimicrobial and antioxidants of natural origin. As *B. mollis* is highly medicinal in traditional healthcare practices, an attempt was made to study an antimicrobial and antioxidant activity from leaf extracts of *B. mollis*.

Materials and methods

Collection of the plant material and preparation of the plant extract

Fresh leaves of *B. mollis* were collected from the Botanical garden of Gauhati University, India. The leaf samples were air-dried at room temperature for about one month. The dried samples were then coarsely powdered in a mechanical grinder. On the other hand, callus obtained from the leaf explants in MS media through tissue culture were extracted following the method of Johnson et al. (2011) with slight modification. These were dried at 40–45 °C in a hot air oven for 7 days till a constant weight was observed. The dried callus thus obtained was powdered in a mechanical grinder (Das et al. 2017).

Microbial strains

For antibacterial activity, two Gram-positive strains viz., *B. subtilis* MTCC441, *S. aureus* MTCC3160 and two Gram-negative strains viz., *P. aeruginosa* MTCC424 and *Serratia marcescens* MTCC2645 and for antifungal activity, *Aspergillus fumigatus* MTCC2550 and *A. niger* MTCC282 were obtained from Microbial Type Culture Collection (MTCC), IMTECH (India). Bacterial strains were maintained in MHA (Muller Hinton agar medium) and fungal strains were maintained in PDA (Potato Dextrose Agar medium) at 4 °C for further experiments.

Solvent extraction

The solvent extraction was done using soxhlet method. 10 g each of coarsely powdered leaf samples and the callus samples was extracted separately in 100 ml of ethanol and methanol using the soxhlet apparatus for 24 h. The extracts were then filtered with Whatman filter paper. The filtrate was evaporated in a hot air oven at 45 ºC. The crude extract thus obtained was lyophilized. The dried extract was then stored at 4 °C for further use (Roy et al. 2017).

Preparation of the standard concentration of the plant extract

Stock solutions of the leaf and callus extracts were prepared by dissolving the dried extract in 10% DMSO (Dimethyl sulfoxide) at the concentration of 200 mg/ml (Parekh and Chanda 2006; Jouda et al. 2016). From the stock solution, different volumes of the extracts were prepared to get the final amount of 20, 10, 5, 2.5 and 1.25 mg/ml concentration. Sterile filter paper discs were loaded with different concentrations of the extracts and allowed to dry at room temperature under aseptic conditions.

Preparation of the microbial suspension

The bacterial slants were prepared in Mueller Hinton Agar (MHA) medium in test tubes and stored at 4 °C. Active cultures were prepared by transferring a loopful of cultures in Mueller Hinton broth medium incubated at 37 °C for 24 h.

The fungal cultures were prepared in Potato Dextrose Agar (PDA) media and incubated at 27 °C for 48 h and stored at 4 °C. Active cultures were prepared by transferring a loopful of fungi in 100 ml Potato Dextrose broth (PDB) in a 250 ml conical flask and incubated at 27 °C for 48–72 h (Buragohain et al. 2013).

Antibacterial activity assay

The antibacterial activity of plant extracts was screened by the disc diffusion method of Kirby-Bauer sensitivity test (Bauer et al. 1966; Murray et al. 1995). The MHA plates were spread uniformly with 100 µl of bacterial cultures (108 CFU/ml) of all the bacterial strains. They were allowed to dry for 10 min. Then, the discs (0.6 cm) were loaded with 20 µl of 20, 10, 5, 2.5 and 1.25 mg/ml extract respectively. The loaded discs were allowed to remain for diffusion for 30 min at room temperature. Ceftazidime disc (30 µg, Hi-Media) was used as a positive control. The plates were incubated at 37 °C for 24–48 h. Zone inhibition formed around the discs were measured in millimeters and recorded. The experiment was repeated twice with three replicas per experiment.

Antifungal activity assay

For the study of antifungal activity of plant extracts (leaf and callus extracts of ethanol, methanol, and water) against two fungal strains, the agar disc diffusion method was used (Bauer et al. 1966; Nordin et al. 2013). Amphotericin B was taken as a positive control. The Potato Dextrose Agar

plates were inoculated with 100 µl of fungal suspension (105 spores/ml) by spreading uniformly over the agar plates. Each disc was loaded with 20 µl of solvent extract containing 20, 10, 5, 2.5 and 1.25 mg/ml respectively so that the extract can diffuse to the medium. One disc was loaded with Amphotericin B (20 µl of 20 mg/ml stock) which served as a positive control. The plates were incubated at 27 °C for 24–72 h. The zone of inhibition created around the discs was measured and recorded in millimeters.

Determination of MIC for antibacterial activity

MIC is defined as the lowest concentration of the compound to inhibit the growth of microorganisms. The MIC was determined in 96 well microtitre plates following the broth microdilution method based on the Clinical Laboratory Standard Institute M07-A8 (CLSI 2009). Two-fold serial dilution of the plant extracts (both leaf and callus extracts prepared in ethanol and methanol separately) were prepared. From the stock solution (2.5 mg/ml) of previously studied zone inhibition test, six different dilutions were prepared as 1.25, 0.625, 0.3125, 0.156, 0.078 and 0.039 mg/ml respectively. 50 µl of MH broth was dispensed into 96 well plates vertically from WA (first well) to WH (eighth well). Then, 50 µl of 2.5, 1.25, 0.625, 0.3125, 0.156 and 0.078 mg/ml extracts were poured in each well from WA to WF. In the seventh well (WG), Ceftazidime was used in place of the plant extract as positive control and the eighth well (WH) was used as negative control which consists of MH broth and extract. 50 µl of bacterial suspension (1 × 105 CFU/ml) was inoculated in each of the wells (from Well A to Well G). The microdilution plates were incubated at 37 ºC for 24 h. After incubation, the bacterial growth was observed by taking absorbance at 405 nm (Taye et al. 2011). The presence of turbidity was considered when the difference of OD value (after incubation-before incubation) of the tested extracts was more than the control (broth + extract) (Taye et al. 2011).

Determination of MIC for antifungal activity

The MIC for antifungal activity was determined in the same procedure as that of antibacterial activity (Nordin et al. 2013). 50 µl of PDB was poured vertically in all the wells from WA to WH. Then, 50 µl of 2.5, 1.25, 0.625, 0.3125, 0.156 and 0.078 mg/ml extracts (both leaf and callus extracts prepared in ethanol and methanol solvent separately) were poured in each well from WA to WF. In the seventh well (WG), Amphotericin B (20 mg/ml) was used as a positive control. 50 µl of fungal suspension (10^5 spores/ml) was added in all the wells. The eighth well (WH) was taken as negative control which consists of PDB and plant extract. The microtitre plate was incubated at 30 ºC for 24 h. The growth inhibition of the fungus in microdilution wells was observed by taking the absorbance at 530 nm and the presence of turbidity was observed by taking the OD value as described by Taye et al. (2011).

Antioxidant activity of the plant extracts

DPPH free radical assay

The antioxidant activity of leaf extracts was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. DPPH solution (0.1 Mm) was prepared by dissolving 8 mg of DPPH in methanol and the remaining volume was made up to 20 ml with methanol. The prepared solution was kept in darkness for 30 min to complete the reaction. The antioxidant activity was measured by following the standard method as described by Blois (1958) wherein the bleaching rate of a stable free radical, DPPH is monitored at a characteristic wavelength in the presence of the sample (Dey et al. 2017; Saikia et al. 2018).

Ascorbic acid was taken as standard and various concentrations of plant extracts (0.25 µg, 0.50 µg, 0.75 µg, and 1 µg) were prepared from the stock solution (1 mg/ml) and the volume was adjusted to 10 ml with methanol. The final volume to be assayed was adjusted to 1 ml by adding 1.25 µl of methanolic DPPH solution to 500 µl of different concentrations of plant extract and 375 µl of methanol in an Eppendorf tube of 1 ml (Tanti et al. 2010). Three independent were performed for the sample. The tubes were allowed to stand for 30 min at 27 ºC. The absorbance of the sample was measured at 517 nm (du 750 Beckman coulters, UV Spectrophotometer). Methanol was taken as blank and the experiment was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

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

Results were expressed as IC_{50} concentration where 50% inhibition of the DPPH radical is obtained. The IC_{50} values were determined graphically.

Hydrogen peroxide radical scavenging activity

The ability to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). Ascorbic acid (standard) was dissolved in distilled water to make different concentrations of 400, 600, 800 and 1000 µg/ml. Sample solutions were prepared by dissolving the extracts in distilled water to final concentrations of 400, 600, 800 and 1000 µg/ml. Test solutions were prepared by adding 0.6 ml of H_2O_2 (40 Mm) for each concentration. For control 0.6 ml of 40 Mm H_2O_2 was added to 1 ml of

phosphate buffer saline (ph 7.4). The reaction mixture was incubated for 10 min and the absorbance value was measured at 230 nm. The percentage of scavenging of hydrogen peroxide was calculated using the following equation:

$$\text{Percent scavenged } \left[H_2O_2\right] = [A_0 - A_t/A_0 \times 100$$

where A_0 is the absorbance of control and A_t is the absorbance of the sample or standard solution.

Reducing power assay

The reducing power was determined according to the method of Oyaizu (1988). The required quantity of ascorbic acid was dissolved in methanol to give a concentration of 400, 600, 800 and 1000 µg/ml. The sample solution was prepared by dissolving extracts in methanol to give a concentration of 200, 400, 600, 800 and 1000 µg/ml. 1 ml of different concentrations of sample solution were mixed with phosphate buffer (2.5 ml, 0.2 m, pH 6.6) and was incubated at 50 ºC for 20 min. After incubation, 10% trichloroacetic acid (2.5 ml) was added to the mixture and centrifuged at 3,000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% FeCl3. The absorbance was measured at 700 nm and compared with standards. The increase in absorbance of the reaction mixture indicated the increase in reducing the power of the sample.

Phenol content

Gallic acid (standard) of 1 mg was dissolved in 1 ml of distilled water and serial dilution was done to make the concentration of 20, 40, 60, 80 and 100 µg/ml. The total phenol content was determined by using the method of Borah et al. (2014).

The calibration curve was prepared by adding 1 ml of different concentrations of gallic acid, 5 ml of 0.2 N Folin ciocalteu and 4 ml of 75 g/l Na_2CO_3. Blank was prepared by 1 ml of distilled water except for gallic acid. Accordingly, 1 ml of sample solution (1 mg/ml) was also mixed with the reagents above and the absorbance was measured at 765 nm to determine the total phenolic compounds in the extract in the gallic acid equivalent (GAE) was calculated by the following formula.

$$T = CV/M$$

where T = total phenol content mg/ml plant extract in GAE, C = the concentration of gallic acid established from the calibration curve in mg/ml, V = the volume of extract in ml, M = the weight of plant extract in mg.

Total flavonoid content

The total flavonoid content was determined by following the method of Borah et al. (2014). The stock solution of quercetin was prepared by dissolving 1 mg of quercetin in 1 ml of methanol and was diluted to different concentrations of 50, 100, 150, 200 and 250 µg/ml. The reaction mixture contains 1 ml of different concentrations of extract, 2 ml of methanol and 0.2 ml of 10% aluminium chloride, 0.2 ml of 1 m potassium acetate and 5.6 ml of distilled water. Blank was prepared by 1 ml of methanol without the plant extract. 1 ml of the sample solution was also mixed accordingly. After 30 min of incubation, the absorbance was measured at 415 nm against the blank. The total flavonoid content in the plant extract in quercetin equivalents was calculated by the formula,

$$T = CV/M$$

where, T = Total flavonoid content in mg/ml of plant extract; C = The concentration of quercetin established from the calibration curve in mg/ml; V = The volume of extract in ml; M = The weight of methanolic plant extract in mg.

The extract concentration providing 50% inhibition (IC_{50}) was calculated from linear regression of plots.

Results

Antibacterial activity of *B. mollis*

The antibacterial activity of leaf and callus derived from leaf extracts prepared in ethanol and methanol is shown in Table 1. In general, the callus ethanol extracts showed higher zone inhibition than leaf extracts of both ethanol and methanol against all the tested bacterial strains. The highest zone inhibition was exhibited by callus ethanol extract against *B. subtilis* with 17.33 ± 0.33 mm inhibition followed by 14.66 ± 0.33 mm inhibition against *S. aureus* at 20 mg/ml concentration. With increasing concentration of the extracts, the zone inhibition was found to be increasing. In the case of positive control (Ceftazidime 30 µg), the highest zone inhibition was observed against *P. aeruginosa* with 17.00 ± 0.57 mm inhibition followed by 14.66 ± 0.66 mm inhibition against *S. marcescens*. The negative control (DMSO) did not show any inhibition. Ethanol extracts of callus showed more activity than ethanol extracts of leaves. Similar findings were also recorded by Johnson et al. (2011) in *Mentha arvensis* which corroborate the fact that antibacterial efficacy of ethanol extract of leaf derived callus was better than the other solvents used.

Table 1 Zone inhibition test (mm) of callus and leaf extract of *B. mollis*

Extract	Concentration (mg/mL)	*B. subtilis* (MTCC 441)	*S. aureus* (MTCC 316)	*P. aeruginosa* (MTCC 424)	*S. marcescens* (MTCC 2645)	*A. fumigatus* (MTCC2550)	*A. niger* (MTCC 282)
Leaf ethanol	1.25	11.33 ± 0.66	10.66 ± 0.33	10.00 ± 1.15	11.00 ± 0.57	12.66 ± 0.33	12.83 ± 0.16
	2.5	12.00 ± 0.57	11.66 ± 0.88	10.66 ± 0.88	11.33 ± 0.66	13.00 ± 0.57	16.33 ± 0.33
	5.0	12.00 ± 0.57	12.00 ± 1.54	10.66 ± 0.66	11.66 ± 0.33	13.66 ± 0.33	16.33 ± 0.88
	10.0	12.33 ± 0.88	12.33 ± 0.66	10.66 ± 0.88	11.66 ± 0.88	14.00 ± 0.57	17.33 ± 0.33
	20.0	12.66 ± 0.66	12.66 ± 0.88	11.33 ± 0.66	12.33 ± 0.66	14.66 ± 0.33	**18.33 ± 0.33**
Leaf methanol	1.25	10.33 ± 0.33	10.33 ± 0.33	9.66 ± 0.33	11.00 ± 0.57	12.00 ± 0.57	14.00 ± 0.57
	2.5	11.00 ± 1.00	10.66 ± 0.33	9.66 ± 0.88	11.33 ± 0.33	15.66 ± 0.66	14.33 ± 0.33
	5.0	11.33 ± 0.66	11.00 ± 0.57	10.00 ± 0.57	11.66 ± 0.33	16.00 ± 0.57	15.33 ± 0.88
	10.0	11.66 ± 0.33	11.66 ± 0.33	10.33 ± 0.66	11.66 ± 0.88	16.66 ± 0.33	15.66 ± 0.33
	20.0	12.00 ± 0.00	12.33 ± 0.66	11.00 ± 0.57	12.33 ± 0.33	**17.66 ± 0.33**	16.00 ± 0.57
Callus ethanol	1.25	13.33 ± 0.88	13.33 ± 0.33	10.66 ± 0.33	12.00 ± 0.57	14.33 ± 0.66	13.66 ± 0.33
	2.5	14.33 ± 0.66	13.66 ± 0.33	11.00 ± 0.57	12.33 ± 0.88	15.66 ± 0.33	13.00 ± 1.00
	5.0	15.00 ± 0.57	14.00 ± 0.57	10.33 ± 0.33	12.33 ± 0.33	16.00 ± 0.57	15.33 ± 0.33
	10.0	15.66 ± 0.33	14.66 ± 0.33	12.00 ± 0.57	12.33 ± 0.66	16.33 ± 0.33	16.00 ± 0.57
	20.0	**17.33 ± 0.33**	**14.66 ± 0.33**	**12.00 ± 1.15**	**13.33 ± 0.66**	17.33 ± 0.33	16..33 ± 0.66
Callus methanol	1.25	10.66 ± 0.33	11.33 ± 0.33	9.66 ± 0.33	11.33 ± 0.88	10.33 ± 0.33	11.66 ± 0.33
	2.5	11.33 ± 0.33	11.33 ± 0.66	11.00 ± 0.57	12.33 ± 0.33	10.66 ± 0.88	13.00 ± 0.57
	5.0	11.66 ± 0.33	11.66 ± 0.33	11.33 ± 0.66	12.33 ± 0.88	12.00 ± 0.57	13.00 ± 0.28
	10.0	11.00 ± 0.57	12.33 ± 0.33	11.33 ± 0.88	12.66 ± 0.33	15.00 ± 1.00	13.33 ± 0.33
	20.0	12.00 ± 0.57	13.00 ± 0.57	11.66 ± 1.20	13.00 ± 0.57	17.00 ± 0.57	13.66 ± 0.33
Ceftadizine	30 µg	12.33 ± 0.88	15.00 ± 0.57	17.00 ± 0.57	14.66 ± 0.66	–	–
Amphotericin B	40 µg	–	–	–	–	21.00 ± 0.57	15.66 ± 0.33

Values are mean ± SD of three replicates repeated thrice per experiment. mm: millimeter; µg: microgram; mg/ml: milligram per milliliter

[a]Zone inhibition test (mm) of callus and leaf extract of *B. mollis* for antibacterial activity prepared in ethanol and methanol

Antifungal activity of *B. mollis*

The antifungal activity of leaf and callus derived from leaf extracts of ethanol and methanol was studied against *A. fumigatus* and *A. niger*. Among all the extracts, the maximum zone inhibition was observed against *A. niger* from leaf ethanol extracts with 18.33 ± 0.33 mm inhibition followed by 17.66 ± 0.33 mm inhibition against *A. fumigatus* from leaf methanol extracts at 20 mg/ml concentration. In the case of positive control (amphotericin 40 µg), the zone inhibition was found to be 21.00 ± 0.57 mm against *A. fumigatus* and 15.66 ± 0.33 against *A. niger*. The negative control (DMSO) did not show any inhibition against the fungal strains. It was also found that with the increase in the concentration of the extract, there was also an increase in antimicrobial activity against the tested fungal strains which is corroborated by the findings of Viswanad et al. (2011).

MIC of the plant extracts against the tested microorganisms

The MIC of the ethanol and methanol extracts of leaves and callus extracts against six experimental test organisms ranged from 0.156 to 0.625 mg/ml except against *P. aeruginosa* which ranged between 0.625 and 1.25 mg/ml. The callus ethanol extracts showed MIC at 0.156 mg/ml against *B. subtilis, S. aureus*, and *(A) fumigatus*. Whereas, the callus methanol extract showed MIC at 0.156 mg/ml against *S. marcescens*. The leaf ethanol extract showed MIC at 0.3125 mg/ml against *(B) subtilis* and *A. niger* whereas leaf methanol extract showed MIC at 0.156 mg/ml against *A. niger* (Table 2).

Antioxidant activity of *B. mollis*

DPPH free radical scavenging activity has been widely used in various edible and medicinal plants for antioxidant activity from plant extracts due to its potent source of natural antioxidants (Moein et al. 2008). DPPH method is widely used to investigate the ability of the plant extracts or the compounds to scavenge free radicals (DPPH) by providing hydrogen atoms or by electron donation. The scavenging activity of the extracts depends upon the reduction of DPPH. The more reduction of DPPH will occur when there are more antioxidants in the extract (Chew et al. 2012). In the present study, the antioxidant activity of the ethanolic

Table 2 Minimum inhibitory concentration of the plant extracts of *B. mollis*

Microorganisms	Minimum inhibitory concentration (mg/ml)			
	Leaf		Callus	
	Ethanol extract	Methanol extract	Ethanol extract	Methanol extract
B. subtilis	0.3125	0.625	0.156*	0.625
S. aureus	0.625	0.625	0.156*	0.3125
P. aeruginosa	1.25	1.25	0.625	0.625
S. marcescens	0.625	0.625	0.3125	0.156*
A. fumigatus	0.625	1.25	0.156*	1.25
A. niger	0.3125	0.156*	0.3125	0.625

*Minimum inhibitory concentration (MIC) of the leaves and callus extracts of *B. mollis* against six experimental test organisms

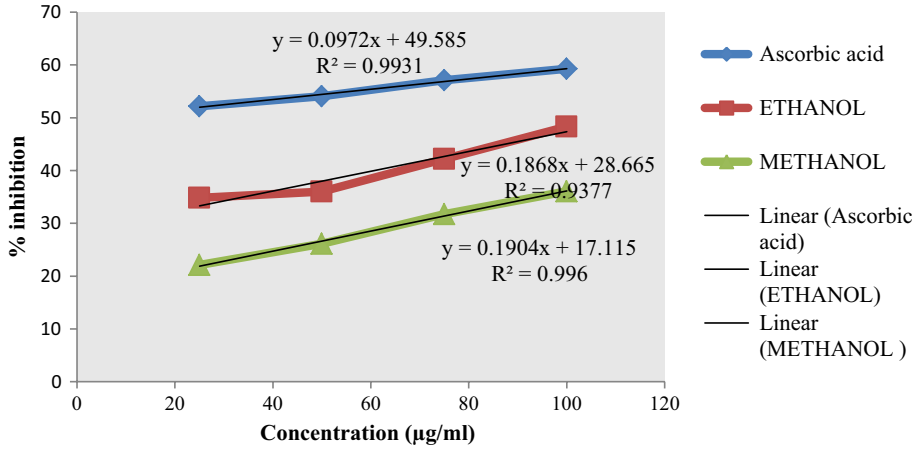

Fig. 1 DPPH scavenging activity by ascorbic acid (standard), ethanolic extract, methanolic extract of *Brucea mollis*

Table 3 DPPH scavenging activity by ascorbic acid (standard), ethanolic extract and methanolic extract *Brucea mollis*

Concentration of ascorbic acid (µg/ml)	% of inhibition ascorbic acid	% of inhibition ethanol extract	% of inhibition methanol extract
25	52.18	34.81	22.1
50	54.09	36.03	26.12
75	57.10	42.21	31.76
100	59.28	48.32	36.09
IC50 (µg/ml)	4.32	114.73	173.10

and methanolic extracts of *B. mollis* was investigated. The free radical scavenging activity of the leaf extracts of *B. mollis* and ascorbic acid (standard) has been shown in Table 3. The ethanol extract of leaves showed better antioxidant activity than the methanol extract. The ascorbic acid and the plant extracts showed DPPH radical scavenging activity in a concentration-dependent manner. With increasing concentration of the extracts, the percentage of inhibition was found to be increasing (Fig. 1). The ethanolic concentration showed the percentage of inhibition of 34.81% at 25 µg/ml to 48.32% at 100 µg/ml concentration. Whereas, the methanolic concentration showed 22.1% inhibition at 25 µg/ml to 36.09% inhibition at 100 µg/ml concentration. The ascorbic acid showed maximum inhibition of 52.18% at 25 µg/ml to 59.28% inhibition at 100 µg/ml concentration. The IC50 value of ascorbic acid was found to be 4.32 µg/ml whereas the ethanol and methanol extract showed 114.73 and 173.10 µg/ml respectively (Table 3).

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to cells because it may give rise to hydroxyl radicals in the cells (Halliwel and Gutteridge 1989; Borah et al. 2014). Thus, the removal of H_2O_2 is very important for antioxidant defense in the cell (Keser et al. 2012). In the study, the H_2O_2 scavenging activity was found to be better in ethanol extract than methanol extract because of its high phenolic content in ethanolic extract than methanolic extract. In H_2O_2 radical scavenging activity, the % of inhibition was found to be concentration-dependent (Fig. 2). The

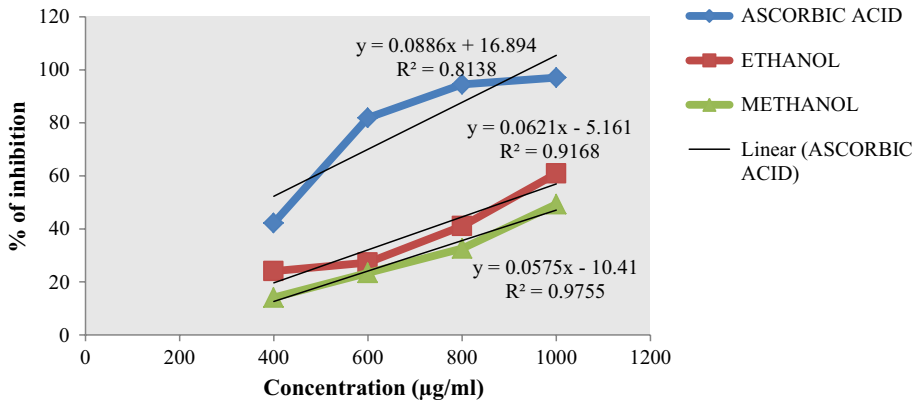

Fig. 2 H_2O_2 radical scavenging activity by ascorbic acid, ethanolic extract and methanolic extract of *B. mollis*

Table 4 H_2O_2 radical scavenging activity by ascorbic acid, ethanolic extract and methanolic extract of *B. mollis*

Concentration (µg/ml)	% of inhibition ascorbic acid	% of inhibition ethanol	% of inhibition methanol
400	42.19 ± 0.01	24.07 ± 0.38	14.07 ± 0.12
600	81.89 ± 0.31	27.29 ± 0.09	23.40 ± 2.9
800	94.47 ± 0.01	41.06 ± 0.20	32.61 ± 0.32
1000	97.05 ± 0.01	60.90 ± 0.17	49.35 ± 0.002
IC50 (µg/ml)	376.25	889.69	1059.82

Table 5 Reducing power assay by absorbance at 700 nm of ascorbic acid (standard), ethanolic extract and methanolic extract of *B. mollis*

Conc. (µg/ml)	Ascorbic acid	Ethanol	Methanol
400	0.209 ± 0.003	0.106 ± 0.008	0.094 ± 0.009
600	0.312 ± 0.008	0.211 ± 0.012	0.156 ± 0.008
800	0.455 ± 0.004	0.334 ± 0.016	0.237 ± 0.050
1000	0.555 ± 0.005	0.436 ± 0.011	0.373 ± 0.007

% of inhibition was found to be 60.90 ± 0.17 from ethanolic extract concentration and 49.35 ± 0.002 from methanolic concentration at 1000 µg/ml as compared to ascorbic acid which showed 97.05 ± 0.01% inhibition at 1000 µg/ml concentration. The IC50 value for ethanolic and methanolic extract showed 889.69 µg/ml and 1059.82 µg/ml as compared with ascorbic acid which showed 376.25 µg/ml concentrations (Table 4).

Reducing power assay

Reducing power of compounds serves as an indicator to have antioxidant activities that are able to reduce Fe^{3+} ferricyanide complex to Fe^{2+} ferrous form. The reducing power of the extracts may be due to the ability of electron donors (Jayanthi and Lalitha 2011). In our assay, the reducing power of the extracts was found to be concentration-dependent which showed highest in 1000 µg/ml ethanol extract than methanol extract compared with the standard ascorbic acid. In reducing power assay, with an increase in the concentration of the extract, the absorbance at 700 nm was found to be increasing which indicated high reducing power. Ethanolic extract showed 0.106 ± 0.008 absorbance at 400 µg/ml to 0.436 ± 0.011 absorbance at 1000 µg/ml. Methanolic extract showed 0.094 ± 0.009 at 400 µg/ml to 0.373 ± 0.007 at 1000 µg/ml. Whereas, ascorbic showed 0.209 ± 0.003 absorbance at 400 µg/ml to 0.555 ± 0.005 absorbance at 1000 µg/ml (Table 5).

Total phenol and flavonoid content

Plants rich in secondary metabolites such as phenols, flavonoids, terpenoids, lignins, tannins, quinines, alkaloids are rich in antioxidant activities (Aiyegoro and Okoh 2010). Flavonoids are the most important phenolics which are responsible for various biological activities. The present study showed maximum phenol and flavonoid contents in ethanolic extract than methanolic extract which attributes to the presence of antioxidant activities by neutralizing free radicals. The total phenolic content was calculated from the calibration graph using gallic acid as standard and was found to be 85.29 and 46.03 µg gallic acid equivalent (GAE) in 1 mg of ethanolic and methanolic extract respectively (Table 6). The total flavonoid content was also calculated from the calibration graph and was found to be 111.55 and 76.34 µg quercetin equivalents in 1 mg of ethanolic and methanolic extracts respectively (Table 7).

Table 6 Total phenolic content (µg/ml)

Extract	Equation	Conc of gallic acid (µg/ml)	Equation	Total phenol content (µg/ml)
Ethanol extract	$Y = 0.009x - 0.061$	85.29	T = CV/M	85.29
Methanol extract	$r^2 = 0.999$	46.03		46.03

Table 7 Total flavonoid content (µg/ml)

Extract	Equation	Conc of quercetin (µg/ml)	Equation	Total flavonoid content (µg/ml)
Ethanol extract	$Y = 0.0039x + 0.0289$	111.55	T = CV/M	111.55
Methanol extract	$r^2 = 0.9997$	76.34		76.34

Discussion

Antimicrobial activity

A number of studies on antimicrobial activity of the plants belonging to Simaroubaceae have been undertaken in *B. antidysenterica* (Taye et al. 2011), *Samadera indica* (Viswanad et al. 2011), *Brucea javanica* (Nordin et al. 2013), *Ailanthus excelsa* (Manikandan et al. 2015). The extracts used in the study were found to exhibit various degrees of antimicrobial effect against the tested microorganisms. Both ethanolic and methanolic extracts of *B. mollis* were found to exhibit broad-spectrum antibacterial and antifungal effects. Similar antimicrobial effects were observed in *Ailanthus excelsa* (Manikandan et al. 2015), *Cassia fistula* (Bhalodia and Shukla 2011), *Acokanthera schimperi* (Taye et al. 2011). It was also found that with the increase in the concentration of the extract, there was also an increase in antimicrobial activity against all the tested bacterial and fungal strains which is corroborated by the findings of Viswanad et al. (2011). Ethanol extracts of callus showed more activity than ethanol extracts of leaves. Similar findings were also recorded by Johnson et al. (2011) in *Mentha arvensis* which corroborate the fact that antibacterial efficacy of ethanol extract of leaf derived callus was better than the other solvents used. Similarly, ethanol extracts of leaf exhibited more antifungal activity against *A. niger* with the highest zone inhibition 18.33 ± 0.33 mm. The differences in the inhibitory effect of both leaf and leaf derived callus extract against the bacterial and fungal strains may be due to the qualitative and quantitative differences in the phytochemical compounds present in them.

Antioxidant properties

DPPH free radical scavenging activity has been widely used in various edible and medicinal plants for antioxidant activity from plant extracts due to its potent source of natural antioxidants (Moein et al. 2008). DPPH method is widely used to investigate the ability of the plant extracts or the compounds to scavenge free radicals (DPPH) by providing hydrogen atoms or by electron donation. The scavenging activity of the extracts depends upon the reduction of DPPH. The more reduction of DPPH will occur when there are more antioxidants in the extract (Chew et al. 2012). In the present study, antioxidant activity of the ethanolic and methanolic extracts of *B. mollis* was investigated. It was observed that the ethanolic extract showed better scavenging activity than the methanolic extract. With increasing concentration of the extracts, it exhibited more scavenging activity. The IC50 value was determined in the extracts compared with the standard ascorbic acid. The IC50 is the concentration of the extract which inhibits 50% DPPH radical formation. The IC50 of ethanolic extract was found to be 114.73 µg/ml and the methanolic extract was found to be 173.10 µg/ml compared to the standard ascorbic acid which exhibited 4.32 µg/ml.

Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to cells because it may give rise to hydroxyl radicals in the cells (Halliwel and Gutteridge 1989; Borah et al. 2014). Thus, the removal of H_2O_2 is very important for antioxidant defense in the cell (Keser et al. 2012). In the study, the H_2O_2 scavenging activity was found to be better in ethanol extract than methanol extract because of its high phenolic content in ethanolic extract than methanolic extract.

Reducing power of compounds serves as an indicator to have antioxidant activity which is able to reduce Fe^{3+} ferricyanide complex to Fe^{2+} ferrous form. The reducing power of the extracts may be due to the ability of electron donors (Jayanthi and Lalitha 2011). In our assay, the reducing power of the extracts was found to be concentration-dependent which showed highest in 1000 µg/ml ethanol extract than methanol extract compared with the standard ascorbic acid.

Plants rich in secondary metabolites such as phenols, flavonoids, terpenoids, lignins, tannins, quinines, alkaloids are rich in antioxidant activities (Aiyegoro and Okoh 2010). Flavonoids are the most important phenolics which are responsible for various biological activities. The present study showed maximum phenol and flavonoid contents in ethanolic extract than methanolic extract which attributes to the presence of antioxidant activities by neutralizing free radicals.

Conclusions

The present study provides evidence that the secondary bioactive compounds present in the plant might have synergistic impact on inhibition of the tested pathogens. Therefore, further studies are necessary to identify the bioactive compounds and evaluate their antimicrobial activity against a wide range of pathogens in order to confirm their efficacy as antimicrobial agent In the present investigation, the different tests used for radical scavenging activity and antioxidant properties of the plant extract confirms the presence of antioxidant properties of the leaf extracts of *B. mollis* which might be benefited in preventing various stress related diseases. Further, isolation and identification of the chemical antioxidant and antimicrobial compounds of the plant may lead to bioprospecting for the welfare of mankind.

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Author contributions SKB was involved conception and design of the study. PD carried out all the experiments and analyzed the data. BT critically analyzed the findings. All authors read and approved the final manuscript.

Compliance with ethical standards

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest Prapty Das has no conflict of interest. Sneha Hasnu has no conflict of interest. Lipika Lahkar has no conflict of interest. Saurov Mahanta has no conflictof interest. Sachin Kumar Borthakur has no conflict of interest. Bhaben Tanti has no conflict of interest.

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