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Full Length Research Paper

In vitro antibacterial, antifungal and cytotoxic activity of three Bangladeshi *Bridelia species*

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The organic soluble extractives of three *Bridelia* species, *B. verrucosa*, *B. stipularis* and *B. tomentosa* growing in Bangladesh were subjected to screening for antibacterial, antifungal and cytotoxic activities. All extractives showed moderate to strong antimicrobial activity against 13 Gram positive and Gram negative bacterial strains and three fungi where the stem bark of *B. tomentosa* demonstrated highest inhibition of growth with zone of inhibition of 23.2 mm against *Bacillus cereus* and 17.5 mm against *Candida albicans*. The crude extractives of all three plants of *Bridelia* species exhibited cytotoxic activity against brine shrimp nauplii having significant LC_{50} and LC_{90}.

Keywords: Antibacterial, antifungal, cytotoxic, *bridelia*, brine shrimp nauplii.

INTRODUCTION

The antimicrobial screening of an agent is essential to ascertain its spectrum of activity against various types of pathogenic microorganisms. In this 21st century majority of antibiotics or antimicrobial agents are becoming resistant to most of the microorganisms (Alanis, 2005). So, discovery of new antimicrobial agents is becoming very essential. Medicinal plants represent a rich source of antimicrobial agents and many potent and powerful drugs (Srivastava et al., 1996). Natural products can also be tested for their bioactivity by the brine shrimp lethality bioassay which is a relatively recent development in the bioassay for bioactive compounds (Mclauglin and Rogers, 1998). This bioassay indicates toxicity as well as a wide range of pharmacological properties of various compounds.

The three *Bridelia* species of the Phyllanthaceae family available in Bangladesh, *Bridelia verrucosa* Haines, *Bridelia stipularis* (L) Blume and *Bridelia tomentosa* Blume are shrubs or small evergreen trees (Kirtikar and Basu, 1980). *Bridelia verrucosa* Haines (Synonym: *B. montana* Willd, *B. sikkimensis* Gehrmann) is a large shrub or straggling tree without thorns which is widely distributed in the Chittagong Hill Tracts (Kirtikar and Basu, 1980; Gricson and Long, 1987). The root and bark are much used as astringent in Bombay and Goa. The plant has been credited with anthelmintic properties (Kirtikar and Basu, 1980; Caicus, 1998; Singh and Ali, 1998). Previous phytochemical studies with the leaves of this plant showed the presence of sitosterol, its glucoside and hexacosanol (Singh and Ali, 1998). *Bridelia stipularis* (L) Blume (Synonym: *Clutia stipularis* L., *B. scandens*, Local name: Pat Khowi) is a large more or less climbing shrub, which grows in shady, moist forest floors. It is distributed in the forest areas of the central and eastern parts of Bangladesh. It is also found in India and Myanmar. The plant is used in the treatment of amoebic dysentery, chest pain, constipation, diarrhea, leucoderma and strangury (Nasir, 2006). Decoction of bark is used for cough, fever and asthma. It also showed hypotensive and hypoglycaemic actions on animals. Leaves are used for jaundice (Krishnan, 1992). *Bridelia tomentosa* Blume (Synonym: *B. lanceaefolia*, *B. monoica*; Local name: Khy, serai) is a large shrub or small evergreen tree and in Bangladesh it is distributed in the forest areas of Srimangal, Sylhet and Chittagong district and also in Dinajpur. It is also found in India, Khasia Mountains, Andaman Islands and distributed in Malay Islands, China, Philipines and Northern Australia (Hooker, 1875). The bark of *B. tomentosa* is astringent and used in colic (Krishnan, 1992) while the leaves are used as herbal medicine for traumatic injury. The roots are used in epidemic influenza and neurasthenia. The bark is known to contain 8% of tannins (Website-Hong

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Table 1. Yield obtained after Kupchan partitioning of the crude extracts of the stem bark and leaf of Bangladeshi *Bridelia* species.

Soluble fractions	*B. verrucosa*		*B. stipularis*		*B. tomentosa*	
	Stem bark (g)	Leaf (g)	Stem bark (g)	Leaf (g)	Stem bark (g)	Leaf (g)
n-hexane	1.75	3.1	2.75	3.3	2.25	2.7
Carbon tetrachloride	0.625	0.625	0.650	0.810	0.625	0.835
Chloroform	0.800	0.620	0.925	0.710	0.850	0.695
Aqueous	6.7	5.0	5.3	5.2	5.3	5.5

Kong Flora and Vegetation).

The inhibitory activity of the water and ethanol extract of *Bridelia ferruginia* stem bark against all 7 hospital strains of bacteria (Irobi et al., 1994) and antibacterial activity of luteoforol from *Bridelia* crenulata (Ramesh et al., 2001) have been reported previously.

As a part of our continuing studies on medicinal plants of Bangladesh we investigated the antibacterial, antifungal and cytotoxic activities of *B. verrucosa*, *B. stipularis* and *B. tomentosa* for the first time, and we, herein, report the results of such studies.

MATERIALS AND METHODS

Plant Materials

Leaf and stem bark of *B. verrucosa*, *B. stipularis* and *B. tomentosa* were collected from the village of Panchouri, Khagrachhori District in February 2007 and identified in Bangladesh National Herbarium where voucher specimens have been maintained representing these collection (Accession No. DACB-31376, 31378 and 31377, respectively). The sun dried leaf and stem bark were cut into small pieces, cleaned, oven dried and pulverized. The powdered stem bark of *B. verrucosa* (550 g), *B. stipularis* (550 g) and *B. tomentosa* (575 g) was separately soaked in 1.5 L methanol and 325 g of powdered leaf of each plant was also separately soaked in 750 mL methanol for seven days, filtered through fresh cotton bed and finally with Whatman No. 1 filter paper and concentrated by using a rotary evaporator at low temperature (36-40ºC) and reduced pressure. A portion (10 g) of the concentrated methanol extract of all the three plants of both stem bark and leaf was separately fractionated by the modified Kupchan partitioning method (Van Wagenen et al., 1993) into *n*-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions and the yields are shown in Table 1.

Antimicrobial screening

Antimicrobial activity of the crude extracts and Kupchan fractions was determined by the disc diffusion method (Bauer et al., 1966) against 13 strains of Gram positive and Gram negative bacteria and 3 fungi (Sathi et al., 2010) as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka (Tables-2-4). Here measured amount of the test sample (8 mg) was dissolved in definite volume (200 µl) of solvent (chloroform or methanol) and applied to sterile paper discs (7 mm diameter) at a concentration of 400 µg/disc and carefully dried to evaporate the residual solvent. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the respective test microorganism. Antibacterial drug Kanamycin (30 µg/disc) and antifungal agent Griseofulvin (20 µg/disc) and blank disc (impregnated with solvent) were used as positive and negative control, respectively. These plates were then kept at low temperature (4ºC) for 24 hours to allow maximum growth of the organisms. The antimicrobial activity of the test agent was determined by measuring the diameter of inhibition zone expressed in millimeter.

Brine shrimp lethality bioassay

For determination of the general toxic properties of the extracts, DMSO solution of the plant extracts was applied against *Artemia salina* for 24 hours in a simplified *in vivo* assay (McLaughin et al., 1998; Meyer et al., 1982). For the experiment, 4 mg of each of the plant extracts was separately dissolved in DMSO and by serial dilution technique solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563 and 0.781 µg/mL were obtained. Then each of this test solution was added to the premarked test tubes containing 10 live shrimp nauplii in 5 mL of simulated brine water. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent of lethality of the brine shrimp nauplii was calculated and the LC_{50} and LC_{90} of the test samples were obtained by plotting percentage of the shrimp killed against the logarithm of the sample concentration. Vincristine sulphate was used as positive

Table 2. Antimicrobial activity of stem bark and leaf of *B. verrucosa* extracts (400 µg/disc), Kanamycin (30 µg/disc) and Griseofulvin (20 µg/disc)

Test bacteria	Diameter of zone of inhibition (mm)										
	MEBV	**HSFBV**	**CSFBV**	**CHSFBV**	**AQSFBV**	**MELV**	**HSFLV**	**CSFLV**	**CHSFLV**	**AQSFLV**	**PC**
Gram positive bacteria											**KAN**
Bacillius cereus	14.3± 1.53	9.9± 1.15	11.6± 1.52	13.8± 2.01	10.7± 1.37	15.6± 0.89	18.2± 0.50	15.6± 0.61	16.7± 0.35	14.9± 0.78	35± 0.58
B. megaterium	10.9± 1.81	12.0± 1.72	14.9± 1.15	12.4± 1.40	11.4± 1.36	15.2± 0.70	17.5± 0.87	16.1± 0.87	15.6± 0.25	15.3± 0.76	35± 0.58
B. subtilis	11.3± 2.08	9.6± 1.53	12.3± 2.52	10.6± 2.08	11.1± 1.60	20.5± 1.12	19.7± 0.85	17.6± 0.61	21.3± 1.91	17.9± 0.65	36± 1.00
Sarcina lutea	12.3± 2.31	12.4± 1.70	11.6± 1.26	14.3± 1.53	12.6± 1.60	21.6± 0.68	18.1± 0.67	19.7± 0.57	17.5± 0.85	15.3± 0.40	27± 0.58
Staphylococcus aereus	10.6± 2.52	11.7± 2.46	12.6± 2.08	14.0± 1.70	13.6± 2.52	19.1± 0.80	21.2± 1.50	19.4± 0.75	18.5± 0.80	17.0± 0.74	32± 0.00
Gram negative bacteria											
Escherichia coli	13.7± 0.76	13.1± 2.43	14.2± 1.67	12.6± 0.80	12.8± 1.73	20.0± 1.52	17.8± 0.61	17.2± 0.47	18.1± 0.38	17.7± 0.55	25± 1.00
Pseudomonas aeruginosa	14.7± 0.50	12.8± 1.29	13.1± 0.87	13.3± 0.78	12.9± 0.31	14.1± 0.60	15.3± 0.61	14.5± 0.15	15.6± 0.50	12.2± 0.70	20± 1.00
Salmonella paratyphi	13.1± 0.76	14.0± 1.59	14.2± 1.67	12.6± 0.80	11.6± 0.36	19.4± 0.35	19.1± 0.73	17.9± 1.15	19.4± 0.70	15.9± 0.40	27± 0.58
S. typhi	13.4± 0.79	13.9± 0.97	15.0± 1.10	13.4± 1.12	11.3± 0.80	19.1± 0.38	17.6± 1.25	19.1± 0.29	16.4± 1.03	15.7± 0.56	22± 0.00
Shigella boydii	12.9± 1.22	13.1± 1.11	12.8± 0.76	14.5± 1.40	11.5± 0.35	19.4± 1.03	17.8± 0.23	18.8± 0.31	17.6± 0.27	15.8± 0.95	27± 0.58
Sh. dysenteriae	12.8± 2.02	13.3± 0.58	13.6± 1.26	11.1± 1.76	14.3± 0.80	19.3± 0.87	20.0± 0.21	18.3± 0.35	18.0± 0.61	15.9± 0.15	25± 0.58
Vibro miniscus	13.1± 1.04	13.5± 2.18	12.6± 1.15	13.5± 1.29	10.1± 0.76	19.6± 0.78	16.2± 1.80	19.8± 2.15	15.6± 0.70	14.8± 0.81	25± 0.58
V. parahemolyticus	14.8± 2.02	13.6± 1.53	12.9± 0.31	14.1± 1.53	11.1± 1.04	17.4± 0.42	18.7± 0.86	18.4± 1.03	16.5± 0.49	15.3± 1.01	20± 0.58
Fungus											**GRI**
Aspergillus niger	12.1± 0.76	13.1± 0.58	11.3± 0.76	12.3± 1.04	12.4± 0.97	11.3± 0.49	11.8± 0.40	15.6± 0.63	11.6± 1.02	11.9± 0.78	20± 0.00
Candida albicans	12.2± 0.75	12.6± 1.53	14.2± 0.44	13.3± 2.08	13.1± 0.76	15.4± 0.42	15.5± 0.65	11.2± 1.46	10.5± 0.79	11.8± 0.85	18± 0.58
Saccaromyces cerevacae	13.6± 1.53	12.8± 1.31	10.6± 1.22	13.4± 0.85	13.1± 0.76	14.0± 0.61	15.4± 0.67	13.1± 0.65	14.4± 0.46	13.1± 0.71	19± 0.58

MEBV: Crude methanolic extract of stem bark; HSFBV: n-hexane soluble fraction of methanol extract of stem bark; CSFBV: Carbon tetrachloride soluble fraction of methanol extract of stem bark; CHSFBV: Chloroform soluble fraction of the methanol extract of stem bark; AQSFBV: Aqueous soluble fraction of the crude methanol extract of stem bark, MELV: Crude methanolic extract of leaf; HSFLV: n-hexane soluble fraction of methanol extract of leaf; CSFLV: Carbon tetrachloride soluble fraction of methanol extract of leaf; CHSFLV: Chloroform soluble fraction of the methanol extract of leaf; AQSFLV: Aqueous soluble fraction of the crude methanol extract of leaf; PC: Positive control; KAN: Kanamycin disc and GRI: Griseofulvin disc

control.

Statistical analysis

For both antimicrobial activity and cytotoxicity screening three replicates of each sample were used for statistical analysis and the values were reported as mean ± SD (n=3).

RESULTS AND DISCUSSION

The organic soluble extracts of both stem bark and leaf

Table 3. Antimicrobial screening of the stem bark and leaf of *B. stipularis* extracts (400 µg/disc), Kanamycin (30 µg/disc) and Griseofulvin (20 µg/disc)

	Diameter of zone of inhibition (mm)										
	MEBS	**HSFBS**	**CSFBS**	**CHSFBS**	**AQSFBS**	**MELS**	**HSFLS**	**CSFLS**	**CHSFLS**	**AQSFLS**	**PC**
Gram positive bacteria											**KAN**
Bacillius cereus	18.5± 0.82	14.8± 0.95	13.8± 0.46	16.7± 1.15	17.2± 1.32	15.6± 0.89	18.2± 0.50	15.6± 0.61	16.7± 0.35	14.9± 0.78	35± 0.58
B. megaterium	17.5± 1.32	18.7± 0.85	21.0± 0.66	17.1± 1.36	17.4± 0.59	15.2± 0.70	17.5± 0.87	16.1± 0.87	15.6± 0.25	15.3± 0.76	35± 0.58
B. subtilis	21.1± 2.05	15.7± 0.35	17.7± 1.11	19.4± 0.50	16.9± 0.42	20.5± 1.12	19.7± 0.85	17.6± 0.61	21.3± 1.91	17.9± 0.65	36± 1.00
Sarcina lutea	21.0± 1.68	17.9± 0.74	18.7± 0.76	17.6± 0.32	16.8± 0.42	21.6± 0.68	18.1± 0.67	19.7± 0.57	17.5± 0.85	15.3± 0.40	27± 0.58
Staphylococcus aereus	23.0± 1.68	17.3± 0.45	15.6± 0.76	20.4± 0.85	17.5± 1.06	19.1± 0.80	21.2± 1.50	19.4± 0.75	18.5± 0.80	17.0± 0.74	32± 0.00
Gram negative bacteria											
Escherichia coli	19.1± 0.63	17.7± 0.25	20.8± 0.60	18.5± 0.77	17.6± 0.40	20.0± 1.52	17.8± 0.61	17.2± 0.47	18.1± 0.38	17.7± 0.55	25± 1.00
Pseudomonas aeruginosa	14.7± 0.50	12.8± 1.29	13.1± 0.87	13.3± 0.78	13.0± 0.31	14.1± 0.60	15.3± 0.61	14.5± 0.15	15.6± 0.50	12.2± 0.70	20± 1.00
Salmonella paratyphi	21.9± 0.86	18.9± 0.42	18.1± 0.67	19.7± 0.74	17.6± 1.26	19.4± 0.35	19.1± 0.73	17.9± 1.15	19.4± 0.70	15.9± 0.40	27± 0.58
S. typhi	20.7± 2.09	18.8± 0.17	18.8± 0.46	16.3± 1.40	17.3± 0.74	19.1± 0.38	17.6± 1.25	19.1± 0.29	16.4± 1.03	15.7± 0.56	22± 0.00
Shigella boydii	20.3± 1.29	18.7± 0.21	17.4± 0.42	18.7± 0.72	16.6± 0.46	19.4± 1.03	17.8± 0.23	18.8± 0.31	17.6± 0.27	15.8± 0.95	27± 0.58
Sh. dysenteriae	20.1± 1.12	17.5± 0.12	15.6± 0.47	20.0± 0.81	16.9± 0.55	19.3± 0.87	20.0± 0.21	18.3± 0.35	18.0± 0.61	15.9± 0.15	25± 0.58
Vibro miniscus	20.3± 0.91	17.9± 0.55	17.5± 0.51	22.3± 0.55	14.9± 1.11	19.6± 0.78	16.2± 1.80	19.8± 2.15	15.6± 0.70	14.8± 0.81	25± 0.58
V. parahemolyticus	17.4± 1.17	16.4± 0.31	16.9± 0.38	17.8± 0.42	11.9± 0.78	17.4± 0.42	18.7± 0.86	18.4± 1.03	16.5± 0.49	15.3± 1.01	20± 0.58
Fungus											**GRI**
Aspergillus niger	13.6± 2.07	11.9± 0.40	16.4± 0.64	15.1± 1.02	9.4± 0.46	11.3± 0.49	11.8± 0.40	15.6± 0.63	11.6± 1.02	11.9± 0.78	20± 0.00
Candida albicans	14.7± 0.4 0	13.0± 0.42	12.4± 1.45	11.1± 0.90	13.4± 0.66	15.4± 0.42	15.5± 0.65	11.2± 1.46	10.5± 0.79	11.8± 0.85	18± 0.58
Saccaromyces cerevacae	11.7± 1.45	14.1± 1.02	12.3± 1.62	12.7± 1.37	10.9± 0.70	14.0± 0.61	15.4± 0.67	13.1± 0.65	14.4± 0.46	13.1± 0.71	19± 0.58

MEBS: Crude methanolic extract of stem bark; HSFBS: n-hexane soluble fraction of methanol extract of stem bark; CSFBS: Carbon tetrachloride soluble fraction of methanol extract of stem bark; CHSFBS: Chloroform soluble fraction of the methanol extract of stem bark; AQSFBS: Aqueous soluble fraction of the crude methanol extract of stem bark, MEL: Crude methanolic extract of leaf; HSFLS: n-hexane soluble fraction of methanol extract of leaf; CSFLS: Carbon tetrachloride soluble fraction of methanol extract of leaf; CHSFLS: Chloroform soluble fraction of the methanol extract of leaf; AQSFLS: Aqueous soluble fraction of the crude methanol extract of leaf; PC: Positive control; KAN: Kanamycin disc and GRI: Griseofulvin disc

of *B. verrucosa*, *B. stipularis* and *B. tomentosa* were subjected to screening for antimicrobial activity by disc diffusion method and cytotoxicity by brine shrimp lethality bioassay. Antimicrobial screening of the methanolic crude extract along with its n-hexane, carbon tetrachloride, chloroform and aqueous soluble materials of the stem bark and leaf of *B. verrucosa*, *B. stipularis* and *B. tomentosa* showed low to strong activity in contrast to standard Kanamycin disc (Tables-2-4). In case of *B. verrucosa*, the inhibition zone was between the ranges of 9.6 to 21.6 mm indicating low to strong activity (Table-2). The methanol extract of the leaf exhibited the highest activity with the 21.6 ± 0.68 mm of the inhibition zone against *S. lutea*. The lowest

Table 4. Antimicrobial screening of the stem bark and leaf of *B. tomentosa* extracts (400 µg/disc), Kanamycin (30 µg/disc) and Griseofulvin (20 µg/disc)

Test bacteria	Diameter of zone of inhibition (mm)										
	MEBT	HSFBT	CSFBT	CHSFBT	AQSFBT	MELT	HSFLT	CSFLT	CHSFLT	AQSFLT	PC
Gram positive bacteria											**KAN**
Bacillius cereus	23.2± 1.47	17.8± 0.66	16.9± 1.10	19.0± 0.31	15.0± 0.49	18.0± 1.71	16.9± 0.75	19.1± 0.15	17.8± 0.56	15.1± 0.70	35± 0.58
B. megaterium	19.1± 0.60	17.5± 0.40	16.4± 0.50	17.3± 0.47	14.4± 0.74	21.6± 0.97	18.5± 0.87	20.1± 0.25	17.4± 0.91	15.6± 0.57	35± 0.58
B. subtilis	20.7± 0.55	16.8± 0.50	18.6± 0.61	19.4± 0.31	14.9± 0.17	22.0± 0.70	18.8± 0.35	18.3± 0.67	18.1± 0.68	13.2± 0.42	36± 1.00
Sarcina lutea	21.4± 0.50	18.1± 0.57	16.8± 0.61	18.9± 0.78	14.8± 0.31	19.4± 0.35	17.6± 0.27	18.1± 0.40	16.0± 0.42	14.6± 0.50	27± 0.58
Staphylococcus aereus	21.0± 0.95	18.1± 0.35	19.6± 0.59	17.6± 0.42	15.8± 0.46	19.9± 1.12	19.6± 0.50	18.8± 0.56	16.9± 0.68	16.7± 0.60	32± 0.00
Gram negative bacteria											
Escherichia coli	22.4± 0.35	17.9± 0.21	18.9± 0.57	17.8± 0.27	15.2± 0.51	20.9± 0.71	17.4± 0.42	16.6± 0.31	19.1± 0.25	16.3± 0.55	25± 1.00
Pseudomonas aeruginosa	16.6± 0.21	14.8± 0.50	13.8± 1.01	13.8± 0.65	12.7± 0.85	15.9± 0.40	14.0± 0.60	13.5± 0.15	14.8± 0.82	15.6± 0.63	20± 1.00
Salmonella paratyphi	21.2± 0.82	18.8± 0.50	17.5± 0.32	20.3± 0.53	15.2± 0.51	20.3± 0.96	18.1± 0.72	17.8± 0.78	17.0± 0.56	15.6± 0.31	27± 0.58
S. typhi	22.3± 0.75	17.6± 0.31	20.5± 0.38	17.3± 0.60	15.0± 0.31	20.9± 0.70	18.3± 0.85	16.9± 0.75	16.8± 0.42	14.8± 0.50	22± 0.00
Shigella boydii	21.3± 1.40	17.7± 0.66	16.9± 0.55	20.1± 0.45	15.4± 0.31	19.8± 0.42	16.8± 0.50	18.8± 0.42	16.8± 0.42	15.3± 0.45	27± 0.58
Sh. dysenteriae	20.9± 0.72	17.7± 0.56	15.9± 0.59	19.5± 1.05	16.8± 0.36	20.8± 0.76	17.5± 1.04	18.1± 0.92	17.3± 0.59	15.7± 0.32	25± 0.58
Vibro miniscus	21.2± 1.10	19.9± 0.47	18.2± 0.50	17.3± 0.40	14.4± 0.50	21.3± 0.72	17.2± 0.31	16.5± 0.32	18.3± 0.78	14.4± 0.23	25± 0.58
V. parahemolyticus	21.3± 0.59	18.7± 0.55	17.1± 0.67	18.1± 0.68	13.8± 1.29	22.1± 0.72	17.9± 0.45	16.9± 0.15	18.1± 0.55	14.2± 0.70	20± 0.58
Fungus											**GRI**
Aspergillus niger	13.6± 0.31	11.4± 0.83	10.2± 0.42	10.8± 0.50	13.2± 0.31	13.9± 0.30	11.9± 0.32	15.8± 0.31	14.7± 0.60	13.8± 0.71	20± 0.00
Candida albicans	13.4± 0.91	14.2± 0.61	15.6± 0.32	13.6± 1.14	10.2± 0.40	17.5± 0.17	15.1± 0.35	11.5± 1.60	12.2± 0.87	10.7± 1.65	18± 0.58
Saccaromyces cerevacae	11.4± 0.66	14.6± 0.31	12.6± 1.10	15.9± 0.17	12.9± 0.72	13.1± 0.72	14.2± 0.60	11.8± 0.56	13.5± 0.38	12.8± 0.46	19± 0.58

MEBT: Crude methanolic extract of stem bark; HSFBT: n-hexane soluble fraction of methanol extract of stem bark; CSFBT: Carbon tetrachloride soluble fraction of methanol extract of stem bark; CHSFBT: Chloroform soluble fraction of the methanol extract of stem bark; AQSFBT: Aqueous soluble fraction of the crude methanol extract of stem bark, MELT: Crude methanolic extract of leaf; HSFLT: n-hexane soluble fraction of methanol extract of leaf; CSFLT: Carbon tetrachloride soluble fraction of methanol extract of leaf; CHSFLT: Chloroform soluble fraction of the methanol extract of leaf; AQSFLT: Aqueous soluble fraction of the crude methanol extract of leaf; PC: Positive control; KAN: Kanamycin disc and GRI: Griseofulvin disc

activity of the inhibition zone 9.6 ± 1.53 mm was given by the *n*-hexane soluble fraction of the stem bark against *B. subtilis*. Again, *B. stipularis* showed mild to strong activity with zone of inhibition between the ranges of 11.9 to 23.0 mm (Table-3). The methanol extract of the stem bark exhibited the highest activity with the 23.0 ± 1.68 mm of the inhibition zone against *St. aereus*. The lowest zone of inhibition 11.9 ± 0.78

Table 5. Results of the brine shrimp lethality bioassay of the test samples of the *Bridelia* species available in Bangladesh.

Sample code	LC_{50} (µg/mL)	LC_{90} (µg/mL)	Sample code	LC_{50} (µg/mL)	LC_{90} (µg/mL)	Sample code	LC_{50} (µg/mL)	LC_{90} (µg/mL)
VS	0.45±0.04	10.0±0.02	MEBS	8.51±0.19	199.5±1.31	MEBT	12.02±0.38	87.1±1.85
MEBV	6.33±0.25	170.0±1.33	HSFBS	7.94±0.43	138.0±1.49	HSFBT	8.13±0.36	141.2±0.65
HSFBV	5.1±0.95	72.4±0.97	CSFBS	4.47±0.65	131.8±0.45	CSFBT	7.08±1.00	117.5±1.31
CSFBV	3.1±0.62	204.2±0.75	CHSFBS	1.2±0.51	112.2±2.2	CHSFBT	1.59±0.22	56.2±0.23
CHSFBV	0.71±0.14	43.7±0.95	AQSFBS	4.7±1.08	70.8±0.9	AQSFBT	4.47±0.73	112.2±0.4
AQSFBV	7.08±0.44	97.7±1.59	MELS	3.16±0.18	91.2±1.36	MELT	5.75±0.42	102.3±0.75
MELV	2.51±0.14	70.8±2.35	HSFLS	6.31±0.18	100.0±1.71	HSFLT	3.55±0.58	75.9±1.93
HSFLV	8.13±0.56	134.9±1.31	CSFLS	1.99±0.25	95.5±1.36	CSFLT	11.22±0.4	128.8±1.12
CSFLV	1.2±0.40	77.6±1.18	CHSFLS	3.98±0.98	102.3±0.76	CHSFLT	4.370.78	69.2±1.24
CHSFLV	3.13±0.36	58.9±0.40	AQSFLS	12.59±1.3	-	AQSFLT	8.51±1.21	107.2±0.41
AQSFLV	7.94±0.36	128.8±0.52						

VS: Vincristine sulphate; **B. verrucosa**, MEBV: Crude methanolic extract of stem bark of the plant; HSFBV: n-hexane soluble fraction of methanol extract of stem bark; CSFBV: Carbon tetrachloride soluble fraction of methanol extract of stem bark; CHSFBV: Chloroform soluble fraction of the methanol extract of stem bark; AQSFBV: Aqueous soluble fraction of the crude methanol extract of stem bark, MELV: Crude methanolic extract of leaf; HSFLV: n-hexane soluble fraction of methanol extract of leaf; CSFLV: Carbon tetrachloride soluble fraction of methanol extract of leaf; CHSFLV: Chloroform soluble fraction of the methanol extract of leaf; AQSFLV: Aqueous soluble fraction of the crude methanol extract of leaf; **B. stipularis,** MEBS: Crude methanolic extract of stem bark of the plant; HSFBS: n-hexane soluble fraction of methanol extract of stem bark; CSFBS: Carbon tetrachloride soluble fraction of methanol extract of stem bark; CHSFBS: Chloroform soluble fraction of the methanol extract of stem bark; AQSFBS: Aqueous soluble fraction of the crude methanol extract of stem bark, MELS: Crude methanolic extract of leaf; HSFLS: n-hexane soluble fraction of methanol extract of leaf; CSFLS: Carbon tetrachloride soluble fraction of methanol extract of leaf; CHSFLS: Chloroform soluble fraction of the methanol extract of leaf; AQSFLS: Aqueous soluble fraction of the crude methanol extract of leaf; **B. tomentosa**, MEBT: Crude methanolic extract of stem bark of the plant; HSFBT: n-hexane soluble fraction of methanol extract of stem bark; CSFBT: Carbon tetrachloride soluble fraction of methanol extract of stem bark; CHSFBT: Chloroform soluble fraction of the methanol extract of stem bark; AQSFBT: Aqueous soluble fraction of the crude methanol extract of stem bark, MELT: Crude methanolic extract of leaf; HSFLT: n-hexane soluble fraction of methanol extract of leaf; CSFLT: Carbon tetrachloride soluble fraction of methanol extract of leaf; CHSFLT: Chloroform soluble fraction of the methanol extract of leaf; AQSFLT: Aqueous soluble fraction of the crude methanol extract of leaf

mm was given by the aqueous soluble fraction of the stem bark against *V. parahemolyticus*. For the plant, *B. tomentosa*, the zone of inhibition was between the ranges of 13.2 to 23.2 mm indicating moderate to strong activity (Table-4). The methanol extract of the stem bark exhibited the highest activity with the zone of inhibition of 23.2 ± 1.47 mm against *B. cereus* and the lowest zone activity 13.2 ± 0.42 mm of inhibition zone was given by the aqueous soluble fraction of the leaf against *B. subtilis*.

During antifungal screening, the extractive of the stem bark and leaf of *B. verrucosa*, *B. stipularis* and *B. tomentosa* showed low to significant activity in comparison to Griseofulvin with inhibiton zone being between the ranges of 9 to 17.5 mm. The activity of most of the test sample against the fungal strain *Candida albicans* was most significant; highest being 17.5 mm with the methanol extract of *B. tomentosa* leaf (Tables-2- 4).

In case of brine shrimp lethality bioassay the LC_{50} and LC_{90} values obtained from the best-fit line slope are shown in Table 5 for the extracts of *B. verrucosa*, *B. stipularis* and *B. tomentosa*. Here, the % mortality was found to increase gradually with the increase in concentration of the test samples. In comparison to positive control (vincristine sulphate), the cytotoxicity exhibited by chloroform soluble fraction of methanol extract of the stem bark and carbon tetrachloride soluble materials of methanol extract of the leaf of *B. verrucosa* and the chloroform soluble materials of methanol extract of the stem bark of *B. stipularis* was highly significant with LC_{50} values of 0.71 ± 0.14, 1.2 ± 0.40 and 1.2 ± 0.51 µg/mL, respectively. On the other hand, the LC_{90} values of the chloroform soluble materials of the methanol extract of the stem bark of *B. verrucosa* and *B. tomentosa* and the leaf of *B. verrucosa* LC_{90} were 43.7 ± 0.95, 58.9 ± 0.40 and 56.2 ± 0.23, respectively.

It is evident from the above study that the extracts of both the leaf and the stem bark of *B. verrucosa* and the leaf of *B. stipularis* have potential antimicrobial and cytotoxic activities. In general, the mechanisms by which microorganisms survive and the action of antimicrobial agents are poorly understood and remain debatable. On the other hand, the chemical constituents of these extracts may have a casual role in the *in vivo* prevention of diseases caused by bacteria, fungi and yeast. Nevertheless, this scientific information can serve as an important platform for the development of safe and effective natural medicine. So, further investi-

igation is underway to isolate the promising bioactive compounds from these three plants.

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