Biological Screening of *Zizyphus rugosa* and *Zizyphus oenoplia* extractives

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ABSTRACT: Different extracts of the leaves and barks of *Zizyphus rugosa* and *Zizyphus oenoplia* were studied for their antibacterial, antifungal, and β-glucuronidase inhibitory activities. The methanol extract of *Z. rugosa* bark showed significant antibacterial activity against *Streptococcus pyogens, Staphylococcus aureus and Pseudomonas aerogenes* whereas the methanol extract of leaves demonstrated moderate activity against *Salmonella typhi*. The chloroform and methanol extracts of *Z. oenoplia* showed good activity against a few bacteria strains. The chloroform extracts of the barks and leaves of *Z. rugosa* also showed antifungal activity. The methanol and ethyl acetate extracts of the bark of *Z. rugosa* revealed significant β-glucuronidase inhibitory activity. Lupeol, betuline, betulinaldehyde and betulinic acid, isolated from *Z. rugosa*, also showed good activity against a few bacteria.

Key words: *Zizyphus rugosa*, *Zizyphus oenoplia*, Antibacterial, Antifungal, β-glucuronidase inhibition

INTRODUCTION

Infectious diseases are one of leading cause of premature death. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world due to indiscriminate use of antibiotics.[1] New therapeutic agents are of great demand. Many infectious diseases are known to be treated with herbal medicines throughout the human civilization. Even today, plant materials continue to play major role in primary health care and higher plants have been shown to be potential sources for the new anti-microbial agents.[2] Different species of the genus *Zizyphus* like *vulgaris*, *sativa*, *jujuba*, *etc*. belong to the family Rhamnaceae showed hypoglycemic activity.[3,4] In continuation of our work on screening of biologically active plant materials *Zizyphus rugosa* and *Z. oenoplia* have been chosen for studies of their antibacterial, antifungal, and β-glucuronidase inhibitory activities for discovery of new therapeutic agent(s) from natural sources. No previous report on biological activity of these two species is available.

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MATERIALS AND METHODS

Plant Materials. Bark and leaves of *Zizyphus rugosa* and *Z. oenoplia* (Rhamnaceae) were collected from the forest of Madhupur, Bangladesh and was identified by the scientist of Bangladesh National Herbarium (BNH), where voucher specimen has been deposited (BNH accession No. 29448). The leaves and bark were cleaned, air-dried followed by drying in an oven at 40° C. The dried leaves and bark were powdered separately by grinding in a cyclotec-grinding machine (200 mesh).

Extraction. The powdered leaves (1.6 kg) was extracted with chloroform (4L x 3, 24 h) followed by methanol (3L x 3, 24 h) at room temperature. The extracts were evaporated to dryness using a rotary

vacuum evaporator and finally freeze-dried to get 34.6 g and 120 g extracts, respectively. A portion of the methanol extract (50 g) was suspended in water (400 mL). The aqueous suspension was treated with ethyl acetate (3 x 200 mL) and the organic layer was separated. The remaining aqueous part was further partitioned with 1-butanol (3 x 200 mL). The ethyl acetate and 1-butanol extracts were evaporated to dryness to obtain 17 g and 10 g extracts, respectively. The powdered bark (2 kg) was also extracted in a similar way to get 37.5 g of chloroform, 130 g of methanol and 14 g of ethyl acetate extracts. The extracts were tested for their antibacterial, antifungal, and β-glucuronidase inhibition activities.

Antibacterial Assay. The agar well diffusion protocol was used to test antibacterial activity[5] against six bacterial strain, *Bacillis subtilis, Streptococcus pyogens, Stapylococcus aureus* (Gram-positive) and *Escherichia coli, Pseudomonas aerogenes, Salmonella typhi* (Gram-negative). Tetracycline (0.5 mg/mL) was used as reference or positive control while DMSO without sample was used as negative control. Antibacterial activity is given in Table 1 and 2.

Test for Antifungal Activity. The agar tube dilution method was applied for determination of the antifungal activity[6] against six pathogenic fungi, *Trichophyton longiformis, Candida albicans, Aspergillus flavis, Microsporum canis, Fusarium solani and Fusarium moniliformis*. Miconazole was used as positive control. Antifungal activity of the extracts is presented in the Table 3.

β-glucuronidase Inhibition Assay. Enzyme inhibitory activity of the testing samples against β-glucuronidase (Sigma) was determined according to Kawasaki[7] *et al* with some modification. The reaction mixture contained 0.9 mL of 0.1 M acetate buffer (pH=5.0), 0.03 mL of *p*-nitrophenyl-β-D-glucuronide (0.4 mM) and 30 units of enzyme. After incubation for 30 min at 37° C the reaction mixture was interrupted by the addition of 0.1 mL sodium carbonate (0.2 M). The increase of absorbance with the release of *p*-nitrophenol from the *p*-nitrophenyl-β-D-glucuronide was measured at 403 nm by means of a spectrophotometer (Spectramax 340). Saccharic acid 1,4-lactone was used as a standard inhibitory agent for positive control. Results are given in the Table 4.

RESULTS AND DISCUSSION

The methanol extract of *Z. rugosa* bark showed significant antibacterial activity against *Streptococcus pyogens, Staphylococcus aureus* and *Pseudomonas aerogenes* by showing zone of inhibition 18, 18 and 20 mm, respectively, whereas the methanol extract of the leaves was significantly active against only one bacteria, *Salmonella typhi* (18 mm) (Table 1). Chloroform extract of bark showed significant inhibition against *Staphylococcus aureus* (25 mm) but good inhibition against *Streptococcus pyogens* (15 mm), *Pseudomonas aerogenes* (15 mm) and *Salmonella typhi* (15 mm). On the other hand the chloroform extract of leaves of *Z. rugosa* showed good inhibition of growth of few bacteria. Methanol and chloroform extracts of *Z. oenoplia* also showed good activity against a few bacteria but none of them was significant.

The chloroform extract of bark gave significant (88%) antifungal activity against *Microsporum canis* and that of leaves against *Fusarium solani* (88%). The ethyl acatate extract of leaves showed significant antifungal activity against *Fusarium solani* (83%). Methanol extracts of both leaves and bark were found to be inactive (Table 3).

β-glucuronidase has been discovered in animals, plants and bacteria[8] which catalyzes the hydrolysis of β-glucuronides produced in the body such as benzo[α]pyreneglucuronides. Pineda *et. al*. demonstrated that liver damage caused an increase in the enzyme in blood and liver cancer could be related to the enzyme[9]. Methanol and ethyl acetate extracts of the bark of *Z. rugosa* showed significant β-glucuronidase inhibition activity (100%) while they were tested using saccharic acid 1,4-lactone as a standard inhibition for positive control (Table 4). It can be expected that these extracts can reduce the risk factor of liver cancer by inhibiting the hydrolysis to

glucuronides of proximate metabolites and active β-glucuronides enzyme inhibitors can be isolated from them. Chloroform and methanol extract of leaves of *Z. oenoplia* showed good activity (60% and 74%, respectively) whereas bark extract was found to be inactive. Lupeol, betuline, betulinaldehyde and betulinic acid isolated[10] earlier from *Z. rugosa* also showed good activity against a few bacteria (Table 2).

The biological activities exhibited by different extracts of *Z. rugosa* and *Z. oenoplia* might be due to the presence of different types of chemical components in the extracts.

Table 1. Antimicrobial activity of plant extracts (zone of inhibition in mm)

Plant	Part	Extract	Microorganisms[b]					
			Bs	Sp	Sa	Ec	Pa	St
Z. rugosa	bark	Chloroform	14[a]	15	25	14	15	15
		Methanol	16	18	18	15	20	14
Z. rugosa	leaves	Chloroform	15	16	14	16	15	--
		Methanol	17	15	14	--	14	18
Z. oenoplia	bark	Chloroform	14	14	13	15	0	0
		Methanol	15	14	15	12	--	15
Z. oenoplia	leaves	Chloroform	16	15	14	14	--	0
		Methanol	15	11	14	15	--	17
Tetracycline			34	30	30	32	28	30

[a]Activity key: -- (no inhibition), 11-14 mm (inactive), 15-17 mm (good), 18-above (significant)
[b]Microorganisms: Bs-*Bacillus subtilis*, Sp-*Streptococcus pyogens*, Sa-*Staphylococcus aureus*, Ec-*Escherichia coli*, Pa-*Pseudomonas aerogenes*, St-*Salmonella typhi*

Table 2. Antimicrobial activity of pure compounds (zone of inhibition in mm)

Compounds	Plant	Microorganisms[b]					
		Bs	Sp	Sa	Ec	Pa	St
Lupeol	*Z. rugosa*	16[a]	13	16	17	17	20
Betuline	*Z. rugosa*	20	15	14	15	17	17
Betulinaldehyde	*Z. rugosa*	14	--	--	16	17	--
Betulinic acid	*Z. rugosa*	14	12	--	15	14	--

[a]Activity key: -- (no inhibition), 11-14 mm (inactive), 15-17 mm (good), 18-above (significant)
[b]Microorganisms: Bs-*Bacillus subtilis*, Sp-*Streptococcus pyogens*, Sa-*Staphylococcus aureus*, Ec-*Escherichia coli*, Pa-*Pseudomonas aerogenes*, St-*Salmonella typhi*

Table 3. Percent inhibition of microbial growth in presence of extracts

Plant	Part	Extract	Microorganisms[b]					
			Af	Fs	Tlon	Fmon	Ca	Mca
Z. rugosa	bark	Chloroform	0[a]	0	0	0	0	88
		Methanol	0	20	5	5	5	11
		EtOAc	11	10	ng	ng	15	0
Z. rugosa	leaves	Chloroform	ng	88	5	5	5	0
		Methanol	ng	20	5	5	5	0
		EtOAc	0	83	ng	ng	15	10
Z. oenoplia	bark	Chloroform	ng	0	0	0	20	0
		Methanol	ng	0	0	ng	0	0
Miconazole			100	100	100	100	100	100

[a]Activity key: ng (not good), 0-25% (no inhibition), 25-50% (not significant), 50-60% (modarate), 60-80% (good), 80-100% (highly significant)
[b]Microorganism: Af-*Aspergillus flavis*, Fs-*Fusarium solani*, Tlon-*Tricohophyton longiformis*, Fmon-*Fusarium moniliformis*, Ca-*Candida albicans*, Mca-*Microsporum canis*

Table 4. Percent inhibitory activity of plant extracts against β-glucuronidase

Plant	Part	Extract	% Inhibition[a]
Z. rugosa	bark	Chloroform	57
		Methanol	100
		EtOAc	100
Z. rugosa	leaves	Chloroform	63
		Methanol	54
		EtOAc	04
Z. oenoplia	bark	Chloroform	36
Z. oenoplia	leaves	Chloroform	60
		Methanol	74

[a]Inhibition: 0-50% (inactive), 50-70% (moderate), 70-85% (good), 85-100% (significant)

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