Antibacterial, Insecticidal and Free radical scavenging activity of methanol extract of *Ziziphus rugosa* Lam. (Rhamnaceae) fruit pericarp

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ABSTRACT

Introduction: *Ziziphus rugosa* Lam. belongs to the family Rhamnaceae and is found chiefly in deciduous and semi-evergreen forest of Western Ghats. The present study was undertaken to determine antibacterial, insecticidal and free radical scavenging activity of methanol extract of *Ziziphus rugosa* Lam. fruit pericarp. **Methods:** The powdered fruit pericarp of *Z. rugosa* was extracted with methanol. Antibacterial activity of methanol extract was determined against *Escherichia coli* and *Staphylococcus aureus* by Agar well diffusion method. Free radical scavenging activity was determined using DPPH assay. The insecticidal activity of extract was tested against second instar larvae of *Aedes aegypti*. **Results:** The extract exhibited dose dependent inhibition of test bacteria. Among bacteria, *E. coli* was found to be more susceptible to extract than *S. aureus*. All the concentrations of extract produced over 50% mortality of larvae and the larvicidal effect was found to be dose dependent. The extract caused 100% mortality of larvae at concentration of 50 mg/ml. The extract exhibited concentration dependent radical scavenging activity with an IC_{50} value of 61.88 µg/ml. The phytochemical analysis of extract showed the presence of alkaloids, saponins, flavonoids and glycosides. **Conclusion:** The extract, in suitable form, may be used to control bacterial diseases, free radical damage and arboviral diseases. The phytoconstituents present in the extract may be responsible for the tested biological efficacies of extract. Further studies on isolation of active constituents from the extract and their biological activity are under investigation.

Key words: *Ziziphus rugosa* Lam., Agar well diffusion, Free radical scavenging activity, DPPH assay, Insecticidal activity, *Aedes aegypti*

INTRODUCTION

Ziziphus rugosa Lam. belongs to the family Rhamnaceae. It is a large straggling scandent armed shrub with large elliptic usually subcordate leaves, paniculate flowers and wood is reddish, moderately hard and fruit is small drupe, glabrous, white when ripe. The plant is found chiefly in deciduous and semi-evergreen forest of Western Ghats and is commonly called as suran in Hindi, chunu koli in Urdu and Badara in Sanskrit and in local language is called as belamarluhanu. Bark is astringent and antidiarrhoeal. Flowers are used in prescriptions for menorrhagia. Stem and fruit are hypotensive. The bark contains vanillic acid, betulin, betulinic acid, kaempferol, quercetin, myricetin, apigenin and apigenin-7-O-glucoside. The bark also contains several N-formyl cyclopeptide alkaloids. The triterpene saponins isolated from the bark showed CNS depressant, tranquilizing and analgesic activity in albino rats and produced no hepatotoxicity. The cyclopeptide alkaloids of the plant show antibacterial as well as antifungal activity.[1,2] The present study was undertaken to determine antibacterial, insecticidal and free radical scavenging activity of methanol extract of fruit pericarp of *Z. rugosa*.

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MATERIALS AND METHODS

Collection and identification

The fruits of *Z. rugosa* were collected from the Doddabetta forest range (located between 12°49'N and 75°57'E longitude) of Sakaleshpura, Hassan district, Karnataka and authenticated by Prof. K.G. Bhat, Udupi, Karnataka. The voucher specimen (KU/AB/KSV/75) deposited in the department of Botany, Jnanasahyadri, Shankaraghatta-577451, Karnataka for future reference.

Extraction and Phytochemical analysis

For extraction, about 50 g of the dried and powdered seed material was taken and added to 100 ml of methanol. The mixture was sonicated for 30 min and then left at room temperature overnight. The extracts were filtered over Whatman No 1 filter paper and the filtrates were concentrated under reduced pressure to pasty mass. The methanol extract was subjected to chemical tests to screen the presence of various secondary metabolites.[3,4]

Antibacterial activity of methanol extract

The antibacterial efficacy of methanol extract of fruit pericarp was tested against bacteria namely *Staphylococcus aureus* MTCC-902 and *Escherichia coli* MTCC-405 obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India by Agar well diffusion method.[5] In this method, 24 hours old Nutrient broth cultures of test bacteria were swabbed uniformly on solidified sterile Nutrient agar plates using sterile cotton swab. Then, aseptically wells of 6 mm diameter were bored in the inoculated plates with the help of gel puncher and the different concentrations of extract (5, 10, 25 and 50 mg/ml of 10% DMSO), Standard (Chloramphenicol 1 mg/ml) and Control (10% DMSO) were added into the respectively labeled wells. The plates were incubated at 37°C for 24 hours in upright position. The experiment was carried in triplicates and the zone of inhibition was recorded.

Insecticidal activity of methanol extract

For determining insecticidal activity, the extract was dissolved in 10% Dimethyl sulfoxide (DMSO) to get different concentrations of extract namely 5, 10, 25 and 50 mg/ml. The insecticidal efficacy of methanol extract was determined against second instar larvae of *Aedes aegypti*. Twenty larvae were placed separately into beakers containing different concentrations of extract. A beaker containing DMSO without extract serves as control. The larvicidal effect of extracts was determined by counting the number of dead larvae after 24 hours, 48 hours and 72 hours. The test was repeated thrice and the percentage of larval mortality was calculated.[6]

Free radical scavenging activity of methanol extract

The antioxidant activity, in terms of radical scavenging ability, of different concentrations of methanol extract and the standard (Ascorbic acid) was tested on the basis of the radical scavenging effect of the stable 1,1-diphenyl-2-picryl-hydrazyl (DPPH)-free radical activity.[7] Different concentrations namely 25, 50, 100, 250 and 500 µg/ml of methanol extract and standard were prepared in methanol. 0.002% of DPPH was prepared in methanol. In clean and labeled test tubes, 2 ml of DPPH solution was mixed with 2 ml of different concentrations of solvent extracts and standard separately. The tubes were incubated at room temperature in dark for 30 minutes and the optical density was measured at 517 nm using UV-Vis Spectrophotometer. The absorbance of the DPPH control (containing no sample) was also noted. The degree of stable DPPH* decolorization to DPPHH (reduced form of DPPH) yellow indicated the scavenging efficiency of the extract. The scavenging activity of the extract against the stable DPPH* was calculated using equation: Scavenging activity in % = A – B / A × 100 [Where A is the absorbance of control and B is the absorbance of test/standard].

RESULTS

The preliminary phytochemical analysis of methanol extract of fruit pericarp showed the presence of alkaloids, saponins, flavonoids and glycosides. The result of antibacterial activity of methanol extract of *Z. rugosa* fruit pericarp is presented in Table 1. Results were recorded as presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of bacterial growth and it as reported as positive and absence of zone as negative. The extract exhibited dose dependent inhibition of test bacteria. Among bacteria, *E. coli* was found to be more susceptible to extract than *S. aureus* as revealed by wider zones of inhibition. The inhibition caused by standard was found to be higher than all the extract concentrations. Control did not reveal any inhibition of test bacteria.

Table 1: Antibacterial activity of methanol extract of *Z. rugosa* fruit pericarp

Treatment	Concentration	Zone of inhibition in mm	
		E. coli	*S. aureus*
Methanol extract	5 mg/ml	14	11
	10 mg/ml	22	16
	25 mg/ml	24	20
	50 mg/ml	26	24
Standard	1 mg/ml	28	27
Control	10%	-	-

Table 2: Insecticidal activity of methanol extract of *Z. rugosa* fruit pericarp

concentration (mg/ml)	Total no. of larvae	No. of dead larvae	% mortality of larvae
0 (Control)	20	00	00.00
5	20	10	50.00
10	20	15	75.00
25	20	18	90.00
50	20	20	100.00

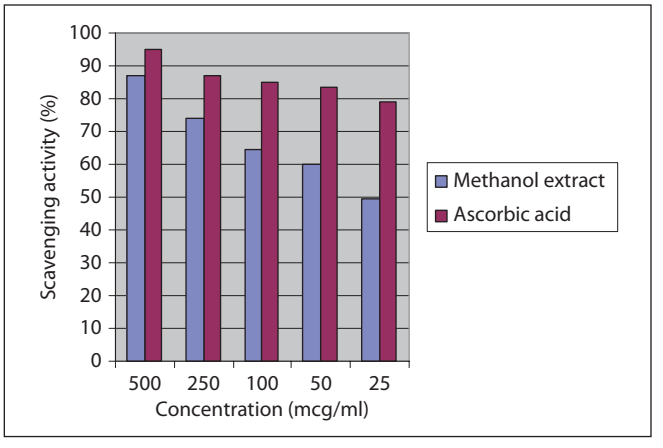

Figure 1: Free radical scavenging activity of methanol extract of *Z. rugosa* fruit pericarp

Insecticidal activity, in terms of larvicidal effect, of methanol extract on second instar larvae of *A. aegypti* is shown in Table 2. The larvicidal effect of extract was determined after 24 hours. Dead larvae were identified when they failed to move after probing with a needle in siphon or cervical region. All the concentrations of extract tested produced over 50% mortality of larvae. The methanol extract caused 100% mortality of larvae at concentration of 50 mg/ml.

The antioxidant activity, in terms of free radical scavenging activity, of different concentrations of seed extract is shown in Figure 1. The extract exhibited marked antioxidant activity by scavenging DPPH* (free radical) and converting into DPPHH. The extract exhibited concentration dependent radical scavenging activity i.e., higher the concentration, more scavenging potential. The methanol extract was able to reduce the stable free radical DPPH to the yellow colored diphenylpicrylhydrazine with an IC_{50} value of 61.88 µg/ml. The scavenging activity of ascorbic acid was greater than extract.

DISCUSSION

The plant *Z. rugosa* has been worked out for novel medical important compounds. Rugosanine-A, a cyclopeptide alkaloid, has been isolated from the stem bark of *Z. rugosa*.[8] A new glycoside zizyphoside has been isolated along with the betulic oleanolic, alphitolic and 2-α-hydroxy xyrusolic acids; zizyphoside on hydrolysis yielded altered aglycone, ebelin lactone.[9] Three flavonolds - kaempferol-4'-methylether, luteolin and luteolin-7-O-glucoside have been isolated from the barks of *Z. rugosa* and their structures were established by spectral evidences.[10] The flowers of *Z. rugosa* are extensively used for the treatment of hemorrhage and menorrhea. Fruit is edible and it also used to treatment of rheumatism and the decoction of the bark is used to heal the wounds and used for diarrhea.[2] 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 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 b-glucuronidase inhibitory activity. Lupeol, betuline, betulinaldehyde and betulinic acid, isolated from *Z. rugosa*, also showed good activity against a few bacteria.[11]

In many developing countries about 80% of available drugs come from medicinal plants and in industrialized countries plants make up the raw material for processes, which synthesize pure chemical derivatives.[12] The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds.[13] Plant derived products have received attention in recent years due to their diverse pharmacological activities.[14] Antimicrobial activity of tannins,[15] flavonoids,[16] saponins,[17] terpenoids[18] alkaloids[19] have been documented. In the present study, phytoconstituents namely alkaloids, saponins and flavonoids were detected in the extract which may account for the antibacterial activity.

Free radicals are found to be a product of normal metabolism. Although oxygen is essential for aerobic forms of life, oxygen metabolites are highly toxic. As a consequence, reactive oxygen species (ROS) are known to be implicated in many cell disorders and in the development of many diseases including cardiovascular diseases, atherosclerosis, chronic inflammation etc.[20,21] Although organisms have endogenous antioxidant defences produced during normal cell aerobic respiration against ROS, other antioxidants are taken both from natural and synthetic origin.[22] Antioxidants that can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction, therefore, appear to be very important.[23] Synthetic antioxidants are widely used but their use is being restricted nowadays because of their toxic and carcinogenic effects. Thus, interest in finding natural antioxidants, without any undesirable effect, has increased greatly.[22] There are several methods available to assess

antioxidant activity of compounds. An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1, diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. The hydrogen atom- or electron donating ability of extract and some pure compounds were measured from the bleaching of the purple colored methanol solution of DPPH. In presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases.[24] DPPH is relatively stable nitrogen centred free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule.[25] DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses color stoichometrically depending on the number of electrons taken up.[26] Though the DPPH radical scavenging abilities of the extracts were less than that of ascorbic acid, the study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. Thus, the methanolic extract of fruit pericarp can be a potential candidate to be explored for the treatment of damage caused by free radicals.

Mosquitoes are considered as a major health menace as they serve as disease transmitting vectors to humans and animals. Several mosquito species belonging to the genera *Anopheles*, *Aedes* and *Culex* are vectors of pathogens of various diseases such as malaria, filariasis, Japanese encephalitis, dengue, chickungunya etc. The approach to combat these diseases largely relied on interruption of the disease transmission cycle by either targeting the mosquito larvae through spraying of stagnant water breeding sites or by killing the adult mosquitoes using insecticides. The control of mosquito borne diseases is becoming difficult nowadays due to increasing resistance to pesticides, lack of vaccines and drugs to treat diseases transmitted by them. Hence, an alternative approach to control mosquitoes is the use of plant extracts. Search for natural insecticides, which are easily degradable and do not have any ill effects on the non-target population, remains one of the top priority issues for many countries. It is observed that the carbohydrates, saponins, phytosterols, phenols, flavonoids and tannins are having mosquito larvicidal activity. Prenylated xanthones, tetracyclic phenols and saponins are reported to be effective in controlling mosquito *A. aegypti*, the vector of yellow fever.[6, 27-30] In this study, the crude extracts have exhibited potent activity in terms of causing mortality of larvae. The presence of phytoconstituents such as saponins, flavonoids and others were detected in this study which might be responsible for the mortality of larvae. The death of larvae was observed within short period of time and thus could be used to control mosquito vectors and diseases transmitted by them.

CONCLUSION

The phytoconstituents present in the extract might be responsible for the tested biological efficacies of extract. The extract, in suitable form, could be used against bacterial diseases, free radical damage and arboviral diseases like chickungunya, dengue etc. Further studies on isolation of active constituents from the extract and their biological activity are under investigation.

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