ISSN 0976 – 3333

Available Online at www.ijpba.info.

International Journal of Pharmaceutical & Biological Archives 2011; 2(3):887-890

ORIGINAL RESEARCH ARTICLE

Evaluation of Pericarp and Seed Extract of *Zizyphus rugosa* Lam. for Cytotoxic Activity

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Received 25 Apr 2011; Revised 28 May 2011; Accepted 13 Jun 2011

ABSTRACT

Ziziphus rugosa Lam. belongs to the family Rhamnaceae. It is commonly called as suran in Hindi, chunu koli in Urdu and Badara in Sanskrit and in local language is called as belamarluhanu. In the present study, we have investigated the cytotoxic potential of different concentrations (10-1000 µg/ml) pericarp and seed extract of *Z. rugosa* in terms of lethal effect on the brine shrimp *Artemia nauplii*. The degree of lethality was found to be directly proportional to the concentration of the extract. Seed extract showed potent cytotoxicity (LC_{50} of 564.73µg/ml) and thus the seed extract is toxic. Highest mortality by pericarp extract was only 22% at 1000µg/ml concentration and thus is non-toxic. The extracts have shown bioactivity in terms of causing mortality of brine shrimps and is an addition to the scientific literature on the plant. The cytotoxic activity could be due to the phytoconstituents present in the extracts.

Key words: *Zizyphus rugosa* Lam., Cytotoxicity, *Artemia nauplii*, LC_{50}

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 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* [3]. 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 [4]. Three flavonoids - 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 [5]. 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 [1]**.** 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

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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 [6]. In a previous study, the methanol extract of pericarp was found to possess marked antibacterial, larvicidal and free radical scavenging activity [7]. Literature survey carried on the cytotoxic activity of *Z. rugosa* fruit by brine shrimp lethality bioassay revealed that this bioactivity remains unexplored. In this study, we have investigated the cytotoxic potential of pericarp and seed extract in terms of its lethal effect on the brine shrimp.

MATERIALS AND METHODS

Collection and Identification

The fruits of *Z. rugosa* were collected from the Doddabetta forest range (located between 12º49'N and 75º57'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 50g of the dried and powdered pericarp and seed material was taken and added to 100 ml of methanol in separate containers. The mixtures were sonicated for 30 min and then left at room temperature overnight. The extracts were filtered through Whatman No 1 filter paper and the filtrates were concentrated under reduced pressure to pasty mass [8].

Cytotoxic Activity of Methanol Extract by Brine Shrimp Lethality Test

The brine shrimp lethality test was conducted according to the method of Raghavendra *et al* [9]. Brine shrimp *Artemia nauplii* eggs (Nihon Animal Pharmaceutical Inc., Tokyo, Japan) were hatched in a container filled with air-bubbled artificial sea water which was prepared with 10g of a commercial salt mixture (GEX Inc., Osaka, Japan) and 500 ml of distilled water. After 36-48 hours, the phototropic shrimps were collected by pipette for bioassay. The different concentrations of methanol extract (10-1000µg/ml) were tested in vials containing 5ml of brine and 25 shrimp in each of three replicates. The vials were incubated at 25°C and surviving shrimps were counted after 24 hours. LC_{50} values greater than 1000µg/ml for plant extracts were considered inactive (non-toxic).

RESULTS AND DISCUSSION

The result of cytotoxic activity of methanol extract of pericarp and seed of *Z. rugosa* in terms of mortality of brine shrimps is presented in (**Table 1**). The degree of lethality was found to be directly proportional to the concentration of the extract. Among extracts, seed extract showed potent cytotoxicity with LC_{50} of 564.73µg/ml and thus the seed extract is toxic. Highest mortality by seed extract was observed at 1000µg/ml concentration (87% mortality) where as no mortality was observed at 10µg/ml. Pericarp extract showed 22% of mortality at 1000µg/ml concentration and thus found to be non-toxic. There was no mortality of shrimps by 10µg/ml extract concentration of both seed and pericarp.

Table 1: Brine shrimp lethality of pericarp and seed extract of *Z. rugosa*

Extract	Concentration (µg/ml)	Mortality (%)	LC_{50} (µg/ml)
Pericarp	10	0.0	-
	100	13.0	
	1000	22.0	
Seed	10	0.0	564.73
	100	18.0	
	1000	87.0	

LC_{50} values were determined by using linear regression.

Bioactive compounds are almost always toxic in high doses. *In vivo* lethality in a simple zoologic organism can be used as a convenient monitor for screening and fractionation in the discovery and monitoring bioactive natural compounds. The brine shrimp lethality test is considered to be very useful in determining various biological activities such as cytotoxic, phototoxic, pesticidal, trypanocidal, enzyme inhibition, and ion regulation activities [10-15]. This is a rapid method utilizing only 24 hours, inexpensive and needs no special equipment. It is so simple that no aseptic technique is required. It utilizes a large number of organisms for validation and a relatively small amount of sample. It does not require animal serum as needed for other methods of cytotoxicity testing [16]. This bioassay has been employed to determine cytotoxic activity of several plant extracts.

The brine shrimp assay is very useful tool and is attractive because it is very simple, inexpensive and low toxin amounts are sufficient to perform the test. The assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumor properties. The brine shrimp lethality assay is based on the ability of the extract to show lethality in laboratory cultured *Artemia nauplii* brine shrimp. It is considered as useful tool for preliminary assessment of toxicity. It has also been suggested for screening pharmacological activities of plant extracts [17,18]. Several studies have been carried on brine shrimp lethality of extracts from natural sources. In a study, Raghavendra *et al*. [9] showed cytotoxic activity of methanol Extract of *Putranjiva roxburghii* Wall (Euphorbiaceae) Seeds. The extract was found to be toxic with LC_{50} of 427.74 µg/ml. The cytotoxicity of methanol extract of leaves of *Abrus pulchellus* Wall (Fabaceae) using brine shrimp lethality bioassay revealed dose dependent activity with LC_{50} of 281.70µg/ml [19].

CONCLUSION

Although the brine shrimp lethality assay is rather inadequate regarding the elucidation of the mechanism of action, it is very useful to assess the bioactivity of the plant extracts. In the course of our studies, the brine shrimp lethality assay actually has proven to be a convenient system for monitoring biological activities. In the present study, the extracts have shown potential bioactivity in terms of causing mortality of brine shrimps which is an addition to the scientific literature on the plant. The cytotoxic activity could be due to the phytoconstituents present in the extracts. Further detailed investigations on pharmacological activities and active ingredients responsible for bioactivity could provide leads to interesting pharmaceuticals.

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