

EVALUATION OF ANTIFUNGAL ACTIVITY OF STONES OF *ZIZIPHUS JUJUBA*

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## ABSTRACT

The plant *Ziziphus jujuba* is widely used in Ayurvedic system of medicine as delicious fruit and an effective herbal remedy. It used as liver tonic, diuretic, emollient, expectorant, anticancer, sedative, as blood purifier and in treatment of diarrhoea. Through literature survey it has been revealed that the paste of the stones of the plant is beneficial in treatment of ringworm infection. Thus, the study is carried out in order to determine the antifungal (ringworm) activity of the stones of the plant. The stones of the plant are subjected to extraction with water and ethanol. The antifungal activity was carried out using agar well diffusion method. All the plant extracts were compared with standard Clotrimazole (1% w/w) as positive control and distilled water, ethanol used as negative control. Accordingly ethanolic extract of the roots was found to more effective against *Trichophyton rubrum* as compared to aqueous extract

**KEYWORDS:** Antifungal, Clotrimazole, *Trichophyton rubrum*, *Ziziphus jujuba*.

## INTRODUCTION

Various species of *Ziziphus* are used medicinally in India, China and Japan. The plant *Ziziphus jujuba* is also known as Ber, jujube. It taxonomically belongs to the family Rhamnaceae. The *Z. jujuba* (L.) Pers. mostly found almost all parts of areas.<sup>[1]</sup> The leaves used for hypoglycemic effects, reduction of sweetness judgements, as diuretic, emollient, expectorant, to promote hair growth, anticancer, sedative, blood purifier and in treatment of diarrhoea.<sup>[2-4]</sup> Fruits used as liver tonic, as an antioxidant, hepatoprotective, protective effect, weight gain, increases stamina and reported to have anticancer effects.<sup>[5-9]</sup> Chemically, *Ziziphus jujuba* contains Flavonoids, Saponins, tannins, Vitamin A, Vitamin B, sugars, mucilage, calcium, phosphate & iron. The pulp contains moisture, protein, fat, carbohydrate, calcium, phosphorus, iron, carotene, thiamine, riboflavin, Vitamin C. Ground seeds on extraction with petroleum ether gave 33% of bright yellowish oil. Fatty acid composition of the oil contains oleic acid- 71.7%, Linoleic acid- 15%.<sup>[10]</sup> Literature survey also reveals that paste of roots is beneficial in the treatment of ringworm infection. Although the antifungal activities of extracts of leaves have been reported, no systematic study has been reported on the stones of the plant.<sup>[11]</sup> Hence the present investigation deals with evaluation of antifungal activity of stones extract *Ziziphus jujuba* against *Trichophyton rubrum*, one of the causative organisms for Ringworm infection.

## MATERIAL AND METHODS

## Collection and authentication

*Ziziphus jujuba* was collected from in and around Chembarambakkam, Chennai India. The plant was identified and authenticated by the taxonomist. The authenticated specimen was deposited in the Department of Pharmacognosy, Sree Sastha Pharmacy College. The authentication specimen number is SSPC/P.CO/002/2020. The aerial parts were dried in room temperature for 2 months. Dried specimen was powdered using mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

## Preparation of extract

The powdered material of stone of *Z. jujuba* (7 kg) was soaked in Ethanol for 15 days, twice, at room temperature, with occasional shaking. Each time, the material was filtered and the filtrate was concentrated at 40°C under vacuum, by rotary evaporator. A blackish crude ethanolic extract of *Z. jujuba* (850 g) was obtained.

## Fractionation

The crude ethanol extract of *Z. jujuba* (1000 g) was suspended in distilled water (5000 ml) and partitioned with n-hexane (3 x 500 ml), chloroform (3 x 500 ml) and ethyl acetate (3 x 500 ml), respectively, to yield the n-hexane (200 g), chloroform (160 g), ethyl acetate (110 g) and aqueous (240g) fractions. 90 g of the crude ethanolic extract and aqueous extract of *Z. jujuba* was left for biological/pharmacological activities. All the fractions

will only contain their particular compounds based on the solubility from the crude extract. For example, the n-hexane fraction will contain only those compounds which are non-polar, and so on.

### Evaluation of Antifungal activity

The in vitro antifungal activity of the *Ziziphus jujuba* stones extract was carried out by Agar well diffusion method.<sup>[12]</sup> Clotrimazole (1mg/ml) used as standard antifungal agent. Antifungal activity was carried out against culture of *Trichophyton rubrum*, using Sabouraud dextrose agar medium<sup>[13]</sup> The 12 mm hole can be made in petridish. The microorganism inoculated plates were maintained at room temperature for 2 hours to allow diffusion of the solution into the medium. The petridishes used for antifungal activity were incubated  $25^{\circ}\pm 1$  for 7 days.<sup>[14]</sup> The diameters of zone of inhibition surrounding each of the wells were recorded.

## RESULT

### Preliminary Phytochemical screening

The extracts were then subjected to preliminary Phytochemical screening to detect the presence of

various Phytoconstituents. The results shows that the ethanolic extract contains saponins, glycosides, phytosterols, tannins, proteins & amino acids and aqueous extract contain saponins, glycosides, tannins, Vitamin A, Vitamin B, sugars & proteins.<sup>[15-16]</sup>

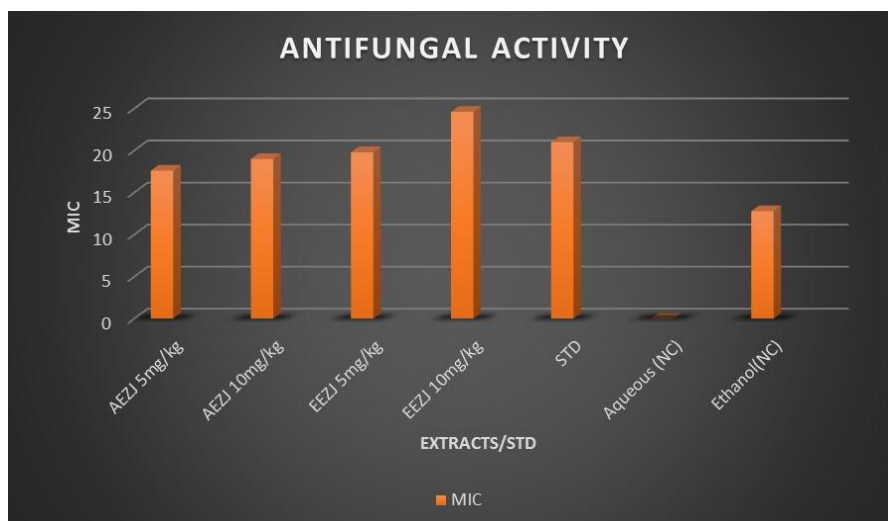
### Antifungal activity

The antifungal results reveal that the activity of the crude extracts of *Ziziphus jujuba* plant is encouraging. Antifungal activity was done by using Agar well diffusion method; Clotrimazole were used as standard for comparing results for antifungal activity. The zone of inhibition of ethanolic extract is 25 mm and aqueous extract has 19 mm taking 10mg/ml of extract. The zone of inhibition of standard drug Clotrimazole has 21 mm. Ethanolic extract shows better antifungal activity. Ethanolic extract shows good antifungal activity against *Trichophyton rubrum* as compare with aqueous extract (Table 1).

**Table 1: Effect of *Ziziphus jujuba* aqueous and alcoholic extract against *Trichophyton rubrum* showing antifungal activity.**

Extracts/drugs	Zone of inhibition (mm) against <i>Trichophyton rubrum</i>							
	1	2	3	4	5	Mean	SD	Result
AEZJ (5mg/ml)	17	18	18	17	18	17.6	0.5	17.6 $\pm$ 0.5
EEZJ (5mg/ml)	20	20	20	19	20	19.8	0.4	19.8 $\pm$ 0.4
AEZJ (10mg/kg)	19	19	19	19	19	19	0	19.0 $\pm$ 0
EEZJ(10mg/kg)	25	24	25	25	24	24.5	0.5	24.5 $\pm$ 0.5
STANDARD	21	21	21	21	21	21	0	21.0 $\pm$ 0
Aqueous (Negative Control)	12	12	12	12	12	12	0	No activity
Ethanol(Negative Control)	13	13	12	13	13	12.8	0.2	12.8 $\pm$ 0.2

Diameter of hole – 12 mm, S.D – Standard Deviation.



**Fig. 1: Effect of *Ziziphus jujuba* aqueous and alcoholic extract against *Trichophyton rubrum* showing antifungal activity.**

### DISCUSSION

Ethanolic extract shows good antifungal activity against *Trichophyton rubrum* as compare with aqueous extract.

The zone of inhibition of ethanolic extract (25mm) shows more than aqueous extract (19 mm) while taken 10 mg/ml extract. Phytoconstituents present in ethanolic

extract are saponins, glycosides, phytosterols, tannins, proteins & amino acids and aqueous extract contain saponins, glycosides, tannins, Vitamin A, Vitamin B, sugars & proteins. The zone of inhibition of standard drug Clotrimazole has 21 mm (1 mg/ml). When 5 mg/ml taken ethanolic extract gives 19 mm and aqueous extract gives 18 mm of zone of inhibition. On the basis of zone of inhibition results, ethanolic extract shows better antifungal activity against *Trichophyton rubrum* as compare with aqueous extract.

## CONCLUSION

From the above results, it is concluded that *Ziziphus jujuba* used traditionally and in backward areas to treat ringworm infection, showed significant antifungal activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plant as antifungal. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for antifungal activity. Thus the present experiments scientifically proved its traditional claim for the beneficial effect in the ringworm infection from aqueous extract and better from ethanolic root extract of *Ziziphus jujuba*.

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