

# THE ANTIFUNGAL, CYTOTOXIC, ANTITERMITE AND INSECTICIDAL ACTIVITIES OF *ZIZYPHUS JUJUBE*

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## ABSTRACT

Plants are very useful, self-generating machines, producing a variety of useful bioactive products. Keeping in view this idea, the crude methanolic extract and various fractions of *Zizyphus jujuba* were screened for antifungal, cytotoxic, antitermite and insecticidal activities. Low activity was shown by the crude methanolic extract (12%), *n*-hexane (9%), chloroform (20%) and ethyl acetate (14%) fraction against *Penicillium notatum*. Low activity was shown by the *n*-hexane fraction against *Aspergillus niger* (10%) and *Trichoderma harzianum* (13%) and inactive against *Aspergillus flavus*, *Fusarium oxysporum* and *Rhizopus stolonifer*. The CHCl<sub>3</sub> fraction exhibited low activity of 10% against *F. oxysporum* while showing no activity against the rest of the test fungi. All the test samples were inactive against *Rhizopus stolonifer*. The crude methanolic extract was highly cytotoxic (73.33%) at the concentration of 1000 (µg/ml) while the rest of the test samples were low in toxicity at the same concentration. The crude methanolic extract of *Zizyphus jujuba* showed significant antitermite activity against *Heterotermes indicola*, among the test samples. Against *Tribolium castaneum*, *Rhizopertha dominica* and *Callosbruchus analis* the insecticidal activity was determined. All the test samples except *n*-hexane showed low activity (20%) against *T. castaneum*. The *n*-hexane fraction showed low activity (20%) against *R. dominica* while the rest of the fractions were inactive against it. Low activity of 40% and 20% was shown by the chloroform and *n*-hexane fraction respectively against *C. analis*. The results of the present study revealed that the plant could be as potent source of cytotoxic drugs.

**Keywords:** *Zizyphus jujuba*, Antifungal, Cytotoxic, Antitermite, Insecticidal, *Tribolium castaneum*, *Rhizopertha dominica* and *Callosbruchus analis*.

## INTRODUCTION

Medicinal plants are an important health and economic component of the biodiversity (Rahman *et al.*, 2004). Most of the world population exclusively relies on medicinal plants for primary health care (Farnsworth, 1994). The importance of medicinal plants for the treatment of various diseases is now being recognized by many people. The main reason behind this is that they have fewer side effects and are economical. Thirteen natural product (NP) derived or semi synthetic NP drugs are available in the market since 2005 for the treatment of ailments like multiple sclerosis, pain, cancer etc (Butler, 2008).

*Z. jujuba* belongs to family *Rhamnaceae* and is commonly called, Red date or Chinese date or Bera (Pushto). 50 genera and more than 900 species constitute this family. This family is almost cosmopolitan and found mainly in tropical to subtropical regions. The leaves, bark and fruit of several species of *Rhamnaceae* notably *Rhamnus cathartica* and *Rhamnus frangula* have been used as laxatives. Edible fruit are produced by many *Zizyphus* species, among them; *Z. mauritiana* and *Z. jujuba* (Indian

*jujube*) are the most important species which are cultivated on a commercial scale (Yilin and Carsten, 2007). For curing jaundice, the juice of fresh leaves of *Z. mauritiana* L and roots of *Z. oxyphylla* Edgew is used (Jan *et al.*, 2009). For prevention of malaria, cold suspension of dried roots powder of *Ampelozizyphus amazonicus* is used (Neto-Andrade *et al.*, 2008). An infusion of fruits of *R. cathartica* L is used as an antiseptic for wounds (Ivancheva and Stantcheva, 2000). *Origanum majorana* L is used in indigestion, asthma, rheumatism and headache (Jun *et al.*, 2001). *Origanum majorana* L protect against Hydroquinone induced cytogenesis and histological changes (Ghaly *et al.*, 2001). N-methyl-D-aspartate (NMDA) induced neuronal cell damage *in vitro* is being prevented by the methanolic extract of *Zizyphi Spinosi* Semen, over a concentration range of 0.05–5 µg/ml (Park *et al.*, 2004).

Seeds of *Z. jujuba* have been used as tranquilizer, convulsant, analgesic, for treating anxiety and insomnia (Peng and Zhu, 2001). *Jujube* is used for liver diseases (Khare, 1995). For treating sore throats, decoctions of *jujube* have often been used in pharmacy. Fruits of *Z. jujuba* yield, Theasinensin A, a polyphenol, which suppresses the antibiotic resistance of Methicillin-resistant *Staphylococcus aureus* (Tsutomu *et al.*, 2005).

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Against *Lycoriella ingenua* and *Coboldia fuscipes*, which are important mushroom pests, extracts of *Z. jujuba* seeds and fruits exhibited moderate activity (Yi *et al.*, 2008).

Keeping in view the traditional importance of *Z. jujuba*, its aerial parts were screened *in vitro* for possible biological/pharmacological activities i.e., Antifungal, Cytotoxic, Antitermite and Insecticidal activities.

## MATERIALS AND METHODS

### *Plant material*

From the native Northern region of Khyber Pakhtunkhwa, Pakistan, the aerial parts of *Z. jujuba* were collected and kindly identified by Prof. Dr. Abdur-Rashid, Department of Botany, University of Peshawar, Pakistan.

### *Extraction*

The collected plant material was kept in shade for drying. After drying they were chopped into small pieces and grounded to powder with an electric grinder. In methanol the powdered material of *Z. jujuba* (7 kg) were soaked for 15 days, twice, at room temperature, with occasional shaking. All the filtrates were concentrated, at 40°C, under vacuum, by rotary evaporator. A blackish crude methanolic extract of *Z. jujuba* (850gm) was obtained, Ahmad *et al.*, 2009.

### *Fractionation*

The crude methanolic extract of *Z. jujuba* (800gm) was suspended in distilled water (500 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 (200gm), CHCl<sub>3</sub> (160gm), EtOAc (110gm) and aqueous (240gm) fractions, respectively. 90gm of the crude methanolic extract of *Z. jujuba* was left for biological/pharmacological activities.

### *Antifungal activity*

The crude methanolic extract and various fractions of *Z. jujuba* were screened for antifungal activity against *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium notatum*, *Rhizopus stolonifer* and *Trichoderma harzianum* following Ahmad *et al.*, 2009. Stock solution was prepared by dissolving 24 mg of test sample in 1 mL sterile DMSO. Sabouraud Dextrose Agar (SDA) was prepared and incubated for 24 hrs at 28±1°C for sterility check. After incubation the sterile plates were used to refresh the above mentioned fungal species followed by incubation for seven days at 28±1°C. 4 ml of SDA was poured in test tubes for making slants followed by autoclaving. After autoclaving when the temperature of the media is about 50°C, 66.6 µL from the stock solutions of test samples were added into respective test tube and kept in slanted position. The tubes were incubated at 28±1°C for 24 hrs to check sterility. Seven days old fungal culture was then introduced into the

labeled test tubes and incubated for seven days at 28±1°C. Miconazole and DMSO served as positive and negative controls. After seven days results were taken by measuring the linear growth on the slanted test tubes in comparison with negative control.

### *Brine Shrimp Lethality Bioassay*

Against *Artemia salina* (brine-shrimp eggs) the cytotoxic effect of the crude methanolic extract and various fractions of *Z. jujuba* were carried following the procedure of Bashir *et al.*, 2009. A good media for hatching of Brine shrimp eggs is sea water. Artificial sea water was prepared by dissolving commercial salt mixture in double distilled water in a shallow rectangular plastic dish of 22 × 32 cm. Using a perforated device the plastic dish was unequally divided. In larger darkened compartment, 50 mg of eggs were sparged. The smaller compartment was open to ordinary light. Keeping this whole setup for 2 days at room temperature allowed eggs to hatch and mature. Using a Pasteur pipette the nauplii were collected. The stock solutions were prepared by dissolving 20 mg of the test samples in 2 mL of volatile organic solvent. 5, 50 and 500 µL were transferred to vials (3 vials/concentration) from the stock solution with final concentration of 10, 100 and 1000 µg/mL respectively. For the evaporation of organic solvents the vials were placed in the hood for half an hour or allowed overnight. 30 larvae and 1 mL of sea water was added to each vial. The final volume of each vial was adjusted to 5ml with sea water. The vials were incubated for 24 hrs, under illumination at 26 ± 1°C. Reference cytotoxic drug (Etoposide) and organic solvents served as positive and negative control. The live brine shrimps were counted using a magnifying glass after incubation period. Using a Finney computer program (Probit analysis) the data were analyzed to determine LD<sub>50</sub> values with 95% confidence interval.

### *Anti-Termite activity*

For determining the anti-termite activity of the crude methanolic extract, CHCl<sub>3</sub> and aqueous fraction of *Z. jujuba* against *Heterotermes indicola* the procedure of Salihah *et al.*, 1993 was employed. Blotting paper and Petri dishes were sterilized and according to the base of the Petri dishes the blotting paper was cut (use uniform size Petri dishes). Test samples were prepared (2mg/ml) in respective solvent, the blotting paper was dipped in it, kept in the Petri dishes and left overnight to allow evaporation of the solvent. To each Petri dish 25 termites were added and observe after 24 hours, till all the termites are dead. Respective solvent served as negative control. All the experiments were performed in triplicate and recording average termites killed each day.

### *Insecticidal activity*

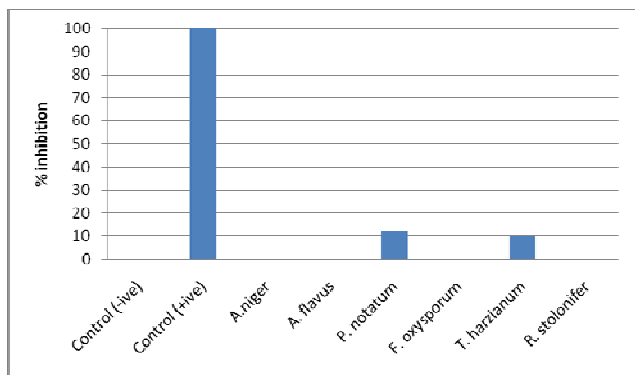
To determine the insecticidal activity of crude methanolic extract and various fractions of *Z. jujuba* against *T.*

*castaneum*, *C. analis* and *R. dominica* the contact toxicity assay Ahn *et al.*, 1995 was used. Under controlled conditions of humidity and temperature the above mentioned pests were reared in plastic bottles in the laboratory in breeding media (sterile). For experimental work insects of uniform size and age were selected. 200 mg of the test samples were dissolved in 3 mL of volatile organic solvent to make stock solution. According to the base size of the Petri dishes (sterile) the filter paper was cut, dipped in stock solution, kept in Petri dishes and left overnight for evaporation of the organic solvent. 10 healthy insects were then transferred to the labeled plates with the help of a clean brush. The plates were then incubated at 27°C with 50 % relative humidity in growth chamber for 24 hours. Results were recorded by counting the number of survivals in each plate. Permethrin served as positive while the organic volatile solvent as negative control.

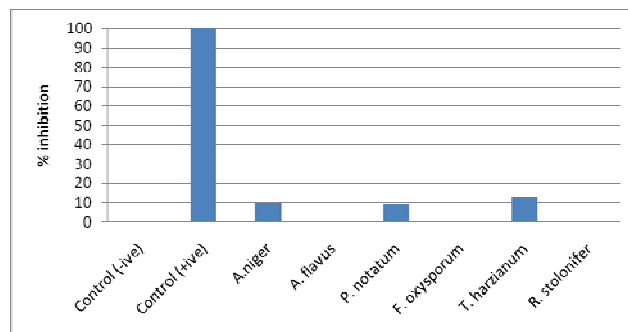
**RESULTS**

**Antifungal activity**

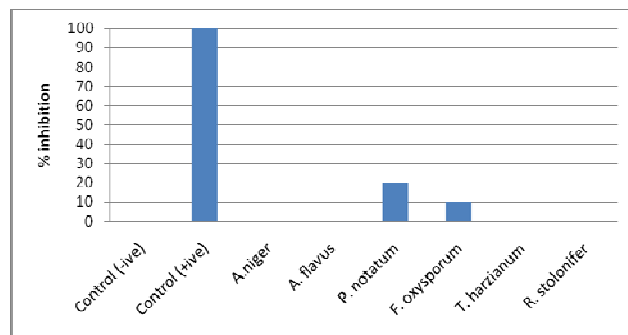
The results of the antifungal activity are given in figs.1-5. Low antifungal activity of 12% and 10% was shown by the crude methanolic extract against *P. notatum* and *T. harzianum* while no activity was observed against *A. flavus*, *A. niger*, *F. oxysporum* and *R. stolonifer*. The *n*-hexane fraction presented 13%, 10% and 9% activity against *T. harzianum*, *A. niger* and *P. notatum* respectively, while inactive against *F. oxysporum*, *A. flavus*, and *R. stolonifer*. 20% and 10% activity was exhibited by the CHCl<sub>3</sub> fraction against *P. notatum* and *F. oxysporum* respectively while it was inactive against the rest of the test fungi. The EtOAc fraction showed 14%, 12% and 11% activity against *P. notatum*, *A. niger*, and *T. harzianum* respectively. It showed no activity against *F. oxysporum*, *A. flavus* and *R. stolonifer*. Against *T. harzianum*, *A. flavus* and *F. oxysporum*, the aqueous fraction exhibited 17%, 15% and 5% activity.



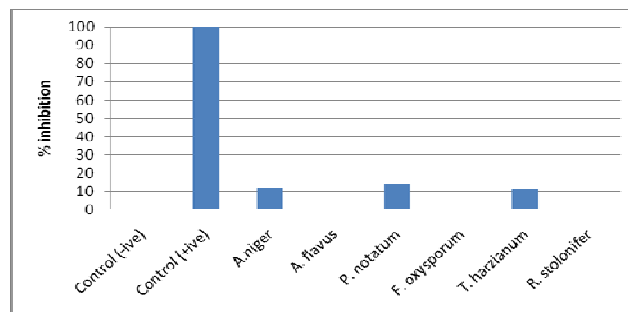
**Fig. 1:** Antifungal activity of the crude methanolic extract of *Zizyphus jujuba* against the test fungi.



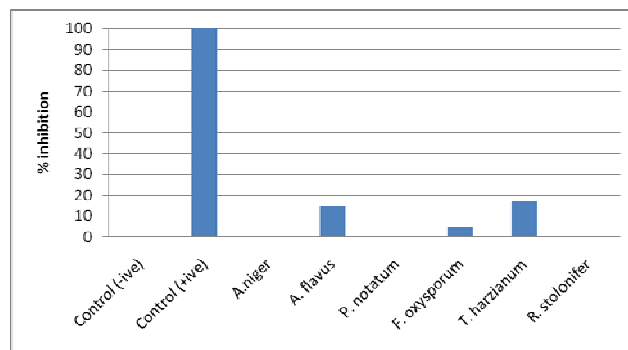
**Fig. 2:** Antifungal activity of the *n*-hexane fraction of *Zizyphus jujuba* against the test fungi



**Fig. 3:** Antifungal activity of the chloroform fraction of *Zizyphus jujuba* against the test fungi.



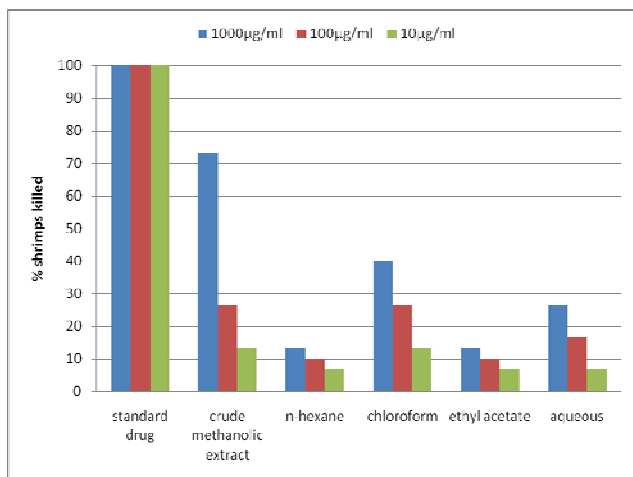
**Fig. 4:** Antifungal activity of the ethyl acetate fraction of *Zizyphus jujuba* against the test fungi.



**Fig. 5:** Antifungal activity of the aqueous fraction of *Zizyphus jujuba* against the test fungi.

**Brine shrimp (*Artemia salina*) lethality bioassay**

The results of this bioassay for the crude methanolic extract and various fractions of *Z. jujube* are presented in fig. 6. At the concentration of 1000µg/ml crude methanolic extract is toxic (73.33%) while 26.66 and 13.33 % cytotoxic effect at the concentration of 100 and 10µg/ml respectively was observed. Value of upper limit was 128.40. Cytotoxic effect of *n*-hexane fraction was 13.33, 10 and 6.66% at 1000, 100 and at 10µg/ml having 0.0019 and 10389.93 values of upper and lower limit. The CHCl<sub>3</sub> fraction showed 40, 26.66 and 13.33 % lethality at 1000, 100 and 10µg/ml respectively. The values of upper limit and lower limit were 0.7137 and 285727 respectively. 13, 10 and 6.66 % cytotoxicity was presented by the EtOAc fraction at 1000, 100 and 10µg/ml. The value of lower limit was 10389.9 and upper limit was 0.0019. The cytotoxic effect of the aqueous fraction was 26.66, 16.66 and 6.66 % at 1000, 100 and 10µg/ml. The value of lower limit was 6.2341 and upper limit was 0.00.



**Fig. 6:** Brine shrimp cytotoxicity of crude methanolic extract and various fractions of *Zizyphus jujube*.

**Anti termite activity**

Anti-termite activity of crude methanolic extract, CHCl<sub>3</sub> and aqueous fractions of *Z. jujube* were carried out against *H. indicola* at "Termites lab at Nuclear Institute of Food and Agriculture (NIFA), Peshawar, Pakistan. All the experiments were performed in triplicates. The results are given in table 1.

The aqueous fraction killed an average of 5 termites on first day. On second day, 7 and third day, 16 termites were killed. On fourth day all termites were dead.

The crude methanolic extract killed an average of 20 termites on first day. On second day 23 and on third day all termites were dead.

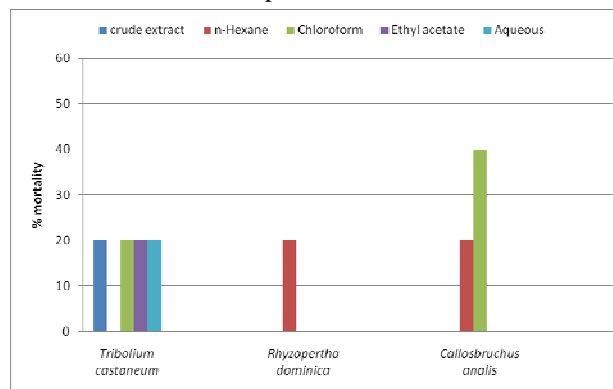
The CHCl<sub>3</sub> fraction killed 12, 16 and 25 termites, on average, on first, second and third day respectively.

**Table 1:** Antitermite activity of the crude methanolic extract, CHCl<sub>3</sub> and aqueous fraction of *Zizyphus jujube*.

Sample	No. of Termites	Day	Average Termites killed
Crude methanolic extract	25	1	20
		2	23
		3	25
Chloroform	25	1	12
		2	16
		3	25
Aqueous	25	1	5
		2	7
		3	16
		4	25

**Insecticidal activity**

As the insecticide from the plant origin are mostly environment friendly therefore insecticidal activity of the crude methanolic extract and various fractions of *Z. jujube* were screened against *R. dominica*, *T. castaneum* and *C. analis*. The results are mentioned in fig.7. Low activity (20%) was exhibited by all test samples except *n*-hexane fraction against *T. castaneum*. The *n*-hexane fraction showed low activity of 20% against *R. dominica*. 40% and 20% activity was shown by the CHCl<sub>3</sub> and *n*-hexane fractions respectively against *C. analis*. Against *C. analis* the other test samples were inactive.



**Fig. 7:** Insecticidal activity of crude extract and fractions of *Zizyphus jujube* by contact toxicity method:

**DISCUSSION**

A number of secondary metabolites (mycotoxins) are produced by fungi of which some have been found to be toxic to humans and animals for example *A. niger* produce Malformins. (Smedsgaard., 1997). In our previous study, antifungal activity of the crude methanolic extract and various fractions of *Onosma griffithii* Vatke were carried out against *A. flavus* and *F. solani*. Moderate antifungal activity was displayed by the crude methanolic extract against the test fungi, Ahmad *et al.*, 2009. The results of the present study indicate that *Z. jujube* lack potent antifungal agents because none of the test sample showed significant activity against the tested fungi.

Previously we studied the cytotoxic effect of *Rumex hastatus*, *Rumex dentatus*, *Rumex nepalensis*, *Rheum australe*, *Polygonum persicaria* and *Polygonum plebejum* (Family *Polygonaceae*) against *A. salina* at 1000, 100 and 10 mg/ml. *R. hastatus*, *R. dentatus* and *R. nepalensis* showed significant activity at a concentration of 1000 mg/ml against *A. salina*, Farrukh et al., 2009. The crude methanolic extract of *Z. jujuba* was cytotoxic (73.33%) at the concentration of 1000µg/ml against brine shrimp, so this shows that this plant contains cytotoxic agents. Termites play an important role in recycling of woody and other plant material. The termites help to aerate soil by their tunneling effect. They are abundant in tropical and subtropical environments and able to decompose cellulose, the main component of wood. But they become pests when they start destroying wood and wooden products of human homes, building materials, forests and other commercial products, causing a great economic loss (Wooten and Elakovich, 1991). The crude methanolic extract was the most potent against the tested termite among the tested samples. Environment friendly Insecticide is one of the most important requirements of the present day. Larvicidal activity of ethanolic extract *Z. joazeiro* Mart was determined against larvae of *Aedes aegypti* showing low activity Omena et al., 2006. Low activity was shown in some cases by the test samples against the test insects. In conclusion, the plant can be used to extract potent cytotoxic agents.

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