

## Comparative Study on Antimicrobial Activities of *Ziziphus jujube* and *Ziziphus nummularia* Fruits Extract

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**Abstract**-The present study was aimed at detecting the phytochemicals and evaluating comparative antimicrobial activities of *Ziziphus jujuba*, and *Z. Nummularia* fruits extract known for their medicinal properties in folk medicine. The comparative assessment of antifungal activity was performed in terms of percentage of radial on solid medium (potatoes dextrose agar PDA) against *Aspergillus flavus*, *M.gypseum*, *T. flavurus clem* and *Penicillium expansum*. The antibacterial effect was studied by the agar direct contact method using *Staphylococcus aureus*, *Pseudomonas aeruginosa* *B.cereus*, *B.pumilus*, *M. luteus* and *Escherichia coli* strains. The results revealed that the methanolic extract and the acetonic extract exhibited significant antimicrobial activity of concentration of 100-500 µg/ml against tested organisms respectively.

**Keywords:** *Ziziphus jujuba*, *Z. nummularia* fruits extract, Antimicrobial activity

## Introduction

### *Ziziphus jujuba*

*Ziziphus jujube* Lam. is also called as Badari, Baer, Bogari is belonging to the family Rhamnaceae<sup>1</sup>. It is a small sub deciduous tree with dense spreading crown, commonly 6 - 8 m high. The Plant is distributed throughout India, Iran, Afghanistan and in China. The bark is blackish to grey or Brown, rough, regularly and deeply furrow, the furrows are at about 1.2 cm apart. Blade 9;13 mm, Branches usually armed with spines, mostly in pairs, one straight ,the other with curved<sup>2-6</sup>. Leaves 3-6.3 by 2.5-5 cm., oblong or ovate, usually minutely serrulate or apex distinctly toothed, obtuse, base oblique 3; nerved, nerves depressed on the glabrous shining upper surface. A survey of literature on *Ziziphus jujube* Lam revealed a few pharmacological activity on the plant were reported, antisteroidogenic activity<sup>7</sup>anti obesity

activity<sup>8</sup> anticancer activity<sup>9</sup>, anxiolytic activity<sup>10-12</sup>. The plant is reported to contain alkaloid Jubanine-E<sup>13</sup> and sedative flavonoids<sup>14</sup> such as swertish and spinosin<sup>15</sup>. Triterpenic acids have been isolated from the fruits of *Ziziphus jujuba*. Betulin, Betulinic acid ,Ursolic acid Ceanothic acid are triterpenes reported by Shoei et al.<sup>16-21</sup>

### *Ziziphus nummularia*

*Ziziphus nummularia* is a common plant of central India and its root, leaves and seed are used by tribes for curing different diseases like allergy, scabies, eczema and pyorrhea etc. The roots of *Ziziphus oxyphylla* Edgew and juice of fresh leaves of *Z. Mauritiana*L are used for curing jaundice<sup>22</sup>. A cold suspension of dried roots powder of *Ampeloziziphusamazonicus* used to prevent malaria<sup>23</sup>. Traditionally, *Origanum majorana*L is used in asthma, indigestion, headache, rheumatism and protect against hydroquinone induced cytogenesis and histological changes<sup>24-25</sup>. The methanolic extract of *Ziziphus spinosissima*, over a concentration range of 0.05–5 µg/ml, prevents N-methyl- D-aspartate (NMDA) induced neuronal cell damage *in vitro*<sup>26</sup>. The seeds of *Z. jujuba* have been used as analgesic, tranquilizer, convulsant and have been prescribed for the treatment of insomnia and anxiety in Asia<sup>27</sup>. Furthermore, traditionally, jujube is used prophylactically for liver diseases<sup>28</sup>. The fruit being mucilaginous is also very soothing to the throat and decoctions of *jujube* have often been used in pharmacy to treat sore throats. *Z. jujuba* extracts exhibited protection against hydroquinone induced cytogenesis<sup>25</sup>. Theasins in a polyphenol obtained from fruits of *Z. jujuba* suppressed the antibiotic resistance of Methicillin resistant *Staphylococcus aureus*<sup>29</sup>. Extracts of *Z.jujuba* fruits and seeds exhibited moderate activity against *Lycoriella ingenua* and *Coboldia fuscipes*, which are important mushroom pests<sup>30</sup>.

## Materials and Methods

### Collection of *Ziziphus jujuba* and *Z. nummularia* fruits

Fruits of *Ziziphus jujube* and *Z. nummularia* fruits were collected from locality of Faridabad, Haryana. Plant material was authenticated by **Dr. Maya Ram Uniyal** (Ex. Advisor medicinal plant UP/UK).

### Extraction of *Ziziphus jujuba* and *Z. nummularia* fruits in different solvents (Nonpolar to Polar)

The collected plant material was washed with water to remove other undesirable material and then dried under shade. The air-dried fruits (200 gm) of all were crushed and remove the seed separately. The crushed fruits extracted with different solvents of increasing polarity viz. petroleum ether, chloroform, acetone, methanol by hot percolation method using soxhlet apparatus. The extract was then evaporated till dryness to obtain residue. These using were concentrated under reduced pressure. The extract was used for antimicrobial activity.

### Anti-microbial activity of different extracts<sup>31</sup>

The **anti-microbial activity** of the fruits of *Ziziphus jujube* and *Z. nummularia* was carried out. The fruits extracts were screened for anti bacterial and anti fungal activities.

#### Anti bacterial activity of fruits extract

In this study, the anti bacterial activity was studied against the micro organism and the bacterial cultures used in the study were: 1. *Escherichia coli*, 2. *Pseudomonas aeruginosa* and 3. *Bacillus cereus*. These bacterial cultures were maintained on nutrient agar slants at first being incubated at 37°C for about 18-24 hours and then stored at 4°C as stock for anti bacterial activity. Fresh cultures were obtained by transferring a loop full of cultures into nutrient broth and then incubated at 37°C overnight. To test anti bacterial activity, the well diffusion method was used.

### Culture media preparation

The microbiological media prepared as standard instruction provided by the HI-Media Laboratories, Mumbai. The media used for anti bacterial activity Muller-Hinton Agar (MHA) and Nutrient broth (NB). They were prepared and sterilized at 121°C at 15psi for 15-20 minutes autoclave.

### Plate preparations

25 ml of pre autoclaved Muller-Hinton agar(MHA) was poured into 90 mm diameter pre sterilized Petri-plates. These Petri-plates were allowed to solidify at room temperature.

### Well diffusion method

After the plates solidified, the freshly prepared microbial growth culture suspension (about 20µl) was spread over the Muller – Hinton agar (MHA) media using L shaped sterilized glass spreader separately under the aseptic condition using laminar air flow. Then, well were made in each plate with the help of borer of 8 mm diameter. In these well, about 100 µl of each fruits extract individually was loaded. This method depends upon the diffusion of fruits extracts from hole through the solidified agar layer of Petri-dish to such an extent that the growth of added microorganism is prevented entirely in a circular area or Zone around the hole containing fruits extract.

**Incubation:** Petri plates were incubated for overnight at 37°C ± 0.5°C in the incubator.

**Inhibition Measurement of Zone of inhibition** After incubation, the diameter of clear zone of incubation produced around the well or holes were measured in mm by ESR Tube and compared with standard drug.

## Results

**Table-1** Antibacterial activity of different extracts of *Ziziphus jujube* and standard drug Chloramphenicol, Streptomycin, Ampicillin.

S.No.	Test Organism	Inhibition zone in mm			Standard Drug		
		Pet. Ether	Chloroform	Methanol	Ampiciline	Streptomycin	Chloram-phenicol
1	E.coli	-	3	12	25	20	24
2	Bacillus cereus	-	5	30	20	20	20
3	pseudomonas	-	15	10	-	22	15

**Table-2** Antibacterial activity of different extracts of *Ziziphusnummularia* and standard drug Chloramphenicol

S.No.	Test Organism	Inhibition zone in mm				Standard Drug		
		Pet. Ether	Chloroform	Acetone	Methanol	Ampiciline	Streptomycin	Chloram-phenicol
1	E.coli	15	24	22	-	22	18	30
2	Bacillus cereus	20	18	27	-	15	17	36
3	B.pumilus	-	20	26	24	-	17	22
4	M.luteus	-	11	15	18	32	25	28

**Table-3** Antifungal activity of different extract of *Ziziphusjujuba* and standard drug Amphotericin-B and Clotrimazole.

S.No.	Test Organism	Inhibition zone in mm			Standard Drug	
		Pet. Ether	Chloroform	Methanol	Amphotericin-B	Clotrimazole
1	<i>Aspergillusniger</i>	-	-	20	-	12
2	<i>Sclerotium</i>	-	10	8	-	-
3	<i>Candida- albicans</i>	-	-	25	-	14
4	<i>Rhizopus</i>	-	-	9	-	-

**Table-4** Antifungal activity of different extract of *Ziziphusnummularia* and standard drug Amphotericin-B and Clotrimazole.

S.No.	Test Organism	Inhibition zone in mm				Standard Drug	
		Pet. Ether	Chloroform	Acetone	Methanol	Amphotericin-B	Clotrimazole
1	<i>Aspergillusniger</i>	-	-	-	-	-	12
2	<i>M.gypseum</i>	20	16	21	-	-	-
3	<i>T.flavurusclem</i>	14	13	23	-	-	11

## Discussion

The antimicrobial activity of fruits extracts of *Ziziphusjujubawas* found active against E.coli, Bacillus cereus, Pseudomonas in chloroform and methanol extracts whereas leaves extracts of Pt.ether was found less active against E.coli, B. cereus, Pseudomonas. The leaves extracts of methanol was found highly active against *Candida albicans* and *Aspergillus niger* while less active against *Rhizopus* and *Sclerotium*. The leaves extracts of Pt. ether did not find any activity. The results revealed that the **methanolic extract** has shown more degree of anti microbial activity than other extract when compared to the

standard drug. It is due to presence of chemical constituents like carbohydrates, phenolic compounds, tannins, triterpenoids, saponins, terpenoids, proteins and aminoacids, this was confirmed by phytochemical studies. While in case of *Ziziphusnummularia* the **acetonic extract** showed good antibacterial activity against B.cereus and B.pumilus and good anti-fungal activity against *T. flavurusclem* in comparison to the standard drug.

## Conclusion

The findings of the present study revealed that *Ziziphus jujube* & *Z.*

*nummularia* different fruit extract contain potent antimicrobial property. The results also support the folkloric usage of the studied plants. These resources have the prospect of finding new clinically efficient antimicrobial compounds and the knowledge can be extended for future investigation into the field of pharmacology for better drug discovery.

## References

1. K.M. Nadkarni, Indian material medica, (Popular prakashan, Bombay, 1986), 1315-1319.
2. K.R. Kritkar and B.D. Basu, Indian medicinal plants, Vol II, 2nd edition.(Bishansingh and Mahendrapal Singh, Dehradun, 1994).
3. Mahajan R.T. and Chopda M.Z., Phyto pharmacology of Zizyphus jujuba Lam- A plant review, 2009, Vol III, (6), 320-329.
4. Dr. Nadkarni K M. Indian Materia Medica. Popular Prakashan Private Limited, Mumbai. 2000, (1):1316-1317.
5. Kirtikar, K.R. and Basu, B. D. Indian Medicinal Plants, *International Book Distributors, Dehradun*. 2005, (1) 589-591.
6. Gaint, K. and Gupta, R. *Phytochemistry*, 1972, 11:1580-95
7. Gupta, M.; Mazumder, U.K.; Vamsi, M.L.; Sivakkumar, T. and Kandar, Cc. Evaluation of antisteroidogenic activity of ethyl acetate extract of Zizyphus jujube bark on mice, *Journal of Ethnopharmacol.*, 2004, Jan, 90(1)25-5.
8. Ganachari, M.S. and Shiv Kumar Effects of Zizyphus jujuba leaf extract of body weight, food intake and serum lipid levels in sucrose induced obese rats. *Indian Journal of Pharmaceutical Sciences*, 2004, 66(3): 363-365.
9. Jiang, J.G.; Huang, X.J. and Chen, J. Isolation and purification of saponins from Zizyphus jujuba and their sedative and hypnotic effects. *Journal of Pharm. Pharmacol.* 2007, Aug, 59(8):1175-1180.
10. Peng, W.H.; Hsieh, M.T.; Lee, Y.S.; Lin, Y.C. and Liao, J. Evaluation of anxiolytic effect of seed of Zizyphus jujuba in mouse models. *Journal of ethnopharmacol.*, 2000, Oct, 72(3):435- 440.
11. Kanika Patel; Neelesh Kumar and N.K. Sharma *Journal of advanced scientific research*, Sciansage Pub., 2011, 2(1): 42-49.
12. Gaint, K. and Gupta, R. *Phytochemistry* 1972, 11:1500-2.
13. Pal, S.; Sen, T. and Nag Chaudhuri, A.K. *J Pharm Pharmacol* 1999, 51: 313-318.
14. Dash, G.K. ; Panda, A.; Patre, C.P. and Ganapaty, S. *Indian J Nat. Prod.* 2003, 19 (3): 24.
15. Huang, X.; Kojima-Yuasa, A.; Norikura, T.; Kennedy, D.O.; Hasuma, T. and Matsui-Uuasa. Mechanism of anti-cancer activity of Zizyphus jujuba in Hep G2 cells. *Am J Chin Med*, 2007, 35 (3):517-32.
16. Pandey, M.B.; Singh, A.K.; Singh, J.P.; Singh, V.P. and Pandey, V.B. Isolation of three new cyclopeptide alkaloids from Zizyphus species. *Journal of Asian Natural products Res.*, 2008, Jul-Aug, 10(7-8): 719-23.
17. Rastogi, R.P. and Mehrotra, B.N. Compendium of Indian Medicinal Plants, *Central Drug Research Institute, Lucknow and National institute of Science Communication*, New Delhi. 1980, (3):694
18. Liu, Q.X.; Wang, B.; Liang, H.; Zhao, Y.Y.; Liu, M.J. Structure identification of jujuboside D from Zizyphus jujuba mill var. Spinosa. *Yao Xue Xue Bao*. 2003, Dec, 38 (12):934
19. Lerner, J. Insulin and hypoglycaemic drug, Glucagon, The pharmacological basis of therapeutics, edited by AG

- Gilman & Goodman, I W Rall&Murad, 7th edition, Macmillan, Newyork 1985, 1490.
20. World Health Organisation: WHO Guidelines for the assessment of herbal medicines, WHO expert committee o specification for pharmaceutical preparation, Technical report series No. 863. Geneva, 1996.
  21. The WHO Expert Committee on Diabetes mellitus, Technical Report Series, 1980
  22. Gul, J.; Mir, A.K. and Farzana, G. Ethno medicinal plants used against Jaundice in DirKohistan Valleys (NWFP), Pakistan. *Ethnobotanical Leaflets*, 2009, 13: 1029-1041.
  23. Neto, V.F.; Andrade Brandao, M.G.L.;Nogueira, F.;Rosário, V.E. and Krettli, A.U. *Ampelozizyphusamazonicus*Ducke (Rhamnaceae), a medicinal plant used to prevent malaria in the Amazon Region, hampers the development of Plasmodium bergheisporozoites. *Int J Parasitol*, 2008, 38(13): 1505-1511.
  24. Jun, W.J.; Han, B.K.; Yu,K.W.and Kim, M.S. Antioxidant effects of *Origanummajorana*L.on superoxide anion radicals.*FoodChem*, 2001, 75(4): 439-444.
  25. Inas, S.G.; Atta, S.;Mosaad, A. and Abdel, W. *Zizyphus jujube* and*Origanummajoran*extracts protect against hydroquinone-induced clastogenicity.*Environ.Toxicol. Pharmacol.*, 2008, 25(1): 10-19.
  26. Jeong, H.P.; Hyun, J.L.; Sang, B.K.;Ju, Y.B. and Yeon, H.S. Protection of NMDA-induced neuronal cell damage by methanol extract of *ZizyphiSpinosi* Semen in cultured rat cerebellar granule cells. *J Ethnopharmacol.*, 2004, 95(1): 39-45.
  27. Peng, Z.C. and Zhu, J. Research advances in chemical constituents and pharmacological effects of semen *ZizyphiSpinosa*. *Med Res.*, 2001, 12: 86-87.
  28. Khare, C.P. and*Zizyphusjujuba*, Encyclopedia of Indian Medicinal Plants.Springer New York. 1995, 493-498.
  29. Tsutomu, H.;Miwako, K.;Kazutoshi, I.; Tomo-omi, O.;Sumiko, S.;Tomofusa, T. and Takashi, Y. Effects of tannins and related polyphenols on methicillin-resistant *Staphylococcus aureus*. *Phytochemistry*, 2005, 66(17): 2047-2055.
  30. Jee-Hwan, Y.; Kwon, P.;Kwang-Sik, C.; Sang-Cheol, S. and Young-Joon, A. Toxicity of medicinal plant extract to *Lycoriella ingénue* and *Coboldiafuscipes*.*J Asia-PacEntomol*, 2008, 11: 221-223.
  31. Suman Rawat; O.P. sati and ArtitomarComparative Study OnAntimicrobial Activities *OCordiaDichotoma*and*SapiumSebiferum* . *Universities' Journal of Phytochemistry and Ayurvedic Heights*,2015 19(2):23-26