

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

*¹Mirza Azim begand²Ragib Ali

¹ and ² QA/QC Department, Himalaya Wellness Company,
Faridabad Haryana India

Email: azim_0088@yahoo.com

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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 acetonc 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 Rhamnaceae¹. 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. Blaze 9;13 mm, Branches usually armed with spines, mostly in pairs, one straight ,the other with curved²⁻⁶. 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 activity⁷anti obesity

activity⁸ anticancer activity⁹, anxiolytic activity¹⁰⁻¹². The plant is reported to contain alkaloid Jubanine-E¹³ and sedative flavonoids¹⁴ such as swertish and spinosin¹⁵. Triterpenic acids have been isolated from the fruits of *Zizyphus jujuba*. Betulin, Betulinic acid ,Ursolic acid Ceanothic acid are triterpenes reported by Shoei et al.¹⁶⁻²¹

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 jaundice²². A cold suspension of dried roots powder of *Ampelozizyphusamazonicus* used to prevent malaria²³. Traditionally, *Origanum majorana*L is used in asthma, indigestion, headache, rheumatism and protect against hydroquinone induced cytogenesis and histological changes²⁴⁻²⁵. The methanolic extract of *Zizyphispinosise*men, over a concentration range of 0.05–5 µg/ml, prevents N-methyl- D-aspartate (NMDA) induced neuronal cell damage *in vitro*²⁶. The seeds of *Z. jujuba* have been used as analgesic, tranquilizer, convulsant and have been prescribed for the treatment of insomnia and anxiety in Asia²⁷. Furthermore, traditionally, jujube is used prophylactically for liver diseases²⁸. 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 cytogenesis²⁵. Theasins in a polyphenol obtained from fruits of *Z. jujuba* suppressed the antibiotic resistance of Methicillin resistant *Staphylococcus aureus*²⁹. Extracts of *Z.jujuba* fruits and seeds exhibited moderate activity against *Lycoriellaingenua* and *Coboldiafuscipes*, which are important mushroom pests³⁰.

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 extracts³¹

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 37⁰C for about 18-24 hours and then stored at 4⁰ 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 37⁰ 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 121⁰C 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 20 μ l) 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 μ l 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 37⁰C \pm 0.5⁰C 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.flavusclem</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. flavusclem* in comparison to the standard drug.

Conclusion

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

nummulariadifferent 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.

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