# In-Vitro Efficacy of Crude Extract of Zizipus Jujuba against Selected Bacterial Strains

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***Abstract-*** The research was assessed to evaluate the efficacy of crude extract of Zizipus jujuba against selected bacterial strains. The extract of *Zizipus jujuba* leaves was obtained by three different methods and the inhibition zones obtained through disc diffusion method. A decent antibacterial activity of *Zizipus jujuba* leaves crude extract of cold water and ethanol was found against *Salmonella typhimurium* and *Staphylococcus aereus*. Maximum zone of inhibition through cold water extract was obtained by *Enterococcus feacalis* (32mm) followed by *Staphylococcus aureus* (28mm), *Salmonella typhimurium* (27.52), *Klebsilla pneumonia* (19mm) and *Escherichia coli* (19mm). Similarly maximum zone of inhibition through ethanol extract was obtained by *Staphylococcus aureus* (28mm) followed by *Salmonella typhimurium* (27.52). The bacterial species showed no sensitivity against hot water extract due to the degradation of alkaloids in hot water. Mean observation taken was that these bacterial species can be inhibited by *Zizipus jujuba* plant. The study showed that *Zizipus jujuba* plant can be used to obtained antibiotics having less or no side effect, especially against *Salmonella* and *staphylococcus aureus* infections.

***Index Terms***- Efficacy, Zizipus jujuba, Antibacterial activity, Cold water extract, zone of Inhibition.

## I. INTRODUCTION

Plants are the fundamental to existence on globe as they directly or indirectly resource around 70-80% of human energy and protein consumption, the rest being resulting from visceral products. They are sparingly significant to man due to their numerous applications, such as antibiotics, analgesic, flavors, perfumes, insecticides, dyes, food additives, poisons etc (Zamin *et al*., 2013). Antimicrobial activity of medicinal plant has turn out to be a worldwide concern. This problem is of great issue particularly in 3rd world countries because are one of the major causes of mortality in these countries is due to these infectious diseases (Majid *et al*., 2013)

The fruit of *jujuba* has been designated as ‘‘fruit of life”. The unique properties were erudite by ancient Chinese over thousands of years. Now a day medical practitioners are now discovering scientific evidence of its extraordinary possessions (Sharif *et al*., 2010). Investigations have exposed that fresh jujubes contain higher anti-oxidant ingredients than apples, strawberries, blueberries, plums, raspberries and blackberries (Soliman and Hegazi., 2013). In Asia the seed of *Z. jujuba* has been utilized as tranquilizer, analgesic and convulsant. These seeds have also been recommended for the cure of sleeplessness, anxiety and restlessness in Asia (Gautam *et al*., 2011) herbal treatment using *Zizyphus jujuba* and other basils ensued more rapid collapsing of jaundice than those who were cured with western remedies (Ebrahimi *et al*., 2011).

*Ziziphus jujuba* is universally known as Red Date (Rao and Lakshmi., 2012) and Chinese date or Bera (Pashto) that belongs to the family of Rhamnaceae (Ahmad *et al*.,2011). Phillip Miller was the man who titled Chinese jujube as *Ziziphus jujuba* in 1768, decided on the type of specimen collected from Austria. (Akhter *et al*., 2013).The family of Rhamnaceae is composed of 50 genera and more than 900 species. (Rao and Lakshmi., 2012). It is disseminated in the low temperature areas (Singh and Arya.,2011) almost multicultural (Ahmad *et al*.,2011) and growing generally in subtropical to tropical areas (Rao and Lakshmi., 2012) and. and found in Asia, Brazil, Nepal (Singh and Arya.,2011) and in several parts of India and Burma (Ganachari *et al*., 2004) . Chinese Jujube required full sun or partial shade for its cultivation and it should be grown on any well-drained soil. This plant does not give good growing outcomes on heavy clay or slushy soils (Edward *et al*., 1994). The vegetative breeding of *Z. jujuba* comprises of numerous techniques of vegetative propagation counting cuttings, grafting cutting and root cutting. Cuttings must be obtained from young vibrant, shoots from a sound mature tree (Singh and Arya., 2011). The leaves of *Z. jujuba* are simple alternate and ovate in shape having green spring color and yellow fall color. The size of the leaves is about 2 to 4 inches. The fruits are oval in shape having red or black color of about 1 to 5 inches size. Fruits have characteristics to attract squirrels and other mammals to cause a significant litter (Edward *et al*., 1994).

It has been investigated that *Z. jujuba* contain saponin, glycosides, alkaloids, steroids, polysaccharides and terpenoids as main ingredients that has a dynamic role in different activities such as hypoglycemic, hypolipidemic, antioxidant, antimicrobial and permeability enhancement activity (Ganachari *et al*., 2004). The alkaloids Coclaurine, Isoboldine, Norisoboldine, Asimilobine, Iusiphine and Iusirine were isolated from *Z. jujuba* leaves (Rao and Lakshmi., 2012).

Current study pursues the evaluation of pathogenicity of bacterial strains against the crude extract of *Zizipus jujuba* that supports the antibiotics preparation for bacterial infections especially Typhoid infection. Those antibiotics will have less or no side effects and adverse effects in comparison with synthetic drugs.

## II. MATERIALS AND METHODS

This research was carried out in the Microbiology Research Laboratory at department of Microbiology in Hazara University Mansehra.

**Plant Collection**

The aerial part (leaves) of the plant was collected from district Mardan Khyber Pukhtoonkhwa, Pakistan. The collected samples were washed out with running tape water, dried by air in the shadow at room temperature for one week. The dried leaves were then grounded into fine powder by an electric grinder.

**Extract Preparation**

*Cold water extract*:

The aqueous extract of dried *Zizipus jujuba* leaves was made in the distilled water. About 5 grams of *Zizipus jujuba* leaves powder were taken and mixed into 50 ml of distilled water. The mixture was taken into 250 ml sterile conical flask, plugged with sterile cotton and kept in shake on electric shaker (K (model: VRN-360) with the 200 rpm for 24 hours. After 24 hours the solution was centrifuged with 4500 rpm for 15 minutes by Eppendorf centrifuge (5702) and filtered through muslin cloth in sterile test tube. This process was repeated three times after which a clear aqueous extract of *Zizipus jujuba* was taken.

*Hot water extract:*

1og of the weighed plant leaves powder was soaked in 100ml of boiled hot water. That mixture was boiled for thirty minutes into a conical flask and put for 24hrs. The extract was filtered using filter paper and evaporated.

*Ethanol Extract*:

The ethanol extract of dried *Zizipus jujuba* leaves was also prepared. The ethanol extract was prepared through the same protocol followed for that of cold water extract.

**Media preparation**

*Nutrient agar*

The enrichment medium used for the growth of microorganisms was Nutrient Agar. Medium was prepared by adding 13g of dehydrated powder using electrical balance into 1 liter of distilled water. PH was adjusted by electrical pH meter at 7.4 and was boiled to dissolve completely.

*Media sterilization*

All Media were sterilized by using automatic autoclave (SANYO) at 121°C for 15 minutes.

*Media pouring and drying*

Media was poured in pre-sterilized glass Petri plates of 90mm in Laminar Flow Hood which was sterilized by overnight exposure of UV light and disinfected with 70% ethanol solution. Media plates were kept open for half an hour in the Laminar Flow Hood for drying and solidifying media.

**Test microorganisms**

The in-vitro activity of the extracts was assayed against the bacterial strains. All the ATCC (Micro BioLogics) against gram positive bacteria *Staphylococcus aerious* ATCC®6538, and *Enterococcus feacalis* and gram negative bacteria *Escherichia coli* ATCC®25922, and *Salmonella typhimurium* ATCC®14028 and *Klebsilla pneumonia*. These strains were kindly provided by Dr. Malik Mujaddad ur Rehman, Assistant Professor, and HOD Department of Microbiology, Hazara University, Mansehra. Strains were maintained on Nutrient Agar Tubes at 4 $^0$C. The antibiotic efficacy of the plant extracts was evaluated against given strains

**Inoculation of test organisms**

After the incubation time, single selective colony of each bacterium from their respective selective agar medium was inoculated into Nutrient agar medium. Sterile wireloop was used for all the bacterial strains. Bacterial strains were picked up by the sterile wireloop and streaked on the Nutrient agar plates inside the ESSCO® (EN 1822.1) streamline horizontal air flow hood. The inoculated plates were kept for 24 hours at 37 $^0$C in incubator (NAPCO). After 24 hours the bacterial growth was observed.

**Disc Diffusion Method**

In order to determine the antimicrobial activity of *Zizipus jujuba* leaf extract, disc diffusion method was used. In this method discs of the plant's extracts were placed aseptically on the Nutrients Agar plates containing the specific bacterial culture. The plates were incubated for 24 hours inside incubator at 37 $^0$C. After incubation the zones of inhibition were observed around the discs on certain nutrient agar plates. The zones of inhibition were measured by the digital Vernier Calliper (Mitutoyo). The experiment was repeated three times in order to confirm the given results.

**Evaluation of antimicrobial activity**

Disc diffusion method was used to determine the antimicrobial of *Zizipus jujuba* leaf extract. Single disc was used for both the cold water and ethanol extract of *Zizipus jujuba* were placed aseptically on the Nutrient agar plates containing the specific bacterial cultures. The plates were incubated for 24 hours at 37 $^0$C. After a good incubation period the zones of inhibition were measured with the help of Digital Verneir Calliper and the values were recorded three times after each experiment. All the antimicrobial assays were performed three times. The mean values of zones of inhibition against each bacterium were documented in this report.

## III. DATA ANALYSIS

The average of all data was recorded and all the data were repeated three times. The statistical data were subjected to Microsoft excel 2010.

## IV. RESULTS

In current research, the antimicrobial activity of *Zizipus jujuba* leaf extract was checked out against two Gram positive and three Gram negative bacteria. The leaf extract was prepared by two ways; one was cold water extract and second was ethanol extract. Their potential antimicrobial activity was qualitative and quantitative, estimated by the presence and absence of zone of inhibition and MIC values.

**Antibacterial activity**

In this investigation, the leaf extract of *Z. jujuba* plant showed the antibacterial activity against all the bacterial cultures

used. Results of the current investigation were recorded in table I and 2 and the Figure 1-4. Among which the graphical result of cold water extract and ethanol has been represented in figure 1 and 2 respectively. Ethanol extract was used against Salmonella typhmurium and Staphylococcus aureus. The graphical comparison of three extracts has been represented in figure 3 and figure 4 presented plate results.

**Table 1: Measurement of zones of inhibition in cold water extract**

| **COLD WATER EXTRACT BY DISC DIFFUSION METHOD** | |
|---|---|
| Bacterial strains | Zone of inhibition |
| *Escherichia coli* | 19mm |
| *Staphylococcus aureus* | 28mm |
| *Klebsella pneumonia* | 19mm |
| *Salmonella typhimurium* | 27.52mm |
| *Enterococcus feacalis* | 32mm |

Table I represents the result of Z. jujuba leaf extract prepared by cold water methods against both the Gram negative and Gram positive. The Gram negative bacteria include *Escherichia coli*, *Klebsilla pneumonia* and *Salmonella typnimurium* giving the zones of inhibition up to 19mm, 19mm and 27.52mm respectively. The Gram positive bacteria include *Enterococcus feacalis and staphylococcus aureus* giving the zones of inhibition up to about 32mm and 28mm.

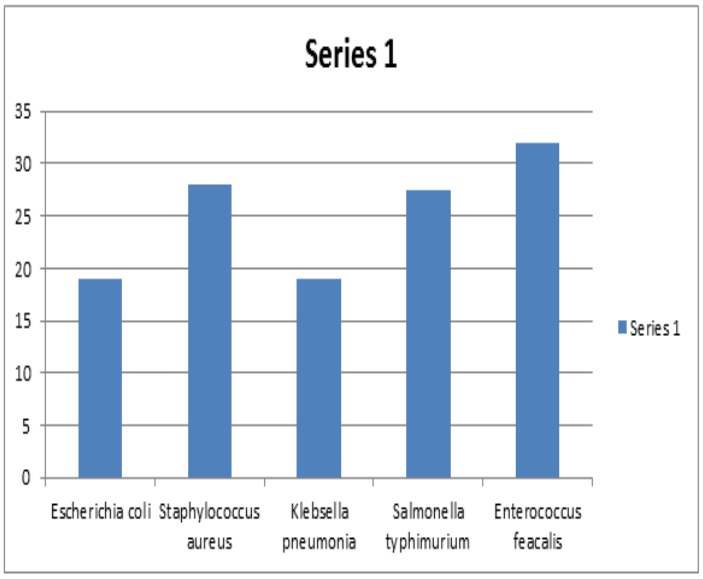


**Figure 1: Graphical result of cold water extract showing zone of inhibition in mm**

**Table 2: Measurement of zones of inhibition in ethanol extract**

| **ETHANOL EXTRACT BY DISC DIFFUSION METHOD** | |
|---|---|
| Bacterial strains | Zone of inhibition |
| *Escherichia coli* | ------ |
| *Staphylococcus aureus* | 28mm |
| *Klebsella pneumonia* | ----- |
| *Salmonella typhimurium* | 27.52mm |
| *E. Feacalis* | ----- |

Table 2 shows the result of Z. jujuba leaf extract prepared through ethanol method. The ethanol extract was used against one Gram negative bacteria and one Gram positive bacteria. The Gram Negative bacteria used in this investigation include *Staphylococcus aureus* and *Salmonella typnimurium* giving the zones of inhibition up to 28mm and 27.52mm respectively.

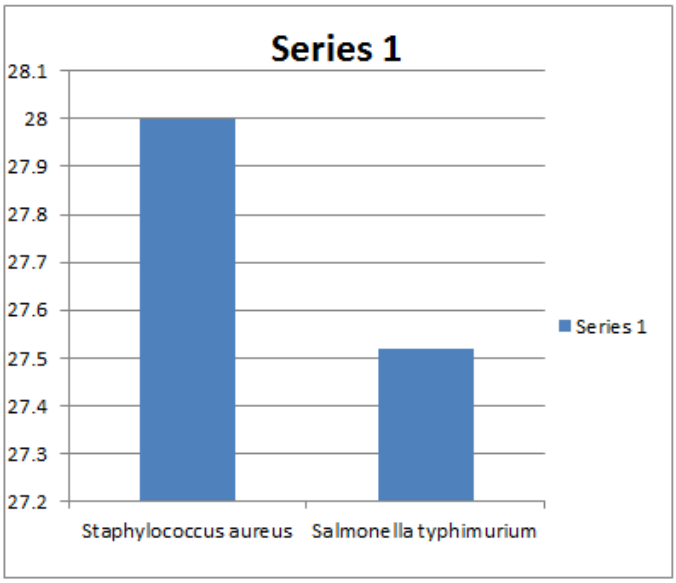


**Figure 2: Graphical result of ethanol extract showing zone of inhibition in mm.**

Hot water extract was used against all the selected bacterial strains except *Staphylococcus aureus.* The hot water extract showed no activity against the bacterial strains. The reason for that were the degradation alkaloids (Coclaurine). Coclaurine has the boiling point less than the boiling point of water therefore that was degraded with the high temperature.

Graphs representing activity of three extracts represented in Figure 3. The cold water and ethanol give same results against *Staphylococcus aureus* and *Salmonella typhiurium* giving zones of inhibition of 28mm and 27.52mm respectively.

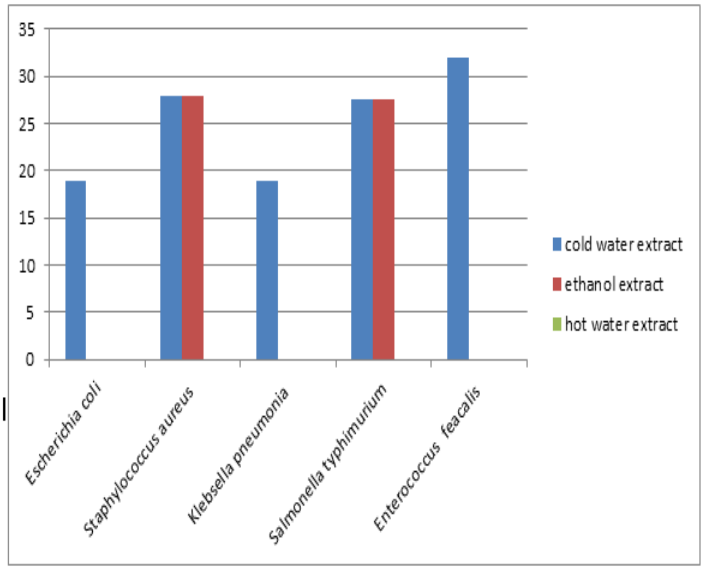


**Figure 3: Graphical representation of result obtained through cold water, ethanol and hot water extract**

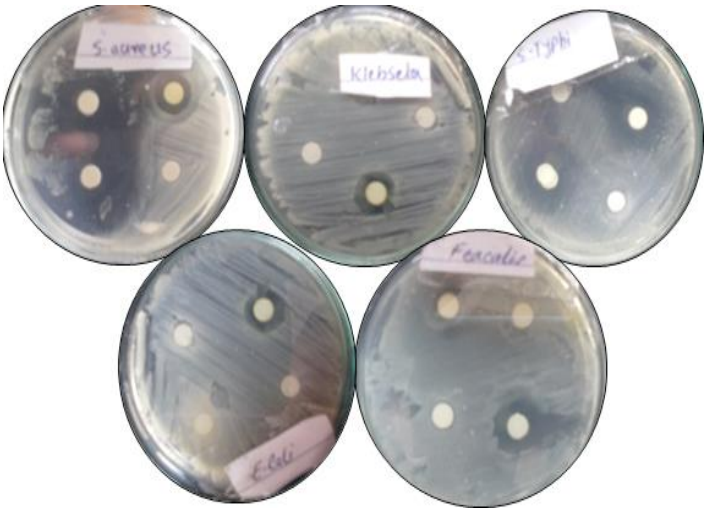

**Figure 4: Plates indicating positive inhibition by *Zizipus jujuba* extracts and also measurement of zones of inhibition**

## V. Discussion

Pharmacologically plants are the main focus of the researchers around the globe. Plants have been used for the preparation of multiple drugs since ancients. The world is facilitated with a great variety of pharmacologically important plants and herbs by the God that has efficient and very potential properties in order to eliminate a variety of diseases and lesser the health problems. On the other hand industrialization and chemically prepared products have damaged the human life on about every aspect, from physical to genetics. The only solution to those problems suggested by scientists is the vast use of plants on different aspects.

Medicinal plants played a vital role against different diseases including microbial infections, cancer and other disorders like diabetes. The antimicrobial activities of most medicinal plants are qualified due to the presence of alkaloids, and flavonoids (Burapedjo and Bunchoo 1995; Fewell and Roddick, 1993).

Zizipus jujuba is one of the useful and important medicinal plants. Ahmad et al has investigated that the crude methanol extract of *Z. jujuba* plant showed moderate activity against *P. aeruginosa, B. pumalis* and *E. aerogens* with 55.55, 52 and 41.37%, low against *S. typhi, S. epidermidis, S. pneumoniae, S. aureus* and *K. pneumoniae* with 37.03, 34.61, 31.03, 30.76 and 28.57% inhibition, respectively.

The present research is the investigation of antimicrobial activity of plant (Z. jujuba). This research suggested that cold water extract, and ethanol extract of *Zizipus jujuba* leaves have a potential activity against Gram Negative bacteria and Gram Positive bacteria. The extract was applied to nutrient agar plates containing different bacterial cultures by the disc diffusion method that interact the extract with bacteria directly and clearly showed its effects on bacterial strains. The discs were kept in the extract for 15 to 20 minutes. The degree of vulnerability of bacterial cultures to the extracts varied among the methods of extraction and strains. Figure 4 shows the comparison between three extracts. It showed the equal potentiality of cold water and ethanol extracts. Pathogenic bacteria *Staphylococcus aureus* and *Salmonella typhimurium* were more vulnerable to both cold water and ethanol extract.

## VI. Conclusions

This investigation suggests the *Zizipus jujuba* to be a good antimicrobial agent against various pathogenic agents. The antimicrobial activity of the plant could be enhancing by synthetic methods. Further investigation is required in this regards that can replace the plant with a very beneficial antimicrobial medicine and enhance its effects.

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