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Evaluation of Antibacterial Activity of *Ziziphusjujuba* Leaf Extracts against *Streptococcus pneumonia* and *Salmonella species*

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Abstract

Ziziphus jujuba is a common medicinal plant traditionally utilised in tropical and subtropical regions for its various biological functions, such as antibacterial, antifungal, and antidiarrheal properties. This study aimed to investigate the antibacterial activity of Ziziphus jujuba leaf extracts against clinical isolates of Streptococcus pneumonia and Salmonella species using the agar well diffusion method. Phytochemical screening, Minimum Inhibitory, and Minimum Bactericidal Concentration Assays (MIC/MBC) were also carried out using standard procedures. The results of phytochemical screening showed that tannins, phenols, saponins, quinines, terpenoids, and steroids were present in ethanolic extract. Similarly, Tannins, saponins, phenols, steroids, phytosteroids, and terpenoids were present in aqueous extract. Salmonella species were sensitive to the aqueous and ethanolic extracts at 500 and 125 mg/mL, respectively, compared to the 250 and 125mg/mL obtained against Streptococcus pneumoniae, respectively. The MIC values of the ethanolic extract were 62.5 and 125 mg/mL for Salmonella species and Streptococcus pneumoniae, while the aqueous extract exhibited MIC values of 62.5 and 125mg/mL for Streptococcus pneumoniae and Salmonella species, respectively. Regarding the MBC values, 125 and 62.5 mg/mL were obtained for the aqueous extract against Streptococcus pneumoniae and Salmonella species, compared to the 62.5 and 125 mg/mL for the aqueous extract. The antibacterial activity exerted by the extract against the test isolates suggests that Ziziphusjujuba leaf can be explored as a source of effective antibacterial medications targeting the test bacteria.

***Keywords:** Antibacterial activity, Phytochemical, Salmonella species, Streptococcus pneumonia, Ziziphus jujuba*

INTRODUCTION

The utilization of medicinal plants is unquestionably a skill that has been practiced since the beginning of humanity, employed as a remedy for illnesses in ancient times and early civilizations worldwide. Additionally, they have the potential to serve as sources of pharmacologically active substances that can be valuable candidates for drug development (Hosseini *et al.*, 2020). Out of the roughly estimated 500,000 plant species, only around 10% are utilized as food by humans and other animals. Furthermore, a limited number of plants have undergone phytochemical investigation, and only a proportion have been exposed to biological or pharmacological screening (Chowdari *et al.*, 2020).

Jujuba, scientifically known as *Ziziphus jujuba* (Magarya), is a species in the buckthorn family Rhamnaceae. It is also commonly referred to as red date, Chinese date, and Chinese *jujuba*. *Ziziphus jujuba* is alternatively known as Badari, Baer, Bogari, or Barihannu. The leaf of *Ziziphus jujuba* is extensively utilized in traditional medicine in Northern Nigeria (Abubakar*et al.*, 2018). The *jujuba* fruit is a delectable fruit that serves as a potent herbal treatment, enhancing endurance and physical power and facilitating weight gain. It enhances liver function and boosts immune system resilience. It serves as an antidote, diuretic, emollient, and expectorant. The leaves are febrifuge, astringent, and claimed to enhance hair development. In the treatment of strangury, they are utilized to form a plaster. The dried fruits have sedative, anodyne, anticancer, refrigerant, styptic, pectoral, tonic, and stomachic properties. They aid in blood filtration and digestion (Venkatachalam *et al.*, 2020). According to Al-Reza *et al.* (2010), the fruits from the Rhamnaceae family have high nutritional values and anti-infectious properties. Generally, Zizyphus species are widely used as medicinal plants in Asian countries, particularly in Taiwan and China, for the treatment of various liver diseases, urinary troubles, allergies, constipation, depression, chronic bronchitis, and insomnia (Afroz *et al*., 2014).

Active substances derived from higher plants have recently come to play a significant role in contemporary medicine. There are currently around 130 chemicals in use that have been isolated from higher plants and their synthetic or modified descendants. Several of these substances are used in conventional medicine and are thought to be effective antibacterial agents. However, a significant obstacle to treating infectious diseases in developing nations is the ongoing growth of bacteria resistant to traditional medications. This makes it necessary to look for novel, highly effective antibacterial compounds in locally grown plants (Abubakar *et al*., 2018).

MATERIALS AND METHODS

Collection, authentication, and preparation of plant material

Leaf samples were acquired from the Botanical Garden, Kaduna State University, Kaduna, Nigeria, and the plant was recognized and verified by a botanist at the Department of Biological Science of the same institution where 190/01 was assigned as herbarium/voucher number for the specimen. The *Ziziphus jujuba* leaves were carefully cleaned under running water and allowed to dry at room temperature for fourteen days. Using a mortar and pestle, it was ground into a coarse powder and kept in several airtight bottles (Periasamy *et al*., 2020).

Extraction of plant material

Plant extraction was done using Periasamy *et al.'s* (2020) procedures. About 100 grams (100g) of powdered *Ziziphus jujuba* were suspended in 1000 milliliters of 70% ethanol in a conical flask, and another 100 grams of powdered *Ziziphus jujuba* were suspended in 1000 milliliters of distilled water in a different conical flask. This was combined and agitated briskly with a sterile glass rod before being stored at room temperature in tightly sealed containers. The mixture was continuously shaken with a rotary shaker for a full day. Whatman No. 1 sterile filter paper was used to filter the mixture and transfer it into a sanitized conical flask. After that, filters were placed in the rotary vacuum evaporator's sample container and left there for 24 hours at 28°C. The resulting semi-solid extract was allowed to dry under a ceiling fan. Using the formula from Periasamy *et al*. (2020), the % yield of the ethanol and aqueous crude extract of *Ziziphusjujuba* was determined.

Percentage Yield of Plant Extract (%) = W_2-W_1/W_0 × 100

Where
W_1= Weight of the container in grams
W_2= Weight of container + Extract
W_0= Weight of powdered leaf, root, or bark

Phytochemical analysis

Phytochemical components of the crude aqueous and ethanolic leaf extracts of *Ziziphus jujuba* were screened using the method described by Periasamy et al. (2020). Phytochemical components analyzed were Alkaloids, Steroids, Saponins, Phenol, Tannins, Glycosides, Terpenoids, Quinine, and Phytosteriod (Periasamy *et al*., 2020).

Test Bacteria

Salmonella species and *Streptococcus pneumonia* were the clinical isolates used. The isolates were obtained from the Department of Microbiology, Kaduna State University, labelled, and put in an icebox before being subjected to further analyses at the Laboratory of the Department.

Reconfirmation of clinical isolates

Gram staining microscopy and biochemical analysis were used to reconfirm the clinical isolates used as described by Cheesbrough (2002).

Antibacterial Susceptibility Test

For the antibacterial screening, Mueller Hinton agar was produced in accordance with the manufacturer's instructions. The antibacterial activity of the aqueous and ethanol extracts of *Ziziphus jujuba* against the clinical isolates of *Streptococcus pneumonia* and *Salmonella* species was determined using the agar well diffusion method, as described by Chowdari *et al*. (2020). A sterile cotton swab stick was used to evenly apply roughly 100µl of standardized inoculums of a bacterial solution across the entire surface of Mueller Hinton agar plates, which were inoculated using a micropipette. After giving the plates ten minutes, sterile cork borer wells of six mm were inserted into the agar. As a positive control, another well was filled with the antibiotic ciprofloxacin. After allowing the extract to diffuse into the agar for ten minutes at room temperature, the plates were incubated for twenty-four hours at 37˚C. Using a meter ruler, the diameter of each zone of growth inhibition for each tested bacterium was measured and recorded in millimeters.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the plant extracts was ascertained by the broth dilution method, as described previously (Bashir *et al*., 2022). Muller-Hinton broth in six tubes, each holding five milliliters, was made. In tubes 1-4, one (1) milliliter of the crude extract from (500, 250, 125, and 62.5 mg/mL) was added, and it was well mixed. After that, four tubes each received 0.5 mL of the test organisms' broth cultures, with the final tube acting as the broth control for each. The inoculation tubes were incubated for twenty-four hours at 37˚C. 500 mg/mL of ciprofloxacin was employed as the positive control. MIC is defined as the lowest concentration at which no growth was observed.

Determination of Minimum Bactericidal Concentration (MBC)

Mueller-Hinton broth that had been produced was poured into sterile test tubes in five-milliliter increments, equal to the number of tubes that had no discernible growth from the MIC. Then, tubes holding the 5 mL Mueller Hinton broth were filled with 0.1 mL of the broth culture. The tubes were stored on a test-tube rack and labeled. A sterile Petri plate was filled with prepared Mueller-Hinton agar, which was then left to harden. A sterile pipette was used to transfer 0.1 mL from each tube onto the agar's surface. A sterile, smooth glass rod that was bent was used to distribute the inoculum. For twenty-four hours, tubes and plates were stored in an incubator at 37˚C. It was noted whether the broth culture was turbid or cloudy, as well as whether bacterial colonies were growing on solid agar media. The MBC was determined by using the lowest concentration of the MIC culture that did not exhibit any apparent bacterial growth (Bashir et al., 2022).

RESULTS

The findings of the phytochemical screening of *Ziziphus jujuba* leaf extracts in ethanol and aqueous form are shown in Table 1. The findings demonstrated that whereas alkaloids, glycosides, and phytosteroids were absent from the ethanolic extract, tannins, phenol, saponin, quinines, terpenoids, and steroids were present. Additionally, the aqueous extract included tannin, saponin, phenols, steroids, phytosteroids, and terpenoids but not alkaloids, glycosides, or quinines.

Table 1: Phytochemical screening of aqueous and ethanol extracts of *Ziziphus jujuba* leaf

S/N	Constituents	Aqueous Extract	Ethanol Extract
1.	Tannin	+	+
2.	Saponin	+	+
3.	Alkaloid	-	-
4.	Glycoside	-	-
5.	Quinines	-	+
6.	Phenols	+	+
7.	Terpenoids	+	+
8.	Steroid	+	+
9.	Phytosteroid	+	-

Key: + = Detected
- = Not detected

The antibacterial activity of an aqueous and ethanol extract of *Ziziphus jujuba* leaf was tested against *Salmonella* species and Streptococcus pneumonia, as shown in Tables 2 and 3. Compared to an aqueous extract, the results of the ethanol extract demonstrated more antibacterial activity.

Table 2: Antibacterial susceptibility of the aqueous leaf extract of *Ziziphus jujuba* against the test isolates solution.

Organism	500mg/ml	250mg/ml	125mg/ml	62.5mg/ml	Positive Control Ciprofloxacin mg/mL
Streptococcus pneumonia	13mm	10mm	R	R	9mm
Salmonella species	14mm	R	R	R	8mm

Key: R= resistant

Table 3: Antibacterial susceptibility of the Ethanolic extract of *Ziziphus jujuba* leaf against the test isolates

Organism	500mg/ml	250mg/ml	125mg/ml	62.5mg/ml	Positive Control Ciprofloxacin mg/mL
Streptococcus	15mm	14mm	12mm	6mm	9mm
Salmonella	16mm	14mm	12mm	6mm	8mm

The results of the Minimum Inhibitory Concentration of the leaf's ethanolic and aqueous extract on the clinical isolates of bacteria are displayed in Table 4.

Table 4: Minimum Inhibitory Concentration Result of *Ziziphus jujuba* against the test Isolates

Organism	Aqueous	Ethanolic
Streptococcus pneumonia	250 mg/mL	125 mg/mL
Salmonella species	500 mg/mL	125 mg/mL

The results of the MBC of the leaf's ethanol and aqueous extract on the clinical isolates of bacteria are displayed in Table 5.

Table 5: Minimum Bactericidal Concentration result of *Ziziphus jujuba* against the test Isolates

Organism	Aqueous	Ethanolic
Streptococcus pneumonia	250 mg/mL	125 mg/mL
Salmonella species	500 mg/mL	125 mg/mL

DISCUSSION

Aqueous and ethanol leaf extracts of *Ziziphus jujuba* were subjected to phytochemical screening, and the results indicated the presence of several phytochemical compounds, including tannins, phenols, saponins, quinines, terpenoids, steroids, and phenols, which have been reported to have antibiotic properties (Deshpande *et al*., 2019; Elshahir *et al*., 2020; Soni *et al*., 2021).

According to the study by Duan *et al*. (2023), the susceptibilities of test bacteria to the active fractions of *Ziziphus jujuba* leaf were likewise shown to be concentration-dependent, meaning that the higher the concentration, the higher the activity.

Ziziphus jujuba extract has strong antibacterial action, while ethanol plant extract demonstrated broad-spectrum efficacy. The study also demonstrates that *Streptococcus pneumonia* and *Salmonella* species were both susceptible to the extract, with the highest zones of inhibition for both species at 500 mg/mL (16 mm for *Salmonella* species and 15 mm for *S. pneumonia* on the ethanolic extract), while the zones of inhibition for both species on the aqueous extract were (13 mm) and (14 mm) for *S. pneumoniae* and *Salmonella* species, respectively. The results of the investigation corroborate those of Babatunde *et al*. (2023), who found that the zone of inhibition increased with concentration. The results showed that the bacterial isolates' susceptibility to *Ziziphus jujuba* leaf extracts was similar to that of ciprofloxacin (positive control). This suggests that *Ziziphus jujuba* leaf extracts could be used as an additional or alternative antibacterial agent to reduce the risk of bacterial vaginal infection in women who are antibiotic-resistant. The results of a previous study by Medini *et al*. (2014), where the selected bacterial isolates used in the study showed good susceptibility to aqueous and ethanolic extracts of *Ziziphus jujuba* at varied concentrations, may explain why the ethanol extract demonstrated more antibacterial activities than the aqueous extract. Ethanol is an organic solvent and can extract more phyto-constituents than water. The differences in the cell walls of the bacteria may be the cause of the isolates' varying susceptibilities to different concentrations. While Gram-negative bacteria have lipopolysaccharides, which may have prevented the active components of extracts from penetrating, Gram-positive bacteria tend to allow diffusion of the active components. This was also consistent with research by Yahia *et al*. (2020), which found that Gram-positive bacteria had a larger zone of inhibition than Gram-negative bacteria. However, it was at odds with research by Abubakar *et al*. (2018), which found that at 150 mg/ml concentration, Gram-negative bacteria were more susceptible than Gram-positive bacteria. All of the extract's actions, however, were dose-dependent, which is consistent with studies by Sakha *et al*. (2018)

that found that the extract's antibacterial activity increased at higher concentrations.

According to earlier studies (Elaloui *et al*., 2017; Abubakar *et al*., 2018; Yahia *et al*., 2020), *Ziziphus jujuba* has excellent antibacterial activity against both Gram-positive and Gram-negative bacterial isolates. The MIC and MBC of the study's results are comparable to these studies' findings. The plant's ability to suppress the isolates with low MIC and MBC values was ascribed to the potency of the plant extracts. This is in contrast to the Dubey *et al*., (2010) findings, which had greater MIC and MBC.

CONCLUSION

This study revealed the presence of tannins, saponins, terpenoids, alkaloids, glycosides, phenol, steroids, quinines, and phytosteroids as the phytochemical constituents of *Ziziphus jujuba* leaf extracts, which underscore its antibacterial action against the test isolate.

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