https://dx.doi.org/10.4314/dujopas.v7i4b.19
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
**Dutse Journal of Pure and Applied Sciences (DUJOPAS),** *Vol. 7 No. 4b December 2021*

# Antibacterial and Phytochemical Screening of *Ziziphus Jujuba* (Jujube/Magarya) Leaf Extract in Kaduna Metropolis

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## Abstract
Ziziphus jujuba *from the family of Rhamnaceae is widely distributed in both tropical and subtropical countries. Different parts of the plant have been used traditionally for several biological purposes including fungal and antibacterial and antidiarrheal. This study was aimed to assess the antibacterial activity of Ziziphus jujuba leaf extract against bacteria isolated from vaginal swab. Preliminary phytochemical screening of the leaves extract of Ziziphus jujuba was carried out using standard analytical methods. The aqueous and ethanol extracts of Ziziphus jujuba leaf were screened for antimicrobial activities against Staphylococcus aureus and Escherichia coli isolated from vaginal swabs using agar well diffusion and broth dilution assay. The results of the phytochemical constituents revealed the presence of alkaloids, steroids, tannins, phenols, cardiac glycosides, and terpenes in the ethanol extract while alkaloids, steroids, tannins, cardiac glycosides, phenols, and saponins were present in the aqueous extract. The inhibitory zones of the ethanol extract against S. aureus ranged between 13.00- 15.00 mm while that of E. coli ranged between 7.00- 10.00 mm at 50 and 100 (mg/ml) respectively. The inhibitory zone of the aqueous extract against the clinical isolates of S. aureus ranged from 9.00- 11.00 and 6.00-8.00 (mm) for E. coli at 50 and 100 (mg/ml) respectively. However, S. aureus was more susceptible to the extract with an MIC of 100 mg/ml. The observed inhibitory activities of the leaf extract against the clinical isolates could be due to phytochemical constituents present in the plant extracts of Ziziphus jujuba.*

**Keywords:** Antibacterial evaluation, *Escherichia coli, In vitro*, Phytochemicals, *Staphylococcus aureus*, *Ziziphus jujuba*,

## INTRODUCTION
Bacterial vaginosis has received little attention since it is considered to be a trivial infection, Though, it is a morbid disease in terms of loss of working hours and cost of treatment (Donders *et al*., 2002). Moreover, it increases the threat to acquiring human immunodeficiency virus (HIV) (Myer *et al*., 2005) and other sexually transmitted diseases (STDs) including gonorrhea, herpes simplex virus type 2 (HSV-2) and trichomoniasis (Cherpes *et al*., 2003). In addition, it increases the risk of miscarriage, preterm labor, preterm delivery, and postpartum complications such as endometritis and wound infections in pregnant women (CDC. 2006; Bittew *et al*., 2017). The existing studies on antimicrobial profile proved that, nosocomial-causing bacteria as well as other infectious bacteria become

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highly resistant to different antibiotic groups. Hence, this situation becomes a clinical threat to human beings (Leopold *et al.*, 2014).

Ethnobotanical knowledge has always guided the search for new medications from medicinal plants. Though, this practice of using medicinal plants as herbal therapies to prevent and cure several diseases differs from one community to another (Kubmarawa *et al.*, 2008). Natural extracts have significant effects and it alternative therapeutic properties due to some bioactive compound they contained enhances effectiveness of diseases treatment (Ogbeba *et al.,* 2017).

Jujube (*Ziziphus jujuba* Mill.) from the family of Rhamnaceae (Chen *et al.*, 2015a) has since gained attention in different fields ranging from nutritional and food sciences credit to its medicinal and nutritional properties (Wang *et al.*, 2016). The fruits of jujube are consumed freshly, though, can also be processed into products, including alcoholic beverage, jam, cake, candied snack, bread and sweetened tea syrup (Zozio *et al.*, 2014; Wojdyło *et al.*, 2016a). Further, the fruits of jujube also contain high level of mineral, organic acids, protein, polyphenols and vitamins (Park *et al.*, 2012; Gao & Wang, 2013). These constituents play a significant role in affecting the nutritional properties of jujube. Jujube fruits for example, content phenolic compounds that possess anti-cancer, anti-obesity, antioxidative and anti-diabetes properties (Yu *et al.*, 2012; Wojdyło *et al.*, 2016b). Ganachari *et al.* (2004) reported that saponin, alkaloids, polysaccharides, steroids, glycosides and terpenoids were detected in the extracts of *Z. jujube*.

The *in vivo* anxiolytic effect had previously been reported in a poly-herbal substance that consists of *Z. jujube* seed extract (Mesaik *et al.,* 2018). Inhibitory assay performed by ethanolic extract of *Z. jujuba* root on fungi *Candida albicans, A. niger, Aspergillus flavus* and *C. tropicalis* showed remarkable antifungal activity (Yuan *et al.,* 2017). Also, the root bark extract of *Z. jujuba* exhibited inhibitory activity against 20 bacteria isolates (Beg et al., 2016). A traditional Chinese preparation (Huangqin Tang) with fruit content from *Z. jujuba* displayed remarkable antispasmodic and anti-inflammatory effect (Triantafillidis *et al.,* 2016). In Nigeria, the leaves of *Z. jujuba* is used traditionally by women after period to wash their private parts and also to cure toilet infections. The aim of the study is to evaluate the antibacterial activities of *Z. jujuba* leaves extract against bacteria isolated from vaginal swab.

## MATERIALS AND METHODS

### Collection of samples

Leaves of *Z. jujuba* were obtained from Kaduna metropolitan area and then conveyed to Biological Science Department, Kaduna State University (KASU) in sterile polythene bags for authentication. Voucher number of V/No: 5332 was assigned to the plant. The leaves were then washed and air dried for 10 days. The dried leaves were ground to powdered form using mortar and pestle. The powder was then placed in clean black polythene bags, labelled and kept until required.

The bacterial isolates including *Escherichia coli* and *Staphylococcus aureus* were collected from Medical Microbiology Laboratory of 44 Nigerian Army Hospital Kaduna, Kaduna State. The Isolates were transferred to the laboratory in ice box and maintained at 4°C in the refrigerator until needed.

**Laboratory procedures**
The aqueous extract of *Z. jujuba* leaf was prepared using distilled water following the methods of Ogbeba *et al.* (2017). 100g of *Z. jujuba* leaves were dissolved in 500 ml of distilled water. The solution was kept for 3 days at room temperature. The solution was filtered using a Whattman (no.1) filter paper in a sterile test tube. The filtrate was then concentrated by evaporation at 50°C using water bath. The concentrated extract was kept in a screw cap bottle at 4°C. The ethanol extract of dried *Z. jujuba* leaves was also prepared via the same procedure as that of aqueous extract.

**Phytochemical Screening**
The phytochemical qualitative screening of *Z. jujuba* leaves extract was carried out according to Ogbeba *et al.* (2017) to determine the presence phytoconstituents such as flavonoids, alkaloids, tannins, glycosides, plabotannins, terpenes, cardiac glycosides, saponins, steroids and terpenoids.

**Standardization of Clinical Isolates**
The method of Oyeleke and Manga (2008) was used to standardize the organisms. The isolates being tested were cultured on Mueller Hilton Agar (MHA) at 37°C overnight. Colonies formed were inoculated into a tube containing Mueller Hinton broth until the turbidity is equal to that of 0.5 McFarland standards.

**Preparation of Extract Concentration**
One gram of the crude extract was dissolved in 10ml of 2% Dimethyl Sulfoxide (DMSO) in a test tube to get 100mg/ml which the highest stock concentration used. This was followed by serial dilution with distilled water to give various concentrations (50mg/ml, 25mg/ml and 12.5mg/ml). Aqueous dilution was also made using the same technique.

**Antibacterial Susceptibility Test of *Z. jujuba***
Agar well diffusion method of Olutiola *et al.* (2016) was used to determine the inhibitory activity of aqueous and ethanolic extracts of *Z. jujube* against the bacterial isolates. The susceptibility test was carried out using 100mg/ml, 50mg/ml, 25mg/ml, and. 12.5mg/ml concentrations. The standardized isolates were spread (0.1 ml) throughout the surface of MHA plate. 6mm diameter wells were made on the agar using a sterile cork borer. 0.1 ml of the leaf extract at the labelled concentration (100mg/ml, 50mg/ml, 25mg/ml, and 12.5mg/ml) was dropped separately in each well using a micropipette. It was then allowed to stand for 4-5 hours for proper diffusion of the extract. Incubation (without inverting the plates) was done for 18-24 hours at 37°C. About 0.1ml of 2% DMSO and ciprofloxacin served as both the negative and positive control respectively. The presence of zone of inhibition around the wells indicated bacterial inhibition by the extracts while the absence of zone indicated no inhibition. The diameter of inhibition zone was then measured using a well calibrated meter ruler.

**Determination of Minimum Inhibitory Concentration (MIC)**
The method of Doughari *et al*. (2017) was used to determined the MIC of the extract. Concentrations (100mg/ml and 50mg/ml) of the extracts were prepared and 1ml each of the varying concentrations was dispensed into test tubes according to the concentration using sterile pipette. About 2ml of sterile Mueller Hilton broth was dispensed into the test tubes. Loopful of the standardized organisms was inoculated into the varying extracts. Inoculum control was prepared by inoculating 1ml of Mueller Hilton Broth (MHB) with the standardized inoculum in a test tube. Broth control was prepared by inoculating 2ml of

MHB in a test tube and extract control was prepared by inoculating 1ml of the crude extract with 1ml of the MHB in a test tube. All the tubes were incubated overnight at 37°C. The turbidity was compared with the controls and the tubes with the minimum concentration showing no growth was taken as the MIC.

## RESULTS

The outcome of the phytochemical screening of ethanol and aqueous extracts of *Z. jujuba* is presented in Table 1. Phytochemicals such as terpenes, tannins, alkaloids, phenols, steroids and cardiac glycosides were present in the ethanol. The phytochemicals including phenols, tannins, saponins, steroids, alkaloids and cardiac glycosides were present in the aqueous extract.

**Table 1: Phytochemical Screening of *Z. jujuba* ethanol and aqueous Extracts.**

| Active Components | Ethanol Extract | Aqueous Extract |
|---|---|---|
| Phenols | + | + |
| Terpenes | + | - |
| Steroids | + | + |
| Cardiac Glycosides | + | + |
| Quinones | - | - |
| Plabotannins | - | - |
| Saponins | - | + |
| Glycosides | - | - |
| Alkaloids | + | + |
| Tannins | + | + |
| Anthraquinones | - | - |

+ = Positive - = Negative

The zone of inhibition of *Z. jujuba* ethanol extract is presented in Table 2. The zone of inhibition against *S. aureus* ranged between 13.00–15.00 (mm) at varied concentration (mg/ml) of 100, 50, 25, and 12.5. Similarly, the zone of inhibition of *Z. jujuba* against *E. coli* ranged between 7.00–10.00 (mm) at varied concentration (mg/ml) of 100, 50, 25, and 12.5. More inhibitory effect of the leaf extract was observed on *S. aureus.*

**Table 2: Inhibitory Activity of Ethanolic Leaf Extract of *Ziziphus jujuba* against the Bacterial Isolates**

| Organism | 100mg/ml | 50mg/ml | 25mg/ml | 12.5mg/ml | Control |
|---|---|---|---|---|---|
| *S. aureus* | 15.00 | 13.00 | – | – | 40.00 |
| *E. coli* | 10.00 | 7.00 | – | – | 23.00 |

The zone of inhibition of *Z. jujuba* aqueous leaf extract is presented in Table 3. The zone of inhibition against *S. aureus* ranged between 9.00–12.00 (mm) at varied concentration (mg/ml) of 100, 50, 25, and 12.5. Similarly, the zone of inhibition of *Z. jujuba* against *E. coli* ranged between 6.00–8.00 (mm) at varied concentration (mg/ml) of 100, 50, 25, and 12.5. More inhibitory effect of the leaf extract was observed against *S. aureus.*

**Table 3: Inhibitory Activity of Aqueous Leaf Extracts of *Z. jujuba* against the Bacterial Isolates**

| Organisms | 100mg/ml | 50mg/ml | 25mg/ml | 12.5mg/ml | Control |
|---|---|---|---|---|---|
| *S. aureus* | 11.00 | 9.00 | – | – | 40.00 |
| *E. coli* | 8.00 | 6.00 | – | – | 23.00 |

The result of MIC is presented in Table 4. The highest MIC of the leaf extract of *Z. jujuba* against *S. aureus* was observed to be 100mg/ml in both aqueous and ethanol extracts. *E. coli* showed no activity in the MIC as turbidity appears at all concentrations. As for the minimum bactericidal concentration, the result indicated presence of growth.

**Table 4: Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Leaf Extract against the Bacterial Isolates.**

| **Organisms** | **MIC (mg/ml)** | **MBC (mg/ml)** |
|---|---|---|
| *S. aureus* | 100 | – |
| *E. coli* | – | – |

MIC = Minimum Inhibitory Concentration MBC = Minimum Bactericidal Concentration

## DISCUSSION

Medicinal plants play very important roles against various diseases such as microbial infections, cancers and other disorders like diabetes. The antimicrobial actions of such plants have been attributed to the presence of certain phytochemical constituents (Ogbeba *et al.,* 2017). Compounds like alkaloids, glycosides, phenols, tannins, sterols and cardiac glycosides were detected from both ethanol and aqueous extracts of *Z. jujuba* leaf. Similar phytochemicals were detected from same leaf by Elaloui *et al.* (2014). The detection of sterol in this study is in line with the study conducted by Elaloui *et al.* (2014) which reported the present of stigmasterol and ß-sitosterol in leaf extract of jujube. Sterol proved to be very active against human pathogenic bacteria (Kavita, 2014). Thus, the antibacterial properties of the extract could be as the result of the sterol compound present. Alkaloids contain analgestic, antibacterial and anti-inflammatory properties which were reported by Pareek (2001). Phenol was also isolated from leaf extracts of both ethanol and aqueous from the leaf of *Z. jujube* (Elaloui *et al*., 2017).

The Ethanol extract exhibited more antibacterial activities than the aqueous, this could be due to the fact that ethanol is an organic solvent and can extract more phytoconstituents than water. This was also confirmed from the previous study conducted by Medini *et al*. (2014). The result of susceptibility of bacterial isolates to leaf extracts of *Z. jujuba* were comparable to ciprofloxacin (positive standard) which highlighted the possibility of using the *Z. jujuba* leaf as either an alternative or complementary antibacterial agent in order to minimize the issue of bacterial vaginal infection of women who are resistant to the antibiotic
The selected bacterial isolates used in this study has showed good susceptibility to aqueous and ethanol extracts of *Z. jujuba* at varied concentrations. This indicated that the activities of the extracts were dose dependent, thus showing concordance with Dubey *et al.* (2010) and Abd-Alrahman *et al.* (2013) that reported the relationship between the antimicrobial efficacy of *Z. jujuba* and *Z. mauritiana* at varied concentrations. The variation in susceptibility of isolates to varied concentrations may be due to the differences in the cell walls of the bacteria where *Staphylococcus aureus* being a Gram positive bacteria with no lipopolysaccharides tend to allow diffusion of the active components, whereas *E. coli* being Gram negative bacteria possess lipopolysaccharides that might have prevented penetration of active components of extracts. The *S. aureus* showed higher susceptibility than *E. coli*

which may be attributed to the fact that *E. coli* is usually resistant to most antibiotics due to permeability barriers afforded by its outer membrane that is composed of lipopolysaccharides. This was also in line with the work of Yahia *et al.* (2020), where higher zone of inhibition was observed on Gram positive bacteria compared to Gram negative bacteria, but contradicted the work of Abubakar *et al*. (2018) where Gram negative bacteria (*E. coli*) showed more susceptibility than Gram positive bacteria (*S. aureus*) at 150 mg/ml concentration. However, all activities of the extract were dose dependent which is similar to the research conducted by Sakha *et al.* (2018) where it was observed that at high concentration, exhibited more antibacterial activity.

The low MIC and MBC exhibited by the plant against the isolates were attributed to effectiveness of the plant extracts. This contradicts the report of Dubey *et al.* (2010) where MIC and MBC of low concentrations were obtained against *E. coli* and *S. aureus*. The differences could be as a result of geographical location where the plants were collected and method of extraction. The results of this work is similar with the previous researches suggesting effective antibacterial activity of *Z. jujuba* against both Gram positive and Gram negative bacterial isolates (Yahia *et al*., 2020; Abubakar *et al*., 2018; Elaloui *et al*., 2017; Dubey *et al*., 2010).

## CONCLUSION

In this study, important phytochemical constituents were detected in both ethanol and aqueous leaf extracts of *Z. jujuba.* The extracts exhibited significant antibacterial activities on clinical isolates including *S. aureus* and *E. coli* at varied concentration. *S. aureus* was more susceptible to the extracts at MIC of 100mg/ml. The phytochemicals detected in the plant extracts could be responsible for the observed antibacterial activities. Further research on fractionation should be done on the leaf extract of *Z. jujuba* due to its high antibacterial activity. The toxicity profile of this plant should be carried out so as to establish its safety/therapeutic index.

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