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Investigation Of Phytochemical Constituents And Therapeutic Potentials Of *Ziziphus Mauritiana* Leaves

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Abstract:*Plants that has medicinal applications represent a rich source of antimicrobial agents. Due to the side effects of antibiotics and the resistance of pathogenic microorganisms against antibiotics, highly much attention paid to plant extracts and biologically chemical active compounds isolated from different plant species. Ziziphus mauritiana is used by local people in Nigeria particularly tin the Northern part of the country as traditional medicine for the treatment of various disorders. This research determined the antibacterial activity from methanolic leaves extract of Ziziphus mauritiana against Staphillococus aureus, Eschericia coli and Bacillus subtillis. The method of extraction used was cold maceration, standard methods were used for the screening of phytochemicals and well diffusion method for the determination of antibacterial activity. The phytochemical result confirms the presence of saponins, tannins, glycosides, alkaloids, phenols, flavonoids, terpenoids and steroids. The antibacterial result indicated that Bacillus subtillis revealed the higher activity ranging from 1.333±0.577 mg/ml to 5.667±0.577 mg/ml. and lowest activity was observed by Eschericia coli 0.000±0.0.00 mg/ml to 3.333±0.577 mg/ml. The antibacterial activity may be due the presence of bioactive compounds in the leaves extract of Ziziphus mauritiana like tannins, alkaloids and terpenoids that have several pharmacological activities.*

Keywords: *Ziziphus mauritiana,* Antibacterial activity, Tannins, Antibiotics, Phytochemicals.

1.0 INTRODUCTION

Plants that has medicinal applications represent a rich source of antimicrobial agents. Due to the side effects of antibiotics and the resistance of pathogenic microorganisms against antibiotics, highly much attention paid to plant extracts and biologically chemical active compounds isolated from different plant species. *Ziziphus mauritiana* is used by local people in Nigeria particularly tin the Northern part of the country as traditional medicine for the treatment of various disorders. The objectives of this research includes extraction of the leaves of *Ziziphus mauritiana* by the used of methanol as a solvent, to screen phytochemical constituents and to determine the anitibacterial activity of *Ziziphus mauritiana* against *Staphillococus aureus, Eschericia coli* and *Bacillus subtillis* from the extracted sample of *Ziziphus mauritiana*.

Regardless of several researches conducted from species of *Ziziphus mauritiana* in Nigeria, in other country like Malaysia the raw materials from this plant were imported from different countries such as Pakistan and India (Yusof and Saat, 2017). This is indicating that investigation about *Ziziphus mauritiana* grown in Nigeria and other country is highly need to be conducted. Hence, to identified, isolates and confirm the exact biochemical compounds that are responsible for antibacterial activity and other activity such as antifungal, anticancer, antioxidant and other pharmacological activity.

Ziziphus mauritiana leaves is generally used for treatment of many diseases such as vomiting, fever, diarrhea, stop nausea asthma (Bukar, *et al*., 2015, Abdallah, *et al*., 2016, Ipandi, *et al*., 2016, Abalaka, *et al*., 2010). Moreover, several researches confirm the presence of secondary metabolites on *Ziziphus mauritiana* leaves such as saponins, terpenoids, anthraquinones, flavonoids, alkaloids, steroids, tannins, glycosides and phenolic compounds Al Ghasham *et al*., (2017), Bukar, *et al*., 2015, Abdallah, *et al*., 2016, Ipandi, *et al*., 2016, Abalaka, *et al*., 2010, Najafi, *et al*., 2012). It also showed that *Ziziphus mauritiana* act as antibacterial agent. Antibiotics are systematic from organic ingredients for inhibitor destroy bacteria certain (Nagumanthri, *et al.,* 2012).

2.0 MTERIALS AND METHODS

2.1 Sample Collection

The fresh and healthy leaves of *Ziziphus mauritiana* were collected from "Aliero" Kebbi in Nigeria and identified at the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero. The sample were cleaned, air-dried at room temperature for three weeks and ground into fine powder using pestle and mortar.

2.2 Extraction of Sample

The fine powder of *Ziziphus mauritiana* leaves was soaked in methanol for about 72 hours in order to accomplished cool extraction method. The crude extracts of *Ziziphus mauritiana* were obtained by using water bath. The yield percentage of the crude extracts was determined by using Equation 1 described by Ramadevi (2005).

$Yield = weight\ of\ the$ crude $extract\ (g)/weight\ of\ ground\ sample\ (g)\ x\ 100$ …… (1)

2.3 Phytochemical Screening

2.3.1 Test for saponins

The extract was dissolved with distilled water. The 2 mL of extract vigorously was shaked in test tube for 2 minutes. The absence of frothing indicates the presence of saponin (Adesegun, *et al*., 2008).

2.3.2 Test for tannins

The crude extract was mixed with 1 % ferric chloride solution. The formation of green black color indicates the presence of tannin (Parmar *et al*., 2012).

2.3.3 Test for glycosides

The extract was mixed with sodium hydroxide solution. The appreance of greenish yellow color solution was confirmed the presence of glycoside.

2.3.4 Test for alkaloids (Wagner's reagent)

The extract solution was mixed with little amount of Wagners's reagent. The formation of reddish or brown precipitate were formed indicate the presence of alkaloid.

2.3.5 Test of phenols

The 2 mL of extract solution was added with 0.5 mL iron (III) chloride solution. The formation of intense color indicates the presence of phenol (Parmar *et al*., 2012).

2.3.6 Test for flavonoids

The crude extract was mixed with sodium hydroxide. The appearance of yellow intense coloration become colorless when added dilute hydrochloric acid indicate the presence of flavonoid.

2.3.7 Test for terpenoids (Salkowski test)

The crude extract was dissolved with chloroform. A few drops of sulphuric acid and acetic anhydride were take placed. The formation of red or violet color indicate the presence of terpenoid (Adesegun *et al*., 2008).

2.3.8 Test for steroids

The crude extract was dissolved in acetic acid. A drop of concentrate sulphuric acid was added alongside of the test tube. Appearance of greenish yellow color indicate the presence of steroid (Talmale *et al*., 2014).

2.4 Antibacterial Activity

Antibacterial assay was tested using disc diffusion method. The pathogenic bacteria were spread on the agar plates with the help of sterile cotton swab. Each petri plate was inoculated and fresh growth of baterial culture was collected for each bacterium by sterile streaked loop method. The well grown bacterial colony was picked from Nutrient Agar (NA) media and sub-cultured in Nutrient broth (NB) media. The bacterium was incubated for 24 hours and maintained at 37ºC. The test was performed on well diffusion method for all bacterial strains. The inhibition zone was measured after incubation period that express as value in mm unit (Yusof and Saat, 2017).

3.0 RESULTS AND DISCUSSION

Table 1: Results of Phytochemical Screening of the Methanol Crude Extracts of *Ziziphus mauritiana* Leaves.

Phytochemicals	Result
Saponins	+
Tannins	+

Glycosides	+
Alkaloids	+
Phenols	+
Flavonoids	+
Terpenoids	+
Steroids	+

Key: + = Present

Table 3: Results of Antibacterial Activity of the Crude Methanol Leaves Extracts of *Ziziphus mauritiana*

Zones of Inhibition (mm)					
Bacterial Isolate	**Distilled H_2O**	**Ciprofloxacin 250 mg/ml**	***Ziziphus mauritiana* leaves extracts**		
			30 mg/ml	**60 mg/ml**	**90 mg/ml**
E. coli	0.000±0.000	21.230±0.030	0.000±0.0.00	1.333±0.577	3.333±0.577
S. aureus	0.000±0.000	18.530±0.030	0.3333±0.577	2.333±0.577	4.333±0.577
B. subtitis	0.000±0.000	19.170±0.030	1.333±0.577	3.000±0.000	5.667±0.577

Key: *E. coli = Escherichia coli, S. aureus = Staphylococcus aureus, B. subtilis = Bacillus subtilis* and H2O = Water. Values are mean inhibition zones (mm) ±SEM of the three replicate experiments.

3.1 DISCUSSION

Plants that has medicinal applications represent a rich source of antimicrobial agents. Due to the side effects of antibiotics and the resistance of pathogenic microorganisms against antibiotics, highly much attention paid to plant extracts and biologically chemical active compounds isolated from different plant species. The results phytochemicals and antibacterial activity of this research is in agreement with several researches regarding *Ziziphus mauritiana* and its species. The presence of phytochemicals in this study is an indication that *Ziziphus mauritiana* has several medicinal applications such as antibacterial activity, antioxidant activity, antifungal activity, anticancer activity antimalarial activity and other pharmacological activities.

The result of phytochemical screening showed the presence of secondary metabolites such as saponins, tannins, glycosides, alkaloids, phenols, flavonoids, terpenoids and steroids. This result is in agreement with result conducted by Febriza, *et al.,* (2022), the result showed the presence of flavonoids, alkaloids, tannins and saponins. The result is also similar to that of Muharrami, *et al.,* (2019), the result indicates the presence of saponins, flavonoids, tanninis, steroids and absence alkaloids and terprnoids. The result is also similar to that of (Yusof and Saat 2017), the result indicates the presence of alkaloids, flavonoids, glycosides, phenols, steroids, sterols, tannins and terpenoids but absence of saponin.

The antibacterial result indicated that *Bacillus subtillis* revealed the higher activity ranging from 1.333±0.577 mg/ml to 5.667±0.577 mg/ml. and lowest activity was observed by *Eschericia coli* 0.000±0.0.00 mg/ml to 3.333±0.577 mg/ml. The positive control (ciprofloxacin) showed the highest activity compared to the tested concentrations of the extracts (30, 60 and 90 mg/ml), while the negative control showed zero activity against the three pathogenic bacterial isolates. The result of antibacterial activity of this study is in agreement to that of Muharrami, *et al.,* (2019) against two pathogenic bacterial isolates (*S. aureus* and *E. Coli*), but *E. coli* showed zero activity for all the tested concentrations. The result of this study is slightly lower to that of (Yusof and Saat 2017), against four pathogenic bacterial isolates (Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Salmonella sp), with *S. aureus* having the the higher activity and *E. coli* with lowest activity. The result of this study is lower than that of Mohamadou *et al.,* (2021), against four pathogenic bacteria (*S. aureus, P. aeruginosa, C. albicans, F. moniliforme*), with *S. aureus* having the higher activity and *F. moniliforme* with lowest activity.

4.0 CONLUSION

The result of phytochemical screening showed the presence of secondary metabolites such as saponins, tannins, glycosides, alkaloids, phenols, flavonoids, terpenoids and steroids with interesting antibacterial activities. They have an effective function on different

types of infectious diseases from fungal and bacterial sources. Hence, they should be used in the preparation of medicinal drugs for the treatment of several intestinal and skin diseases.

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