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Research Article – Phytomedicine

Phytochemical analysis, proximate composition and antibacterial activities of *Ziziphus* species (*Z. jujube* and *Z. spina-christi*)

Kamalu Abdullahi Alhassan, Aisha Shuaibu Indabawa, M. Manjur Shah*
Department of Biological Sciences, Faculty of Science, Yusuf Maitama Sule University, Kano State, Nigeria

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*Corresponding author (E-mail.: mmanjurshah@gmail.com)

Abstract

Despite tremendous progress in human medicines infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. Their impact is particularly large in developing countries due to relative unavailability of medicines and emergence of widespread drugs resistance.The aim and objectives of this research work was designed to carried outthe Phytochemical analysis, proximate composition and to evaluate the antimicrobial activities of the *Ziziphus jujube* and *Ziziphus spina-christi* leaves against clinical bacterial isolates (*Escherichia coli*, *Staphloccoccus aureus* and *Klebsiella pneumoniae*)as they were found out that since ancient times to date, they are used in treating various antimicrobial, ailment and disorders etc. The results of the research shows that, the effect of antibacterial activities of both aqueous and ethanolic extracts of *Ziziphus jujube* (e extract conc. aqueous; F=119.37, Bacterial extract conc. aqueous; F=1.00 and extract conc. ethanol; F=15.74, Bacterial extract conc. ethanol; F=0.59) are reciprocally proportional to their counterpart*, Ziziphus spina-christi* (extract conc. aqueous; F=54.96, Bacterial extract conc. aqueous; F=0.94 and extract conc. ethanol; F=81.11, Bacterial extract conc. ethanol; F=1.37). In sum, the minimum inhibitory concentration of *Ziziphus jujube* shows that the aqueous extract has MIC at range of 11.7 to 8.7mg/ml on all tested bacteria but the ethanolic extract has M.I.C of 14.8 to 8.2mg/ml range on *E.coli*, *Klepsiella*spp and *S. aureus*. While, the minimum inhibitory concentration of *Ziziphus spina-christi* shows MIC of aqueous extract range of 12.8 to 8.3mg/ml on E. coli, *Klepsiella* spp and *S.aureus*. But, *Ziziphus spina-christi* MIC of ethanolic extract is 13.5 to 8.8mg/ml on all the tested bacteria. In sum, *Zizuphus spina-christi* has lower nutritional content and low MIC ethanolic extract than that of *Ziziphus jujube*.

Keywords: *Ziziphus spina-christi, Ziziphus jujube, E. coli, Klepsiella* spp and *S. aureus*

Introduction

Despite tremendous progress in human medicines infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. Their impact is particularly large in developing countries due to relative unavailability of medicines and emergence of widespread drugs resistance (Zampini *et al*., 2009). During the last decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has led to the search for new antimicrobial agents mainly among plants extracts with the goal to discover new chemical structures, which overcome the above disadvantages (Bouamama *et al.,* 2006). Current research on natural [illegible] ethno-medicinal uses (Arora and Kaur, 2007). Furthermore, the current trends in drug development process are focussed on natural sources, especially sources of plant origin due to some proven correlation between the folkloric medicinal uses of some of these plants to biological activity. Hence, the use of plant materials to prevent and treat infectious diseases successfully over the years has continued to attract the attention of scientists worldwide.

Ziziphus is a genus of about 40 species of spiny shrubs and small trees in the buckthorn family, Rhamnacea, distributed in the warm temperature and subtropical regions throughout the world. The leaves are alternate, entire with three prominent basal veins and 2-7cm (0.79-2.76m) long; some species are deciduous, other evergreen. The leaves can either be deciduous or evergreen depending on species, and are aromatic.The flowers are small inconspicuous yellow green. The fruit is an edible drupe, yellow-brown, red or black, globose or oblong, 1-5cm (0.39-1.97m) long, often very sweet and sugary, reminiscent of a date in texture and flavor (S.W.G.,1995).The fruits are an important source for birds, which eat the whole fruit and regurgitate the seeds intact, expanding the seeds in the best conditions for germination (ornitochory). Secondly, seed dispersal is carried out by mammals or fishes. The fruit is energy rich because of the large amount of sugar it contains. It is cultivated and eaten fresh, dry, and jam. It is also added as a base in meals and in the manufacture of candy. *Ziziphus* species can grow either as [illegible]nd are used as a hedge to form defensive fences for animals (Cherry, 1985). Some species, like *Ziziphus spina-christi* (L.) Wild occur on nearly every continent. They are temperate or tropical plants, having a great range. They are most abundant where annual average temperatures are between 12 and 350c and minimum winter temperature are not lower than -20°C. They prefer locations with a high temperature coupled with humidity. They require a deep soil, fresh, soft, siliceous- calcareous nature or limestone – clay-silica-clay and subsurface permeable, with pH between 5.5 and 7.8. In excessively sandy or clay soils which may be affected by standing water, the plants do not grow well. Many species are very sensitive to drought, and if the land is excessively dry and of calcareous nature, they may resent the lack of moisture. At the slightest drought premature fruit drop is frequent. The ecological requirements of the genus are mostly those of vigorous species with a great ability to propagate in conductive habitats. This genus is adapted mostly to high rainfall and humidity, but some species are deciduous, living in Mediterranean humid climate. The deciduous *Ziziphus* species lose all of their leaves for part of the year depending on variations in rainfall. In deciduous species in tropical, subtropical, and arid regions, leaf loss coincides with the dry season. They grow mostly in tropical forests but have also been found in stubbles, pastures, coastal ranges, tropical mountain areas and wet to dry interior regions. The family is distributed throughout tropical and subtropical areas and in cloud forest. The differences are ecological adaptations to different environments over a relatively dry-wet climate. Species in less humid environments are smaller or less robust, with less abundant and thinner foliage and have oleifera cells that produce trees with amore fragrant aroma. *Z. spina-christi* has very nutritious fruits and are usually eaten fresh. The fruits are applied on cuts and ulcers. They are also used to treat pulmonary ailments and fevers and to promote the healing of fresh wounds, for dysentery (Adzu *et al*., 2001). The leaves are applied locally to sores, and the roots are used to cure and prevent skin diseases (Adzu *et al.,* 2001). The seeds are sedative and are taken sometime with buttermilk to halt nausea, vomiting and abdominal pains associated with pregnancy (Kaaria, 1998). The leaves are applied as poultices and are helpful in liver troubles, asthma and fever (Michel, 2002).

Several researches have been conducted on these plants like phytochemical analysis and proximate composition)*e.g.* research carried out by Mogadam *et al.* (2010) on antibactrial activities of eight Iranian plants extracts against methicillin and cefixime resistant *S. aureus* stains. And series of problems are associated to it, examples are: depending on the methods of extracting extracts in the plant, some compounds are degraded when soxhlet extraction method is used, which always shows that the plants lack some thermo liable compounds. Again, depending on the method of drying the plant samples prolong sun drying or oven drying method also cause degradation of some compounds (Prashant *et al.*, 2011). This research work was carried out in order to determine the effectiveness of *Ziziphus jujube* and *Ziziphus spina-christi*, as they were are used in treating various ailment and disorders i.e. they are used as antibacterial, antifungal, antivirus, antioxidants, antiulcer, anticancer etc. therefore, research is needed to be conducted to further ascertain their phytochemical content, antibacterial activities and their minimum inhibitory concentrations for pharmacological use.

Material and Methods

Collection and Identification of the Plant

The *Ziziphus* species leaves were collected from Tattarawa in DawakinTofa local government area of Kano state, Nigeria, in a sterilised polythenbag; the plants were identified in the herbarium of department of Plant Biology, Faculty of life Science, Bayero University Kano. The leaves were washed thoroughly with sterile distilled water and then allowed to Shade dried. The plant was grinded to powdered form and sieves to have a fine powdered plant sample.

Extraction of leaves extract

The extraction was carried out according to the procedure explained by Luque de Castro and Garcia-Ayuso (1998) with little modification. Exactly 500 ml of each solvent (ethanol and aqueous) was transferred to the round bottom flask of the Soxhlet extractor. Thereafter, 50g each of *Z. jujube* and *Z. spina-christi* leaves were loaded into the thimble and placed inside the Soxhlet extractor separately. The apparatus was set for extraction on the heating mantle for about 7 hours. The liquid extracts were then transferred to beakers, and the solvent (ethanol) was allowed to evaporate. The crude extracts were then covered for further analysis.

Phytochemical Screening

Phytochemical screening was carried out on the leaves extracts in order to determine the presence of different kinds of secondary metabolites using method adopted by Tiwari *et al*. (2011).

Proximate analysis

Proximate analysis was carried out on the leaves extracts in order to determine the quantative analysis of Moisture content, Ash content, Crude Protein, and Crude Fibre using method adopted by Shumaila Gul and Mahphara Safdar (2009).

Preparation of extract Impregnated Paper Disc

Disc of 6mm was punched out from Whatman No. 1 filter paper with the aid of paper puncher and placed into bijou bottles in batches of 100 discs; the disc was sterilized by autoclaving at 121^0C for 15 minutes and then allowed to cool. The concentrations of the of the plant extracts to be tested was 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml. The positive control was Chloramphenicol 250mg/ml while the negative control was D.M.S.O. In each concentration of the plant extract prepared, 100 paper discs was inserted inside and impregnated with extract. Therefore, in each 500mg/ml concentration of extract, each disc was impregnated with 50mg/ml extract in 125mg/ml each disc was impregnated with 5mg/ml extract, in 125mg/ml each disc was impregnated with 1.25mg/ml extract and in 62.5mg/ml, each disc was impregnated with 0.625mg/ml extract (Sofowora, 1993).

Collection and Identification of Clinical Isolates

The test organisms (*E. coli, S. aureu*s and *Klebsiella* spp) were collected form Malam Aminu Kano Teaching Hospital Kano state, Nigeria. The isolates were put into fresh inoculation on Nutrient agar, gram staining identification and biochemical test was also carried out.

Preparation of Turbidity Standard

The turbidity standard was prepared as described by Cheesbrough, (2000), Mukhtar (2007) and Cakir *et al.* (2004).

Determination of Minimum Inhibitory Concentration (MIC)

The lowest zone of inhibition of the bioassay studies was used as the power one (10-1) of the MIC. Six test tubes were used in this study. First four test tubes are for the concentrations while the last two are for positive and negative control. All test tubes contain 1ml of nutrient broth. 10-1 test tube will contain the lowest concentrations of the bioassay result. The serial dilution was carried out and was incubated at 37^0C for 24 hours, before the result was taken.

Results

Table 1. Physical characteristic of aqueous and ethanol leaves extracts.

Plant sample	Quantity of plant used (g)	quantity of solvent use (ml)	extract obtained (g)	Color	Odor	Texture
Z. spina Christi aqeous	50	500	7.8	Red	Fruity	Soft
Z. spina Christi ethanol	50	500	7.6	Brown	Fruity	Soft
Z. jujube Aqeous	50	500	7.2	Brown	Woody	Soft
Z. jujube ethanol	50	500	6.0	Brown	woody	Soft

Table 2. Phytochemical Analysis of *Ziziphus jujube* and *Ziziphus spina-christi* Leave extract

Plant sample	Saponin	Tannin	Flavonoid	Alkaloid	Phenol	Cardiac glycoside	Terpenoid
Z.spina-christi (aqeous)	+++	++	++	++	+++	++	+++
Z.spina-christi(ethanol)	+++	++	+++	+++	+++	+++	++
Z.jujube (aqeous)	+++	+++	+++	+++	+++	+++	+++
Z.jujube (ethanol)	+++	++	+++	+++	+++	+++	+++

Key: + =Present- =Absent.

Table 3. Proximate Analysis of *Ziziphus jujube and Ziziphus spins-christi* leaves extract

Plant sample	Moisture (%)	Ash (%)	Fiber (%)	Protein (%)	Carbohydrate (%)
Ziziphus spina-christi	46	3.8	6.3	2.41	41.49
Ziziphus jujube	51	4.25	5.21	4.58	34.76

Table 4. Antibacterial activities of aqueous and ethanolicleaveextract of *Ziziphus jujube* and *Ziziphus spina-christi* against some selected bacteria

Plant sample	Solvent	Bacteria	500	250	125	62.5	Positive	Negative
Z.spina-christi	Ethanol	*E.coli*	13.5	11.5	10.2	9.0	32	00
		Klebsiella spp	11.8	11.0	10.0	8.8	35	00
		S.aureus	12.8	11.3	10.2	9.2	38	00
Z.spina-christi	Aqeous	*E. coli*	12.5	11.3	10.2	9.2	32	00
		Klebsiella spp	11.0	10.3	9.5	8.8	35	00
		S. aureus	11.5	10.2	9.3	8.3	38	00
Z.jujube	Ethanol	*E. coli*	14.8	11.0	10.1	9.3	32	00
		Klebsiella spp	12.6	10.9	10.5	8.2	35	00
		S. aureus	14.0	12.4	11.5	8.9	38	00
Z.jujube	Aqeous	*E. coli*	11.5	10.5	9.7	8.7	32	00
		Klebsiella spp	13.0	11.5	10.2	9.5	35	00
		S. aureus	11.7	10.5	9.5	8.7	38	00

+ = chloramphenicol 250mg/ml, -= dimethylsulfoxide

Table 5. Minimum Inhibitory Concentration (MIC) of the leaves extracts of *Ziziphus jujube and Ziziphus spina-christi* against the isolated bacteria [extract concentration in mg/ml]

Plant sample	Bacteria	62.5	31.25	15.625	7.8125
Z.spina- christi	*E. coli*	_	_	+	+
	S. aureus	_	_	+	+
	Klebsiella spp	_	+	+	+
Z.spina-christi	*E. coli*	_	_	+	+
	S. aureus	_	_	+	+
	Klebsiella spp	_	_	+	+
Z.jujube	*E. coli*	_	+	+	+
	S. aureus	_	+	+	+
	Klebsiella spp	_	+	+	+
Z.jujube	*E. coli*	_	_	+	+
	S. aureus	_	+	+	+
	Klebsiella spp	_	+	+	+

Key: + =growth (bacteriocidal),- =(no growth (bacteriostatic)

Table 6. Minimum Bactericidal Concentration (MBC) of the leaves extract of *Ziziphus jujube and Ziziphus spina-christi* against the isolated bacteria [extract concentrations in mg/ml]

Plant sample	Bacteria	500	250	125	62.5	31.25
Z.spina- christi	*E.coli*	_	_	+	+	+
	S.caureus	_	_	_	+	+
	Klebsiella spp	_	+	+	+	+
Z.spina- christi	*E.coli*	_	_	+	+	+
	S.aureus	_	+	+	+	+
	Klebsiella spp	_	_	+	+	+

Table 6 Conted....

Z.jujube	*E.coli*	_	_	+	+	+
	S.aureus	_	_	_	+	+
	Klebsiella spp	_	_	+	+	+
Z.jujube	*E.coli*	_	_	+	+	+
	S.aureus	_	_	+	+	+
	Klebsiella spp	_	_	+	+	+

Key: += turbid- =non turbid

Table 7. Effect of antibacterial activities of ethanol extract on Z. *spina-christi*

Source	DF	Sum of Square	Mean Square	F Value	Pr(>F)
Bacterial	2	2.5139	1.2569	0.62	0.5672
Error(a)	6	12.0833	2.0139		
Extract Concentration	3	68.7222	22.9074	81.11	0.0000
Bacterial :extract concentration	6	2.3194	0.3866	1.37	0.2796
Error(b)	18	5.0833	0.2824		
Total	35	90.7222			

The result shows a p=value of 0.567 ($p < 0.05$) this implies that antibacterial activities of ethanol extract on *Z.spina-christi* have no significant impact on the tested bacteria (*E. colli*, *Klebsiella* spp and *S. aureou*s).

Table 8. Effect of antibacterial activities of aqueous extract on Z.*spina-christi*.

Source	Df	Sum of square	Mean square	F value	Pr (>f)
Bacterial	2	7.0417	3.5208	6.34	0.0332
Error (a)	6	3.3333	0.5556		
Extract concentration	3	41.2222	13.7407	54.96	0
Bacterial extract concentration	6	1.4028	o.2338	0.94	0.4941
Error (a)	18	4.5	0.25		
Total	35	57.5			

A p-value of 0.03 ($p < 0.05$) signifies that the activity of aqueous extract as an antibacterial on *Z. spina-christi* has a significant effect on the bacterial. However, the level of significant differs among the bacterial.

Table 9. Effect of antibacterial activities of ethanol extraction *Ziziphus jujube*.

Source	DF	Sum of square	Mean square	F value	Pr (>f)
bacterial	2	6.74	3.37	2.18	0.1937
Error (a)	6	9.225	1.5425		
Extract concentration	3	107.21	35.7367	15.74	0
Bacterial extract concentration	6	8.0733	1.3456	0.59	0.7323
Error (a)	18	40.8717	2.2706		
Total	35	172.15			

The result shows a p-value of 0.194 ($p < 0.05$) this implies that antibacterial activities of ethanol extract on Z. *jujube* have no significant impact on the tested bacteria (E. *coli*, *Klebsiella* spp and S. *auerous*).

Table10. Effect of antibacterial activities of aqueous extract on *Ziziphus jujube*

Source	DF	Sum of square	Mean square	F value	Pr (>f)
Bacterial	2	7.3472	3.6736	1.86	0.2350
Error (a)	6	11.875	1.9792		
Extract concentration	3	48.9097	16.3032	1	0.4552
Bacterial extract concentration	6	0.8194	0.1366	1	
Error (a)	18	2.4583	0.1366		
Total	35	71.4017			

The result shows a p-value of 0.236 ($p < 0.05$) this implies that antibacterial activities of aqeous extract on Z. *jujube* have no significant impact on the tested bacteria (*E. colli*, *Klebsiella* spp and *S. auerous*).

Discussion

The proximate analysis of aqueous and ethanolic extract of *Ziziphus spina-christi* and *Ziziphus jujube* shows that, *Ziziphus spina-christi* (aqueous extract) yielded the highest quantity of extract (7.8g) followed by ethanolic extract of *Ziziphus spina-christi* (7.6g). This indicates that *Ziziphus spina-christi* yielded high quantity of extract than aqueous and ethanolic extracts of *Ziziphus jujube*, 7.2g and 6.0g respectively. The proximate analysis showed that *Ziziphus spina-christi* has lower moisture level (46%) than *Ziziphus jujube* (51%) also lower ash content (3.8%) than *Ziziphus jujube* (4.25%), but has higher crude fiber content (6.3%) than *Ziziphus jujube* (5.41%). Still *Ziziphus spina-christi* has lower protein and carbohydrate content (2.41% & 41.49%) *than Ziziphus jujube* (4.58% & 34.76%

The phytochemical screening shows that both aqueous and ethanolic extracts of both plants have all secondary metabolites tested present. This is in accordance with the findings of Abd-Alrahman*et al* (2013) that alkaloid, tannins, flavonoid, saponin, triterpenes, glycoside are present in *Ziziphus jujube* and also the work of Ermias *et al.,* (2011) that steroid, flavonoid, tannin, lipid, anthraquinone, saponin and alkaloid are present in *Ziziphus spina-christi*.

The antibacterialactivities of both plants show that they have high activities at 500mg/ml and low activities at 62.5 mg/ml. All the plants are most effective on E. coli at 500mg/ml followed by S. *aureus* then *Klepsiella* spp. This is in accordance with the work of Bukar *et al.* (2015) who reported that *Ziziphus spina-christi* has antibacterial activities against S. *aureus*, E. *coli, Shigella* spp and

Pseudomonas aeruginossa. This is also in accordance with the work of Abd-Alrahman *et al.* (2013) who reported that *Ziziphus jujube* has antibacterial effect on *Baccilus cereus*, S. *aureus,* E. *coli*, *Pseudomonas aeruginosa*, *Klepsiella pneumonia* and *Listeria monocytogenesis*.

The minimum inhibitory concentration of *Ziziphus jujube* shows that the aqueous extract has MIC at 62.5 mg/ml on all tested bacteria but the ethanolic extract has MIC of 31.25 mg/ml on *E.coli* and 62.5 mg/ml on *Klepsiella* spp and *S. aureus*, but Ahmad *et al.* (2010) reported that the MIC of aqueous extract of *Ziziphus jujube* on *E. coli* is 3.5 mg/ml, 3.0 mg/ml on *S. aureus* and 3.8 mg/ml on *Klepsiella* spp. While the ethanolic extract has 3.0mg/ml, 2.3 mg/ml and 3.3mg/ml on *E. coli*, *S. aureus* and *Klepsiella* spp respectively. MIC are significantly lower than MIC, this could be due to substandard solvent, contamination or wrong preparation of extract concentration he used. The minimum inhibitory concentration of *Ziziphus spina-christi* shows that the M.I.C of aqueous extract on *E. coli* and *Klepsiella* spp is 31.25mg/ml while it is 62.5 mg/ml on *S.aureus*. The MIC ofethanolic extract is 31.25 mg/ml on all the tested bacteria. Motamedi *et al.* (2014) reported that the MIC of ethanolic extract of *Ziziphus spina-christi* on *E. coli*, *S. aureus* and *Klepsiella* spp are all 18 mg/ml.

Conclusion

Conclusively, both *Ziziphus spina-christi and Ziziphus jujube* has antibacterial activities against E. *coli*, *Klepsiella* spp and *S. aureus,* due to the fact that the qualitative phytochemical screening of both plants shows that they contained saponin, tannin, flavonoid, alkaloid, phenol, cardiac glycoside and terpenoid. Both leaves extracts have high antibacterial effect at 500mg/ml and low antibacterial effect at 62.5mg/ml. It can also be concluded that proximate analysis of *Ziziphus spina-christi* has higher nutritional value than *Ziziphus jujube*, except in crude fiber and carbohydrate, where *Ziziphus jujube* has higher value.This indicated that both leaves can be used as good antibacterial agents due to their phytochemical content and antibacterial activities.

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