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Antimicrobial screening and effect of the pulp extracts of *Zizyphus spina-christi* (Linnaeus Desf) on some biochemical parameters in rats

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Abstract

The pulp extract of *Zizyphus spina-christi* which have ethnomedicinal value was studied. The in vitro antimicrobial activity of the pulp extract (aqueous) was assayed using the agar plate diffusion and broth dilution methods. Test microorganisms were *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Streptococcus pyogenes, Escherichia coli*, all the organisms were laboratory isolates. The effect of gastrically administered aqueous Pulp extract of *Zizyphus spina-christi* was investigated in rats. Groups of rats were orally administered 200mg/kg, 400mg/kg and 800mg/kg body weight of extracts daily for three (3) weeks. The animals were sacrificed by decapitation and blood collected for biochemical analysis. The pulp aqueous extract showed more susceptibility on *E. coli*, *P. aeruginosa* and *C. albicans* which is a fungal suggesting that the extract possess antifungal activity. The pulp aqueous extract showed minimum inhibitory concentration (MIC) of 6.25mg/ml against *E .coli* and *C. albicans.* The minimum bactericidal concentration of the pulp aqueous extract was 12.5mg/ml for *S. pyogenes*. The result of biochemical analysis showed that no significant difference existed between the treated group and the control group for the liver enzymes; Alanine transaminase (ALT) Aspartate transaminase (AST) Alkaline phosphatase (ALK) Serum protein and Serum albumin. Also, Serum electrolyte (Na^+, K^+, HCO_3^-, Cl^-) equally showed no significant difference ($P > 0.05$) to the control group. Similar results were obtained for Serum Urea and Creatinine Levels. The result obtained indicates that the extract is neither hepatotoxic nor nephrotic.

Keywords: Antibacterial activity, *Zizyphus spina-christi* extracts (aqueous), phytochemical screening, Kidney function test, Liver function test, Rhamnaceae.

Introduction

The plant *Zizyphus spina-christi*, a deciduous tree is found in Borno State and locally called kurna in Hausa and Kanuri. The Shuwa Arab of Nigeria, Chad and Cameroun call it Nabak (Arndt *et al*., 2005). It is a quick growing, strong and long lived tree. It flowers from January-February in Nigeria. The pleasant smelling flowers are hermaphrodite (Adzu *et al.*, 2002). *Zizyphus spina-christi* has been reported to have activity against bacteria, fungal and other pathogens that are normally quite resistant (Nazif *et al*., 2002). Plant leaves of *Zizyphus* are used in Iranian folk medicine as an antiseptic, antifungal and anti-inflammatory agent and for healing skin

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diseases such as atopic dermatitis (Amin, 1991). *Zizyphus* species such as *Zizyphus jujube* has been clinically tried on hepatic infections and in treating nephrosis syndrome and other auto-immune diseases (Evans, 1989). The aim of this study is to subject the crude extract of the pulp of this plant to antimicrobial study and also, determine the effect of the extracts on serum biochemical parameters which are potent indicators of hepatic and nephritic abnormalities with a view to establishing their ethnomedicinal value or otherwise.

Experimental

Plant material. Fresh samples of the ripe fruit of *Z. Spina-christi* were collected in March, 2007 from Jiddari, polo area of Maiduguri, Borno state. The plant specimen was identified by a taxonomist Prof. S .S. Sanusi of the Department of Biological Science, University of Maiduguri. A voucher specimen was deposited and labeled 544[a] at the herbarium.

Preparation of plant extract. The fruit was air-dried for two weeks. The dried fruits were peeled and separated into Pulp. The dried pulp were pulverized with mortar and pestle. Eight hundred grams of the powdered samples were placed in a thimble and extracted with two liters of distilled water. The aqueous layer were concentrated *in vacuo*, a dark brown solid mass were obtained for the Pulp (18.36%w/w).

Phytochemical screening. The crude aqueous extract was phytochemically screened for the determination of constituents utilizing standard methods of analysis (Harborne, 1973; Evans, 1989; and Sofowora, 1993).

Bacterial and Fungal strains. The Gram-positive organisms used in the study were *Staphylococcus aureus (Sa)* and *Streptococcus pyogenes (Sp)*. The gram-negative organisms were *Escherichia coli (Ec) Pseudomonas aeruginosa (Pa)* and a fungi *Candida albicans (Ca)* which were clinical isolates. All the organisms were obtained from the veterinary research laboratory, University of Maiduguri, Borno State.

Antimicrobial testing. Preliminary antimicrobial analysis of the extract were carried out using stock concentration of 100mg/ml respectively, prepared by dissolving 1g of each extract in 10ml of sterilized distilled water. The micro-organisms were maintained on agar slants. The inocula were prepared by inoculating the test organisms in nutrient broth and incubating for 24 hours at 37°c. After incubating, the broth cultures were diluted to 1:1000 for Gram-positive bacteria and 1:5000 for Gram-negative bacteria. One millilitre of the diluted cultures was inoculated into a 19ml sterile molten nutrient agar (48°c) and pour into sterile Petri dishes. These were gently swirled and allowed to solidify. Afterwards, wells were bored into the solidified and inoculated nutrient agar plates using a sterile cork borer about 20mm in diameter. A reconstituted solution of 5ml of the aqueous extract of various concentrations (1000mg/ml, 800mg/ml, 600mg/ml and 400mg/ml) were added through the corresponding hole on nutrient agar in different plates. Tetracycline, a standard drug (250mg/ml) was placed on the agar plates. One hour was allowed for the extract to diffuse into the agar plate after which the plates were incubated overnight at 25°c and 37°c for fungi and bacteria strain respectively. At the end of the incubation period, diameters of inhibition zones were measured and recorded in millimetres.

Minimum inhibitory concentration (MIC). MIC was recorded as the lowest concentration where no visible turbidity was observed in the test tubes. It was determined using the broth dilution technique (Baker and Breach, 1980., Vollekova *et al*., 2001). The extract was first

diluted to the highest concentration (100mg/ml) in sterile distilled water and then two fold serial dilution of each extract was made to concentrations ranging from 0.3125-50mg/ml using nutrient broth. The extract was inoculated into 0.2ml suspension of the organisms respectively.

Minimum bactericidal concentration (MBC). MBC was determined by using the broth dilution technique previously described by Vollekova *et al*. (2001) by assaying the test tubes resulting from MIC determination. A loopful of the content of each test tube was inoculated by streaking on a solidified nutrient plate incubated at 37°c for 24 hours and observed for bacterial growth. The lowest concentration of the sub culture with no growth was considered the minimum bacterial concentration.

Animal Treatment. Forty (40) white Wistar strain rats of both sexes weighing between 120-160g were purchased for the study. They were divided into four (4) groups of Ten (10) rats each, allowed free access to drinking water and standard diet (Nutrifeed Nigeria Ltd). The animals were administered doses ranging from 200, 400 and 800mg/kg of aqueous extract respectively with a separate group serving as control which was administered orally a single dose of normal saline (0.9%Nacl). The rats were administered the doses orally for three weeks using a feeding tube. The rats were sacrificed by humane decapitation 24 hours after the last treatment and blood collected was allowed to clot, centrifuged at 3000g and serum harvested.

Biochemical Analysis. Alanine transaminase (ALT) Aspartate transaminase (AST) Alkaline Phosphatase (ALK Phos) were estimated calorimetrically using reagents Kits, Randox, Lab. Ltd crumlin, U.K. Protein was estimated by the Biuret test and creatinine by the Jaffe reaction method as described by Henry *et al.* (1974). Albumin was assayed using the dye bromocresol green binding method while urea was assayed by the diacetylmonoxime reaction method as described by Harold (1958). Sodium and potassium level were estimated using flame photometry. Serum bi-carbonate was estimated by volumetric method of Vanslyke and Aullen (1977) and the chlorides were estimated by titrimetric method of Scales and Scales (1971).

Statistical Analysis. Data collected from the biochemical parameters were summarized as mean ±S.E.M. Analysis of variance was used to test the mean. P value less or equal to 0.5 was considered statistically significant.

Results

Table 1.shows the result of preliminary phytochemical tests of the Pulp aqueous extract. The Pulp extract (aqueous) contained alkaloids, carbohydrates, saponins, tannins and flavonoids which are potential phytochemical for drugs. The antibacterial and antifungal susceptibility of the Pulp extract of *Zizyphus spina-christi* are shown in Table 2. The *P. aeruginosa* was found to be more susceptible to the extract at lower concentrations. From Table.3 PEE had an MIC of 6.25mg/ml against *E. coli and C. albicans*. Table 4 shows that the PAE had bactericidal concentration of 12.5mg/ml against *S. pyogene*.

The biochemical test results (Table 5) showed that the serum enzymes (AST, ALT and ALK) were not significantly ($P \geq 0.05$) elevated by the extract administration. The group administered 800mg/kg body weight aqueous extract recorded the least concentration of AST, ALT and Alkaline phosphatase. Serum albumin and Serum protein equally showed no significant ($P \geq 0.05$) changes to the control. The group treated with 400mg/kg body weight aqueous extract recorded the least concentration of Serum albumin and Serum protein. Similarly, biochemical test results (Table 6) shows that no significant elevation ($P \geq 0.05$) occurred on

the Serum electrolytes (Na^+, K^+, $HCO3^-$, Cl^-)
to that of the control.

Table 1: Preliminary phytochemical tests of the Pulp aqueous extract

Constituent	Test	PAE
Flavonoids	Lead acetate test	+
	NaOH	+
	Iron (iii) chloride	+
Alkaloids	Dragendorff's	+
	Mayer's	+
	Wagner's	+
Saponins	Froth test	+
Carbohydrates	Molisch's Test	+
	Barfoed's Test	+
	Fehling Test	+
Tannins	Iron (iii) chloride Test	+
	Lead acetate Test	+
Steroidal nucleus	Salkowski	+
	Liebermann	+

Key: PAE – Pulp aqueous extract, + = Positive, - = Negative

Table 2: Antimicrobial susceptibility test of PAE of *Zizyphus spina-christi. L*

	zone of inhibition (mm)				
Conc of extract (mg/ml)	*E. coli*	*S. aureus*	*P. aeruginosa*	*C. albicans*	*S. pyogen*
400	21	18	R	R	18
600	30	26	R	R	26
800	36	30	16	R	35
1000	41	35	26	R	37
T. C (250)	30	35	25	50	40

Key: PAE- Pulp aqueous extract, T. C- Tetracycline

Table 3: Determination of Minimum inhibitory concentration (MIC) of *Zizyphus spina-christi. L*

Conc of Extract	Conc. of micro-organism				
Mg/ml	*E. coli*	*S. aureus*	*P. aeruginosa*	*C. albicans*	*S. pyogene*
100	–	–	–	–	–
50	–	–	–	–	–
25	–	–	–	–	–
12.5	–	β	β	–	β
6.25	β	+	+	β	+
3.25	+	+	+	+	+
1.625	+	+	+	+	+
0.3125	+	+	+	+	+
MIC	6.*25*	12.5	12.5	6.25	12.5

Key: + = Turbidity observed – == No turbidity observed β = MIC value.

Table 4: Determination of Minimum bactericidal concentration (MBC) of *Zizyphus spina-christi. L*

	Conc. of micro-organism				
Conc (mg/ml)	E. coli	S. aureus	P. aeruginosa	C. albicans	S. pyogene
0.3125	+	+	+	+	+
1.625	+	+	+	+	+
3.125	+	+	+	+	+
6.25	+	+	+	+	+
12.5	+	+	+	+	β
25	–	–	–	–	–
50	–	–	–	–	–
100	–	–	–	–	–
MBC	25	25	25	25	12.5

Key: + = Turbidity observed – == No turbidity observed β = MBC value.

Table 5: Effects of Pulp (aqueous) extract of *Z. spina-christi L* on Serum enzymes, Protein and Albumin following prolonged (21 days) administration on rats.

		Dose of Pulp Aqueous Extract (mg/kg)		
Parameters	Control	200	400	800
AST (lU/L)	11.50±2.12 a	13.00±0.00 a	12.50±6.36 a	10.00±4.24 a
ALT (IU/L)	6.00±2.82 a	6.00±0.00 a	6.00±2.82 a	4.50±0.00 a
ALK.P(IU/L)	30.50±2.12 a	34.00±0.00 a	33.50±0.70 a	32.50±4.95 a
PROTEIN (g/dl)	37.50±3.52 a	38.50±2.12 a	35.00±1.41 a	37.50±4.95 a
ALBUMIN (g/l)	59.00±1.41 a	58.00±8.42 a	58.50±2.12 a	61.50±3.54 a

a P≥ 0.05; Values show no Significant difference compared to control

Table 6: Effects of Pulp (aqueous) extract of *Z. spina-Christi L* on Serum electrolytes, Urea and Creatinine following prolonged (21 days) administration on rats

		Dose of Pulp Aqueous Extract (mg/kg)		
Parameters	Control	200	400	800
Na^- (mmol/L)	140.00±2.82 a	136.00±1.41 a	133.50±4.95 a	138.00±2.83 a
K^- (mmol/L)	4.950±0.07a	5.200±0.00 a	4.750±0.21 a	4.950± 0.21a
HCO_3^-(mmol/L)	21.50±2.12 a	21.00±1.41 a	22.00±0.00 a	20.00±2.88 a
Cl^- (mmol/L)	108.00±2.82 a	109.00±1.41 a	107.00±7.07 a	105.00±7.07 a
Urea (mmol/L)	5.300±0.14 a	5.850±0.07 a	5.550±0.21 a	5.400±0.29a
Creatinine (μmol/L)	87.50±2.12a	83.50±6.36a	85.50±0.70a	89.50±0.71a

a P≥ 0.05; Values show no Significant difference compared to control

However, the group administered 400mg/kg body weight aqueous extract showed the least concentration of Na^+ ion and K^+ ion. The least concentration of Cl^- ion was observed in the group administered 800mg/kg body weight aqueous extract. HC03$^-$ equally showed its least concentration at 800mg/kg body weight aqueous extract treatment. Urea and Creatinine followed similar trend as they showed no significant elevation (P≥0.05) to the control. The group administered 800mg/kg body weight aqueous extract showed the least concentration of Urea while the group treated with 200mg/kg body weight aqueous extract showed the least concentration of Creatinine.

Discussion

The result of the phytochemical analysis of the pulp of *Z. Spina-christi* showed that it contained carbohydrates, flavonoids, saponins and tannins. These compounds are known to have curative properties against several pathogens (Hassan *et al*., 2004). This therefore explains its possible use in the treatment of a number of ailments. The antimicrobial screening recorded express zones of inhibition on most of the tested organisms. However, it has been suggested that plant extracts exhibiting diameters of zone of inhibition > 10mm were considered active (Zwadyk, 1972, Usman *et al*., 2005). From the result on MIC and MBC presented on Tables 3 and 4, it was observed that the PAE exhibited inhibitory effect on the Gram positive bacterium *S. aureus* at 12.5mg/ml. These organisms are known to play significant role in causing skin diseases including superficial deep follicular legions (Srinivasan *et al*., 2001). Also, the PAE showed increased activity on the Gram-negative organisms *E. coli* and *P. aeruginosa*. *E. coli* is the common cause of urinary tract infection and accounts for approximately 90% of most urinary tract infections in young women (Brooks *et al*., 2002).

Administration of the Pulp aqueous extract of *Z. spina-christi* to rats did not elevate the biochemical parameters observed. ALT, the more specific indicator for the liver function (Kaplan *et al.*, 1988) was not significantly increased. This shows that no hepatocellular damage was recorded. The levels of Creatinine, Urea and Serum electrolytes were also not statistically increased. The insignificant changes in Serum urea suggest the absence of renal impairment.

The present study, indicates that *Zizyphus spina-christi* can be very useful in the control of hepatic and nephrotic abnormalities and therefore should be subjected to clinical trials

Conclusion

This study exposes the ethnomedical value of the fruit of *Z. spina-christi L*. for the treatment of wounds , burns, stomach discomfort and urinary tract infections whose causative agents are some of the test organisms used for the study. The study also suggest that *Zizyphus spina-christi* can be very useful in the control of hepatic and nephrotic abnormalities and therefore should be subjected to clinical trials.

We therefore suggest further research using more purified samples to ascertain our claim.

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