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Phytochemical and Antimicrobial Studies of *Ficus religiosa Seed* Extracts

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Abstract: The seed of *Ficus religiosa* (family of Moraceae) was collected from Kumbotso, Kano State of Nigeria and is one of the plants seed frequently used among traditional medicine healers in some parts of Nigeria for the treatment of chest and stomach infections. Due to these claims, the seed was analysed to ascertain the efficacy or otherwise of its ethno medicinal value. The seed of the plant was extracted by continuous extraction first, using petroleum ether (60-80^{0}C) followed by methanol solvent. The phytochemical screening of these seed extracts showed the presence of steroids, tannins, glycosides, cardiac glycosides, flavonoids, saponins, anthraquinones, and carbohydrate. The disc diffusion assay was performed at 40mg/ml to evaluate antimicrobial activity of the extracts. The crude extracts of petroleum-ether proved sensitive with significant zone of inhibition ranging from 20-30 mm against Staphylococcus aureus, Salmonella typhi and E. coli, while methanol extract was sensitive with significant zones of inhibition ranging from 19-20 mm against Salmonella typhi, and E. coli. The activity of these extracts were compared favourably with that of standard antibiotic Amoxicillin (30gdic[1]) The minimum inhibitory concentration (MIC) of petroleum ether extract against the stated organisms were 5 mg/ml, 7.5 mg/ml and 10.5 mg/ml, while that of methanol were 7 mg/ml and 5.5 mg/ml respectively. The spectra of antimicrobial activities displayed by the extracts could be attributed to the presence of these Phytochemicals substances and this signifies the potential of the seed as a source of therapeutic agents.

Keywords: Ficus religiosa, Seed, anti-microbial, extracts.

1.0 Introduction

Plants have been the basis for medicinal treatment through much of human history, and such traditional medicine is widely practiced today. Herbal medicine is the oldest form of health care known to humankind. Herbs have been used by all culture throughout history. It was an integral part of the development of modern civilization. Primitive man observed and appreciated the great diversity of plants available to him. The plants provide food, clothing, shelter and medicine [1],[2].

In recent times, herbal medicine was indispensable and formed an important part of the primary health care system of many nations [3]. Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition. They have been playing important role in the lives of rural people particularly in remote parts of developing countries with few health facilities.

The plant *Ficus religiosa*is used traditionally as antiulcer, anti diabetes, inflammatory disorder, gastric problems, and treatment of gonorrhoea, skin diseases and infectious diseases. The bark is used as an antiprotozoal, antiviral, astringent, and antibacterial. The leaves are used for skin diseases, anti venom activity and regulate the menstrual cycle of women. The seeds of the plant have laxative, cooling and refrigerant properties while the tender branches are used as toothbrush in case of high fever and fruits serve as laxative and treatment of asthma [4][5]. It is against this background the *Ficus religiosa, which* is one of the plants that are used in herbal preparation in some part of Nigeria. *Ficus religiosa*commonly known as sacred fig is a largely perennial tree belonging to the family **moraceae** and largely found in the tropical and sub-tropical regions around the world**..** It is one of the most revered trees in Asia due to its mythological and traditional background [6],[7]

2.0 Materials and Methods

2.1 Collection and preparation of plant material

The seeds of *Ficus religiosa*were collected from "Yar-Fulani" garden along "Kwankwaso" road, Madobi Local Government Area, Kano state.The identity of the plant was confirmed and authenticated at the Herbarium unit of Department of Plant Biology, Bayero University Kano.The seeds of *Ficus religiosa*were dried at room temperature under the shade then pounded using porcelain mortar and pestle.

2.11 Extraction

The powdered material (500g) was extracted exhaustively using continuous extraction method with (700 ml) each of chloroform and methanol solvents in a Soxhlet extractor [8]. The extracts were concentrated in vacuum at 40 °c using a rotary vapour, after which 3.42g and 11.34g of the extracts of chloroform and methanol were obtained respectively.

2.2 Phytochemical Screening

Phytochemical screening of the extracts were carried out to identify the phytochemicals constituents (secondary metabolites) using standard phytochemical methods [9],[10]. The screening involves detection of Carbohydrates, Flavonoids, Steroids, Terpenoids, Saponins, Tannins/ cardiac glycoside, Alkaloids, Steroids.

2.3 Test organisms

The microbes that were used in this study were obtained from the Department of Medical Microbiology Laboratory, Ahmadu Bello University Teaching Hospital, Zaria. The test organisms were *Staphylococcus aureus, Salmonella typhi and E. coli*. The bacterial isolates were maintained on nutrient agar and sub - cultured every three days. An inoculum of each isolate was suspended in 5ml of Mueller Hinton broth (MHB) and incubated overnight at 37 °c. The overnight cultures were diluted with Mueller Hilton broth and adjusted to give a concentration of bacterial cells equivalent to a Mc Farland 0.5 standard, prior to the bacterial testing [11]

2.4 Determination of antimicrobial activity

Mueller Hilton agar was the media used as the growth media for the microbes and the media was prepared according to the manufacturer's instructions, boiled to dissolve and sterilized at 121^{o}c for 15minutes, then allowed to cool at room temperature. About 20ml of the media was then poured into sterile Petri dishes and allowed to solidify. 6mm discs were then cut and sterilized at 100^{o}c for 60 minutes.The discs were then soaked into the solution of the extracts. The media was then seeded with0.5ml standard inoculums of the test organisms. The inoculums were spread evenly over the surface of the media by the use of sterile swap sticks. The seeded plates were dried at room temperature for 15-20 minutes, then the discs were planted at the side of each seeded plates and incubated at 37^{o}c for 24hours. Zone of inhibition were measured with a transparent ruler and compared with the control used which was amoxicillin. The results were recorded in millimetre (mm)as indicated in table 2.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined on the test organisms that were sensitive to the extract and was done by broth dilution method [12].McFarland's turbidity standard scale was prepared to give a turbid suspension of the microorganism. Incubation was made at 37 ^{0}C for 6 hours. The broth culture was diluted with normalsaline until the turbidity matched that of the McFarland scale by visual comparison. The two fold serial dilution of the extracts were done to give concentrations of 5 mg/ml, 7.5 mg/ml 10.5 mg/ml for pet-ether, while that of methanol were 7 mg/ml and 5.5 mg/ml respectively. The initial concentration was obtained by dissolving 0.2 g of the extract in the nutrient broth. 0.1 ml of the organism suspension was inoculated into different concentration of the extract in the test tubes. The test tubes were then incubated at 37 ^{0}C for 24 and 48 hours for the bacteria and fungi respectively. The test tubes were observed for turbidity i.e. presence or absence of growth. Minimum inhibitory concentration was recorded as the least concentration of the extract, where inhibition growth started. The results are recorded in Table 3.

3.0 Results

The results of the analysis is indicated in the tables below and each table contains result of specific analysis.

The phytochemical screening showed that, both the pet-ether extract showed the presence of Steroids, Saponins, anthraquinones, glycosides and cardiac glycosides while methanol extract revealed the presence of steroids, flavonoids, saponins, anthraquinones, glycosides, cardiac glycosides and Carbohydrates as shown in Table 1.

Table 1Phytochemical screening of the petroleum ether and methanol extracts of the seed *Ficus religiosa.*

Constituents	**Tests**	**Extracts**	
		Petroleum Ether	**Methanol**
Steroids	Liebermann Bustard's test.	+	+
Flavonoids:	Ferric chloride test	-	+
Alkaloids:	Meyer's reagent	-	-
Saponins		-	+
Tannins		+	-
Anthroquinones	Free Anthroquinones	+	+
Glycosides:	a.)Fehling's solution test	+	+
	b.)Ferric chloride test	+	+
Cardiac glycoside	Salkowaski test	+	+
Carbohydrate	Molisch's test	-	+

KEY: + Present; - Absent

Table 2: Result of Antimicrobial Sensitivity Tests of seedsof *Ficus religiosa* extracts

Test organism	**Extracts**	
	Petroleum ether	**Methanol**
Salmonella typhi	S	S
Escherichia coli	S	S
Staphylococcus aureus	S	R

Key: R= Resistant, S= Sensitive

Table 3: Zone of Inhibitions (mm) of Petroleum-ether and Methanol, Extracts of seeds of*Ficus religiosa*.

Test organism	**Extracts**		
	Pet-ether	**Methanol**	**Control**
Salmonella typhi	26mm	20mm	40mm
Escherichia coli	20mm	19mm	35mm
Staphylococcus aureus	30mm	0	40 mm

Key: 0 = no zone of inhibition

Table 4: Minimum Inhibitor Concentration of the Extract against the Microbes

Test organism	**Extracts**	
	Petroleum-ether	**Methanol**
	12.5 mg/l 10mgl/ml 7.5 mg/ml 5mg/ml 2.5mg/ml	12.5 mg/l 10mgl/ml 7.5mg/ml 5mg/ml 2.5mg/ml
Salmonella typhi	- -- * +	- - * + ++
Escherichia coli	- - * + ++	- - - - -
Staphylococcus aureus	- - - *+	- --* ++

Key: - = No growth, * = MIC, + = scanty colonies growth, ++ = moderate colonies growth.

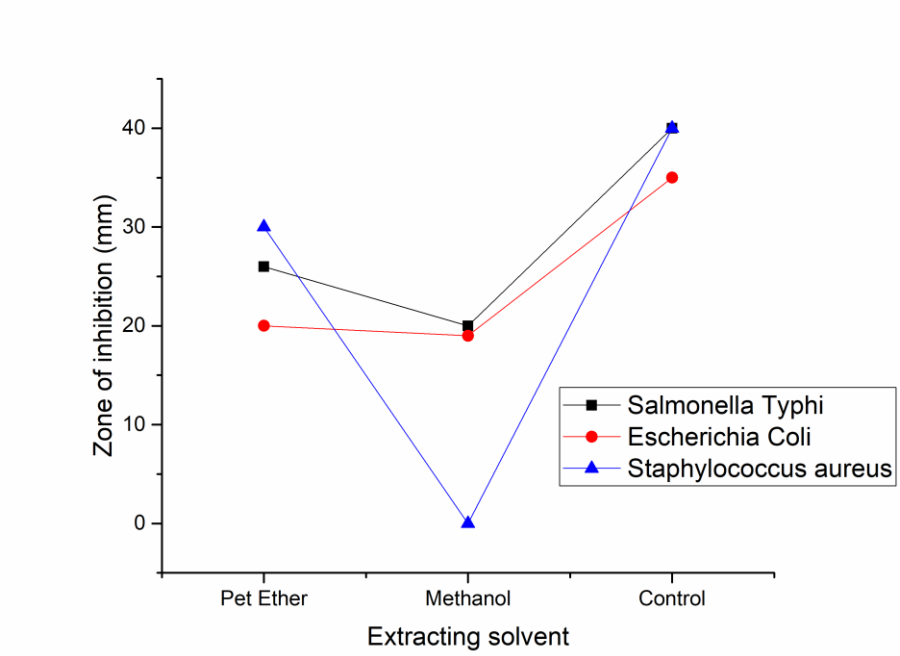

Fig. 1 Variation of zone of inhibition of the microbes due to different extracting solvents

The *Ficus religiosa*extracts that were sensitive to the tested organism (Table 2.) were further tested for zone of inhibition at concentrations of $4x10^{-4}$ µg/ml. The methanol extract showed diameter of zones of inhibition of 20 mm and 19 mm against *S. typhi and E.coli*, while petroleum-ether extract showed diameter of 26 mm, 20 mm and 30 mm for *S. typhi*, *E. coli* and S. *aureus* (Table 3) respectively. As shown in Fig. 1, the highest diameter of zone of inhibition of the extracts on *S. aureus* was 30 mm and the lowest was 20 mm for *S. typhi*.

The Minimum Inhibitory Concentration (MIC) (i.e. the lowest concentration of antimicrobial required to prevent visible growth) of the extracts also followed the same trend with the zones of inhibition. The crude pet-ether extract was found to have the minimum inhibitory concentration of 5mg/ml, 7.5 mg/mland 5 mg/ml for *S. typhi*, *E. Coli* and *S. Auriu*swhile methanol extract was found to have the minimum inhibitory concentration of 5 mg/ml and 7.5 mg./mlfor*S. aureus and S. typhi*(Table 4) thus indicating high potency.

Discussion

The solvent extracts had demonstrated antimicrobial activity against the test organisms. The pet-ether extract was more potent with activity against all the test organisms especially against S. *typhi* (30mm),*E. coli* (26mm) and *S. aureus* (20mm). The methanol extract however, demonstrated the least activity against all the test organisms. It is hypothesised that differences in polarity between the two solvents could be responsible for the differences in solubility of plant's active portion, hence variation in degree of activity. This is because of Methanol is highly polar while petether is considered amongst the most non polar solvents [13]. The non-activity demonstrated by the methanol extract against the *S. aureus* may be responsible for higher intrinsic resistance to most antimicrobial agents[14]. The demonstration of low MIC value (19 mg/ml) by methanol extracts (Table 3) is an indication that the phytoconstituents of the plant seed have therapeutic potential as compared with the standard antibiotic *Amoxicillin*as shown in Table 2.

Flavonoids, saponins/ tannins, steroids and terpenoids were present in one extract or the other (Table 1). Flavonoids are known to be synthesized by plants in response to microbial attack. Hence/ it should not be surprising that they have been found to be effective antimicrobial substances against a wide array of microorganisms when tested *in-vitro.* Their activity is probably due to their ability to react with extracellular and soluble proteins and to complex with bacterial cell waits leading to the death of the bacteria [15]. Tannins are also reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects. Phytotherapeutically, tannin-containing plants are used to treat nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins [16]

The spectra of antimicrobial activities displayed by these extracts could be explained by the presence of flavonoids, tannins, saponins and steroids. The purified components may have even more potency with respect to inhibition of microbes. Further studies on the phytoconstituents and purification of individual groups of

bioactive components can reveal the exact potential(s) of the plant to inhibit several pathogenic microbes. In conclusion, the activity exhibited by the extracts against clinical microbial isolates that are associated with various infectious diseases provides scientific justification for the ethno medicinal uses of the plant.

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