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Phytochemical and *in vitro* antibacterial activity of crude extracts of the seeds of *Parinari polyandra (Rosaceae)*

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ABSTRACT: Phytochemical and antibacterial properties of aqueous and methanolic extracts of the *Parinari polyandra Seed* (*Rosaceae*) used in herbal medicine by Traditional Medicine Practitioners (TMPs) in Ilorin; Kwara State were screened and tested against five pathogenic bacterial.

Phytochemical screening of the crude extracts of the seeds *Parinari polyandra* showed the presence of tannin, saponin, cardiac glycoside, terpenoids, flavoniods and volatile oil. The in-vitro antibacterial properties of aqueous and methanolic extracts of the plants was determined by agar diffusion technique, using pure isolates of *Staphylococcus aureus (ATCC 259230), Esherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus specie.* The results of the zone of inhibition showed that the aqueous and methanolic extracts of *Parinari polyandra* have antibacterial activity (25.33 ± 1.24 mm and 29.67 ± 1.20 mm respectively) on standard *Staphylococcus aureus (ATCC 259230);* while methanolic extract of *Parinari polyandra* showed bioactivity on *Esherichia coli* (18.00 ± 0.94 mm) at concentration of 15mg/ml.

Comparatively, methanolic extract of the seeds of *Parinari polyandra has* a significant higher in-vitro antibacterial activity than the aqueous extract and compared favourably with Ampicillin trihydrate control. The minimum inhibitory concentrations (MIC) of the aqueous and methanolic extracts of *the seeds of Parinari polyandra* on standard *Staphylococcus aureus (ATCC 259230)* are 40mg/ml and 25mg/ml respectively. The crude aqueous and methanolic extracts of these plants possess antibacterial properties to justify their traditional use in herbal medicine.

Keywords: Phytochemical properties, antibacterial activity, zone of inhibition, agar diffusion technique, aqueous extracts, methanolic extracts, Ampicillin trihydrate

Introduction

Plants have for ages been used by different cultures and civilization in the treatment of prevailing diseases even as the World Health Organization has encouraged the incorporation of traditional medicine into conventional medicine to improve healthcare of people. NNMDA(2008),WHO(1980)

Parinari polyandra (Rosaceae) is a tropical plant commonly found in some West African countries including Nigeria, Senegal, Ghana and Ivory Coast(Cote-de-voire). Abolaji (2007). *Parinari polyandra* is known locally in Nigeria as Gwanja Kwasa (Hausa), Abere (Yoruba) and Abbadima (Nupe).

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The leaves and stem of this plant is used ethno- botanically in Southwest Nigeria for the treatment of infertility, painful and inflammatory conditions, erectile dysfunction and diabetes mellitus. Vongtau (2004), Vongtau et al (2007).

It was observed that Traditional Medicine Practitioners (TMP) in Ilorin, Kwara State use the powdered seeds of *Parinari polyandra* as herbal recipe for the treatment of bacterial infection and venereal diseases.

The present study aims at investigating the phytochemical and in-vitro antibacterial properties of *Polyandra* polyandra.

Material and Methods

Collection of plant material

Seeds of *Parinari polyandra* were bought from traditional herbal shop located in Gegele area, Ilorin,Kwara State. The plant material was identified by Professor L.lajide, Chemistry Department, Federal University of Technology Akure, Ondo State. It was further authenticated by Professor F.A.Oladele of Plant Science Department, University of Ilorin, Ilorin Kwara State. The plant material was given voucher specimen number UIH/582 and deposited at University of Ilorin herbarium.

Processing of plant material

The identified plant material was air-dried for six weeks and pulverised into fine powder using clean and sterile Mortar and Blender. The powdered sample was sieved and quartered to obtain a representative of 400g used for this study.

Extraction of plant material

One hundred grams of the powdered seeds was macerated with 1L of absolute methanol (BDH) and allowed to extract at room temperature for 72h with intermittent shaking .The solution was filtered and the filtrate was concentrated and freeze-dried at Central Science Laboratory, Obafemi Awolowo University, Ile-Ife, Ife ;Osun State.

Preliminary Phytochemical screening

Qualitative screening of methanolic extracts of the seeds of *Parinari polyandra* (MEPPS) was carried-out by standard procedures described by Harbone (1982), Sofowora (1982) and Edeoga (2005).

Collection and Screening of test Organisms .

The pathogenic organisms used (*Staphylococcus aureus, Esherichia coli Pseudomonas aeruginosa, and Klebsiella pneumoniae*) were collected as pure isolates from Department of Medical microbiology and Parasitology, University of Ilorin Teaching Hospital. The pure bacterial isolates were further identified biochemically and morphologically to confirm their identity. They were then inoculated into nutrient agar slants and stored at 4° C.

Screening of crude extracts AQPPS and MEPPS for antibacterial activity

Standard procedures described by WHO (1991) was used to standardize the test organisms and carryout agar diffusion technique. The test organisms were subcultured in nutrient broth and incubated at 37°C for 24h to obtain confluent growth. Sterile pipettes were used to seed each of the test organisms into 5ml sterile normal saline (0.85% NaCl) and their turbidity was compared with 1% Barium Chloride dihydrate for appropriate inoculum's density.

Sterile swab sticks (Evepon) was used to inoculate pure isolates of the test organisms on the prepared nutrient agar plates and labelled properly. Sterile cork borer (5mm) was used to bore holes on inoculated nutrient agar plates and incubated appropriately.

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0.3ml of each plant extracts solution was dispensed aseptically into the holes bored on the inoculated agar plates and incubated 37°C for 24h.). 0.3ml of the controls (Ampicllin trihydrate and sterile normal saline) were also dispensed into the holes of the inoculated agar plates and incubated appropriately. Zone of inhibition of the crude extracts and the controls were measured using a transparent plastic ruler as indices for potency and antimicrobial activity.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the bioactive plant extracts was determined by tube dilution technique.

Results

The phytochemical analysis of the seeds of Parinari polyandra is presented in Table 1. Table 2 also shows the results of the tests carried out to show the antibacterial properties of the plant.

Table 1: Phytochemicals present methanolic extracts of Parinari polyandra Seeds

Secondary Metabolites	X. aethiopica	
Saponin	Positive	
Cardiac glycoside	positive	
Alkaloids	negative	
Tannin	positive	
Volatile Oil	positive	
Flavonoids	positive	

Table 2: Antibacterial activity of extracts of Parinari polyandra Seeds

AOGENS					
	ZONE DIAMETER (mm) OF GROWTH INHIBITION				
	AQPPS	MEPPS	Ampicilline	Sterile	Sodium
			Control	Chloride	
				Control	
Staphylococcus aureus	25.33±1.24	29.87±1.20	33.30±1.20	0	
Pseudomonas aeruginosa	0	0	0	0	
	0	18.00±0.94	0	0	
Escherichia coli					
	0	0	21.33±0.72	0	
Klebsiella pneumonia					
	0	0	51.00±1.70	0	
Proteus Specie					

AQPPS: Aqueous crude extracts of *Parinari polyandra seeds* MEPPS:Methanolic crude extracts of *Parinari polyandra seeds* Each value is Mean±SEM

PARINARI POLYANDRA LEAVES

PARINARI POLYANDRA SEEDS





Plate 1: Photographs of the leaves and seeds of *Parinari polyandra*.

PLANT EXTRACTS	CONCENTRATION (mg/ml)	BACTERIAL GROWTH	REMARK
AQPS I	80	-VE	 1
AQPS II	40	-VE	
AQPS III	20	+VE	MIC (40
AQPS IV	10	+VE	mg/ml)
AQPS V	5	+VE	

Table 3: Mic Of Aqueous Extract Of Parinari Polyandra Seed (AQPPS) On *Staphylococcus aureus* (ATCC 25925 Control)

Table 4:MIC Of Methanolic Extract Of *Parinari Polyandra* Seed (MEPPS) On *Staphylococcus Aureus* (ATCC 25925 Control)

PLANT EXTRACTS	CONCENTRATION (mg/ml)	BACTERIAL GROWTH	REMARK
MEPP I	100	-VE	
MEPP II	50	-VE	
MEPPIII	25	-VE	MIC (25mg/ml)
MEPPIV	12.5	+VE	
MEPSPV	6.25	+VE	

Antimicrobial activity of Aqueous extract of *Parinari polyandra* on Standard Staphylococcus aureus(ATCC 25923)



Antimicrobial activity of Ampicillin trihydrate control on Standard Staphylococcus aureus (ATCC25923)



Plate 2 : ANTBACTERIAL ACTIVITY OF AQPPS AND ANTIBIOTIC CONTROL



ANTIBACTERIAL ACTIVITY OF MEPP (50mg/ml) ON PURE ISOLATE OF STAPHYLOCOCCUS AUREUS

ANTIBACTERIAL ACTIVITY OF AMPICLLIN TRIHYDRATE CONTROL(12.5mg/ml) ON STAPHYLOCOCCUS AUREUS



Plate 3: ANTIBACTERIAL ACTIVITY OF MEPPS AND ANTIBIOTIC CONTROL

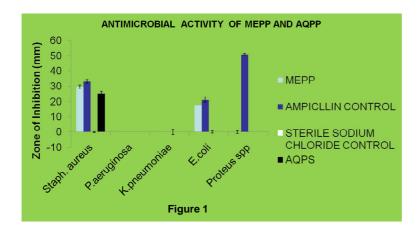
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ANTIBACTERIAL ACTIVITY OF MEPP (15mg/ml) ON PURE ISOLATE STAPHYLOCOCCUS AUREUS ANTIBACTERIAL ACTIVITY OF STERILE SODIUM CHLORIDE SOLUTION (9mg/ml) ON PURE ISOLATE OF STAPHYLOCOCCUS AUREUS



Plate 4: ANTIBACTERIAL ACTIVITY OF MEPPS AND STERILE SODIUM CHLORIDE CONTROL



Antimicrobial activity(zone of inhibition) of aqueous (AQPS) and methanolic extracts of Parinari polyandra Seeds (MEPPS)

Discussion

Phytochemical screening of the crude extracts of the seeds *Parinari polyandra* showed the presence of tannin, saponin, cardiac glycoside, terpenoids, flavoniods and *volatile* oil (Table1). Phytochemicals present as mineral elements and secondary metabolites play vital roles in the bioactivity exhibited by medicinal plants used ethno - botanically. Kar and Choudhary (1994).

The results from the zones of inhibition showed that the aqueous and methanolic extracts of *Parinari polyandra* have antibacterial activity (25.33 \pm 1.24mm and 29.67 \pm 1.20 mm respectively) on standard *Staphylococcus aureus* (*ATCC 259230*); while methanolic extract of *Parinari polyandra* seed showed bioactivity on *Esherichia coli* (18.00 \pm 0.94 mm) at concentration of 15mg/ml.(Table 2)

Comparatively, methanolic extract of the seeds of *Parinari polyandra has* a significant higher in-vitro antibacterial activity than the aqueous extract and compared favourably with Ampicillin trihydrate control (Figures 1 and 2).

The minimum inhibitory concentrations (MIC) of the aqueous and methanolic extracts of *the seeds of Parinari polyandra* on standard *Staphylococcus aureus* (*ATCC 259230*) are 40mg/ml and 25mg/ml respectively(Table 3 and 4).

The crude aqueous and methanolic extracts of these plants possess antibacterial properties to justify their traditional use in herbal medicine.

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