Phytochemical and Antibacterial Evaluation of *Parinari curatetellifolia* Planch Ex Benth (*Chrysobalanaceae*)

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ABSTRACT: *Parinari curatellifolia* Planch ex Benth (*Chrysobalanaceae)* is used in traditional medicine for the treatment of pneumonia, wound infections, dressing of fractures and dislocation. *P. curatellifolia* stem bark extracts in methanol, ethylacetate and n-butanol were evaluated for antibacterial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Bacillus subtilis* using cup plate method. The extracts were used at 50mg/ml concentration. The extracts were also screened for the presence of some secondary metabolites. The result of the antibacterial screening produced zones of inhibition ranging from 12-21mm for the methanolic extract while the ethylacetate and n-butanol fractions showed inhibition zones of 16-24mm respectively. The aqueous extract showed inhibition zones ranging from 12-20mm. Ampicillin (0.01mg/ml) used as positive control, showed zones of inhibition ranging from 14-34mm. Ethylacetate fraction was the most active of the extracts on the test bacterial species. Water was used as negative control. The extracts in most cases compared favorably with the ampicillin. The activity of the extracts was more on the gram positive bacteria than on the gram negative ones. The minimum inhibitory concentrations (MICs) of the ethylacetate fraction for *B. subtilis, P. aeruginosa* were 1.56mg/ml each, for *E.coli and S. aureus* were 3.13 mg/ml and 0.78mg/ml respectively. The minimum bactericidal concentrations (MBCs) of the ethylacetate fraction for *B.subtili*s and *S. aureus* were 6.25mg/ml each, for *P. aeruginosa* and *E.coli* were 12.50mg/ml each. The phytochemical screening revealed the presence of anthraquinones, tannins, saponins, flavonoids, cardiacglycosides, terpenoids, and carbohydrates. The antibacterial activity of the extracts may be attributable to the presence of these compounds in the extracts. The findings of this work lend support to the ethnomedical use of the plant.

Key Words: Antibacterial; Phytochemistry; MIC; MBC; *Parinari curatellifolia*

INTRODUCTION

Nature has been a source of medicinal agents for many years and since the origin of man. In Nigeria, almost all plants are medicinal and the application in medicine is currently well acknowledged and established as a viable profession (Kafaru, 1994). *P. curatellifolia* Planck ex Benth (Chrysobalanaceae) is a large ever green spreading tree which grows up to 20m tall with a single bare stem (Mark and Bart, 2002). Hot infusion of th *P. curatellifolia* e stem bark is used in the treatment of Pneumonia. A leaf decoction is either drunk or used in bath as fever remedy. Crushed or pulp leaves are used in dressing fractures or dislocations and for wounds, sores and cuts. After being sripped, the twigs can be used as a tooth brush. Three ent- kuarene diterpenoids have been isolated as cytotoxic constituents of the root bark of *P. curatellifolia* collected from Zimbabwe. These are known compounds 15-oxozoaptlin and novel analogs 13-methxy-15-oxozoaptlin and 13-hydroxy-15-oxozoaptlin. These compounds were broadly cytotoxic when tested in the human tumor cell panel with the most potent cytotoxic activity being observed in each case in the A431 human epidermoid carcinoma cell line (Kraft *et al.*, 2003). Natalie et al., (2001) also reported the isolation and identification of 13-hydroxy-15-oxozoaptlin from methanol extract of *P. cu ratellifolia* root bark from South Africa. The study showed that 13-hydroxy-15-oxozoaptlin showed G_2 DNA damage check inhibition and antimitotic activity. The elemental composition of *P. curatellifolia* has also been investigated. It was found to contain N, P, K, Ca, Mg, Zn, Na and Cu (Kapu and Niger, 1975). To our

knowledge, information on antibacterial and phytochemical properties of this plant has not been reported. This present study seeks to document the antibacterial activities of the extracts of the plant.

MATERIALS AND METHODS

Plant material

The plant material was collected in February, 2006 in Zaria, Kaduna state- Nigeria. The plant material was identified and authenticated at the Herbarium unit, Department of Biological Science, Ahmadu Bello University, Zaria, Nigeria. The parts of plant collected were: fresh leaves, stem, root and unripe fruits for the purpose of identification and authentication. The voucher number 903 is available at the unit for reference.

The stem bark of the plant was collected, and then pulverized into coarse powder with the aid of pestle and mortar. The powder was then stored in an appropriate container until required for use.

Preparation of plant extracts

The extraction was carried out using the soxhlet extractor. 170g of the powered material was extracted with 1.4 litres of methanol. The extract was concentrated over water bath. The yield obtained was 40% (Brain and Turner, 1975)

The extraction was also carried out by maceration process. Sixty gram (60g) of powered material was extracted with 500ml of distilled water. The extract obtained was concentrated over water bath. The yield obtained was 25.3% (Brain and Turner, 1975). The ethylacetate was then fractionated sequentially using ethylacetate and butanol. The fractions obtained were concentrated over water bath.

Test organisms

The test organisms used were standard strains of *Bacillus subtilis (*NCTC 10342 A76*), Staphylococcus aureus (*CATCC 13969*), Escherichia Coli (*NCTC 10418*) and Pseudomonas aeruginosa* (ATCC 1853*)*. They were obtained from the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The bacterial species were grown for 24hous in nutrient broth and diluted to 1:5000 for *E.coli* and *P.aeruginosa* and 1:1000 for *S.aureus* and *B. subtilis*.

Susceptibility Test

The cup plate method was used. Sterile nutrient agar plates were flooded with the various dilution of the test bacteria and drained with sterile Pasteur pipette. Wells measuring 8.0mm in diameter were bored into the inoculated plates using cork borer (No.4). The wells were filled with the extracts (50mg/ml) and ampicillin (0.01mg/ml). The plates were allowed to stand for pre-diffusion time for 2 hours and then incubated for 24 hours at 37^{o}C. After incubation, diameters of zones of inhibitions were measured to the nearest millimeter using metric rule (Carter, 1972)

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The ethyl acetate fraction was chosen for the tests because; it showed higher activity against the bacterial species tested. Two-fold serial dilution of 2mls of the ethyl acetate fraction (50mg/ml) was made in nutrient broth (5mls). Ten dilutions were made and all were inoculated with 0.5ml suspensions of the various diluted bacterial species and incubated for 24 hours at 37^{o}C. After incubation subcultures of the mixtures were made unto nutrient agar plates and incubated for 24 hours at 37^{o}C. At the end of which the least concentration that showed no detectable growth was considered the MIC. To determine the MBC, the plates were further incubated for 24 hours at the same temperature. After incubation, the least concentration that showed no growth was considered the MBC (Carter, 1972).

Phytochemical Screening

All the extracts were screened for the presence of some secondary metabolites using the methods of Sofowora (1989), Brain and Turner (1975) and Trease and Evans (1996).

RESULTS AND DISCUSSION

The results obtained showed that the stem bark had antibacterial activity. At 50mg/ml

concentraton the aqueous extract produced inhibition zone diameter of 12-20mm while the methanol extract produced zone diameter of 12-21mm against the bacteria. The ethyl acetate fraction produced inhibition zones of 16-23mm while the n-butanol fraction showed zones of inhibition ranging from 12-18mm. Ampicillin at 0.01mg/ml concentration produced inhibition zones ranging from 14-34mm against the test bacteria (Table 1). The MICs of the ethyl acetate fraction ranges from 0.78-3.13mg/ml while the MBCs ranges from 6.25-12.50mg/ml for the bacterial species tested (Table 2).

Table1: Antibacterial activities of aqueous and organic solvent stem bark of *Parinari curatellifolia* .

Test bacteria	**Zone of inhibition (mm)/ Extract in different solvents**				
	Methanolic Extract	**Aqueous Extract**	**Ethylacetate Fraction**	**n- Butanol Fraction**	**Ampicillin 0.01mg/ml**
B. subtilis	21.00	20.00	22.00	18.00	34.00
S. aureus	18.00	18.00	24.00	17.00	22.00
P. aeruginosa	12.00	17.00	19.00	16.00	14.00
E. coli	17.00	12.00	16.00	12.00	17.00

Values greater than 8.00mm indicates some activity.

The ethylacetate fraction appears to be the most active component of the extract, showing the highest activity against the bacteria species (Table 1).The ethylacetate fraction is the most active on *S. aureus* (24mm) and its activity on *S. aureus* compares favourably with the activity of 0.01mg/ml Ampicillin on the same bacterial species. The activity of the extracts were higher on the gram positive bacteria, *B. subtilis* and *S .aureus* than the gram negative ones, *P. aeruginosa* and *E. coli* as the zones produced by the extracts against the gram positive bacteria are higher than the zones against the gram negative bacteria. This apparent difference in their susceptibilities to the extracts might be related to the structural differences in the cell envelope compositions of the gram positive and gram-negative bacteria. The gram positive cell envelope is simple while that of gram negative is complex consisting of lipoproteins outer membrane and lipopolysaccharides (Jawetze, *et al* 1978). The outer membrane of the gram negative cell outer envelope does block the penetration of large molecules and hence the relative resistance of gram negative bacteria to some antimicrobial agents (Jawetze *et al*., 1978).

The results of phytochemical screening revealed the presence of anthraquinones, saponins, flavonoids, cardiac glycosides, tannins and carbohydrates (Table 3). All these are secondary metabolites that have been noted to have antimicrobial activities (Cowan, 1999). The observed antimicrobial activities of the extracts can be attributed to the presence of these secondary metabolites.

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Ethyl acetate Fraction of *Parinari curatellifolia* in mg/ml

Test Bacteria	**MIC**	**MBC**
B. subtilis	1.56	6.26
S. aureus	0.78	6.25
P. aeruginosa	1.56	12.50
E. coli	3.13	12.50

Table 3: Phytochemical Constituents of the Stem bark extract of *Parinari. curatellifolia*

Constituents	Methanolic Extract	Aqueous extract	Ethylacetate Fraction	n-Butanol fraction
1. Alkaloids				
a. Mayer's reagent	-	-	-	-
b. Dragendoff's reagent	-	-	-	-
2. Anthraquinone				
a. Borntrager's test	+	+	+	+
3.Terpenoids				
Liebermann-Burchard's test	+	+	+	+
4. Saponin				
a. Frothing test	+	+	+	+
b. Haemolysis test	+	+	+	+
5. Flavonoids				
a. Ferric chloride test	+	+	+	+
b. Shinoda's test	+	+	+	+
6. Cardiac glycoside				
a. Keller- Killiani's test	+	+	+	+
7. Cyanogenetic glycoside				
a. Guignard's test	-	-	-	-
8. Tannins				
a. Ferric chloride test	+	+	+	+
b. Lead sub-acetate test	+	+	+	+
9. Carbohydrate test				
a. Molisch's test	+	+	+	+
b. Fehling's test (Reducing Sugars)	+	+	+	+

+ = Positive, - = negative

CONCLUSION

The result of the phytochemical screening revealed the presence of the following these secondary metabolites anthraquinones, tannins, saponins, flavonoids, cardiac-glycosides, and terpenoids. The result of this work lends support to the use of the plant in treating pneumonia and wounds as the test bacterial species can be involve in any of the health conditions.

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