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The biological activities of secondary metabolites of *Parinari macrophylla* Sabine

O. T. Audu*, A. O. Oyewale and J. O. Amupitan.
Department of chemistry, Ahmadu Bello University, P. M. B. 1045, Zaria – Nigeria.

ABSTRACT

The secondary metabolites of *Parinari macrophylla* Sabine of the family Chrysobalanaceae was examined. The fruits and stem were successively extracted with hexane, ethyl acetate and methanol. Phytochemical analyses of plant extracts revealed the presence of phenolic compounds, alkaloid and glycosides. Plant extracts were evaluated for biological activity using the brine shrimp lethality bioassay method. The results showed the presence of cyctotoxic compounds and the following LC_{50} values (at 95% confidence interval) of 201, 241 and 500 were obtained for hexane, ethyl acetate and methanol extracts respectively. Chromatographic purification of the ethyl acetate extract gave six fractions of which fractions A, B and F LC_{50} 20, 125 and 300 respectively. Antimicrobial test showed plants extracts to be active against *Staphylocccus aureus, Escherichia coli, Salmonella typhi, candidas albicans* and *Pseudomonas aeruginosa* with m.i.c value 20 μg/ml. IR studies of plant extracts showed presence of hydroxyl and carbonyl functional groups.

*Author for correspondence

INTRODUCTION

In our continued search for medicinal plants in Nigeria, *Parinari macrophylla* sabine which belongs to the family Chrysobalanceae was investigated. The plant family has 17 genera and 350 species [1,2]. Chrysobalanceae is a family of trees and shrubs distributed throughout the tropical regions especially in Africa. *Parinari macrophylla* sabine has been reported to contain sugar, protein and steroid [3]. Pharmacologically the decoction of the bark and leaves are used as mouth wash, internal troubles and for inflammed eye [3]. An antioxidant has been found in the plant, which may be a soluble flavanoid glycoside [4]. Diterpenoid has been isolated from other species of *Parinari* [5,6]. In this paper we report our findings on the investigation and isolation of bioactive compounds from *Parinari macrophylla* sabine.

EXPERIMENTAL

General Experimental Procedures

Infrared Spectra were run on a Perkin Elmer 1600 FT –IR spectrometer. *Artemiania salina* leach (Aquarium system U.S.A.) was used for Brine Shrimp lethality bioassay. *Escherichia coli* NCTC 10418, *Salmonella typhi* NCTC 52311, *Staphylococcus auerus* NCTC 6571 *Klebsiela pneumonia* ATCC 10031, *Pseudomonas aeruginosa* NCTC 6750 and *Candida albicans* ATCC 10231 were obtained from the Department of Microbiology Ahmadu Bello University Hospital, Zaria. All the organisms were checked for purity and maintained in nutrient agar. Silica gel 230 – 400 mesh (by Merck) TLC and precoated TLC (Merck, Kieslgel 60 F254) were used.

Plant material

Parinari macrophylla sabine was collected from Kogi State, Nigeria. The plant was identified at the Herbarium of Department of Biological Sciences Ahmadu Bello University Zaria. A voucher specimen was deposited at the Herbarium with number 2621. Plant was air-dried, powdered and stored for use.

Extraction

The plant material (120g) was packed into a thimble of a soxhlet extractor and soxhlet extracted successively with hexane (900ml), ethyl acetate (900ml) and methanol (900ml). The extracts were dried under vacuum using rotavapor at 40^0C. The yields of extracts obtained are as follows hexane (2.7g), ethyl acetate (2.1g) and methanol (1.1g). These extracts were stored in the refrigerator until tested.

Phytochemical analysis

This was carried out as described by Vishnor [7], Sofowora [8] and Trease and Evans [9]. Extracts were tested for Alkaloid, Glycoside and Steroid.

Table 1: Result of Phytochemical, Brine shrimp test and Antimicrobial screening

Extract	Secondary Metabolite present	LC_{50} Value at 95% confidence interval	Microorganism Active against
Hexane	Glycoside	201	*Salmonella typhi* *Staphylococcus aureus*
Ethyl acetate	Glycoside Alkaloid	242	*Escherichia coli* *Salrnonella typhi* 20μgml
Methanol	Steroid Glycoside Alkaloid	500	*Staphylococcus aureus* 20μ g1ml *Pseudomona aeruginosa* *Candida albican*
Fraction A			
Fraction B		20	
Fraction C		125	
Fraction D		ND	
Fraction E		ND	
Fraction F		300	

ND: Not determined.

Table 2: Result of Infrared analysis

Extract	Frequency cm^{-1}	Functional group.
Fraction E	3374.83	OH bonded
	2935.50	$>CH_2$, $-CH_3$ stretching
	1729.39	>C= O carbonyl
	1633.06	>C=C< bond
	1453.40	$>CH_2$
	1266.75	O-H bending
	1042.41	C – O stretching
Fraction F	4307.62	-OH
	2938	$>CH_2$, CH stretching
	1729.88	>C= O carbonyl
	1633.69	>C=C< bond
	1453.32	$>CH_2$
	1214.26	O-H bending
	1044.21	C- O stretching
	633.24	=C-H out of plane

Brine Shrimp lethality bioassay: Standard protocol was used [10]

Antimicrobial test

The paper disc diffusion method as described by Ericson *et al*. [11] was used.

Chromatographic purification

The Ethyl acetate fraction was hydrolysed and subjected to silica gel chromatography and eluted with cyclohexane: pet ether (60-80) (1:1), Dichloromethane: pet ether (1:1), Ethyl acetate and methanol to give fractions A-F

RESULTS AND DISCUSSION

Parinari macrophylla sabine has been used by natives in traditional medicine. To date, no detail of the chemical constituents of this plant has been reported. The antimicrobial activities of extracts of this plant were studied against the following pathogens: *Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, Candida albicans* and *Staphylococcus aureus*. The result is showed in Table 1.

Hexane extract showed activity against *S. typhi* and *S. aureus*; ethyl acetate extract was active agaunst *E. coli*, while the methanol extract was active against *S. aureus, P. aeruginosa* and *C. albicans*. Our observation showed that it is possible to check the growth of these pathogens with principle of these extracts. The result of the Brine shrimp lethality bioassay shows the extracts to be moderately toxic to Brine shrimp. This suggest that the extracts contain compounds that are cyctotoxic since LC_{50} value are moderately low (see Table 1). Phytochemial analysis had revealed the presence of secondary metabolites in these extracts, which has been widely reported to have biological activity. These include glycosides and alkaloids whose presence may account for the biological activity of the extracts of *Parinari macrophylla* sabine.

Column chromahography of the active dried ethyl acetate extract gave fractions A-F. The Brine shrimp lethality bioassay carried out on fraction obtained from the column showed fraction A to be the most potent with LC_{50} value of 20. IR spectra data of some fractions obtained from the column are presented in Table 2. The

data reveal the presence of carbonyl and hydroxyl functional group.

We have investigated the secondary metabolites of *Parinari macrophylla* sabine and found that they are biologically active and this may explain the use of extracts of the plant by natives to treat disease like wounds, stomach and respiratory troubles.

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