Full Length Research Paper

Phytochemical compounds and antimicrobial activity of three medicinal plants (*Alchornea hirtella*, *Morinda geminata* and *Craterispermum laurinum*) from Sierra Leone

Lahai Koroma¹ and Basil N. Ita^{2*}

¹Department of Basic Sciences, Eastern Polytechnic, Kenema, Sierra Leone. ²Department of Chemistry, University of Uyo, Uyo Nigeria.

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Petroleum ether, acetone, ethanolic and aqueous crude extracts of various plant organs of Alchornea hirtella, Morinda geminata and Craterispermum laurinum used in Sierra Leone exhibited variable degree of antimicrobial activity against four bacterial species. Compared with the standard drug ciprofloxacin, the extracts exhibited low to moderate antibacterial activity. Generally, the tested microorganisms were resistant to the petroleum ether and acetone extracts. The aqueous extract of the stem bark of *M. geminata* was sensitive to *Streptococcus pyogenes* (61% inhibition) and leaf extract of *A. hirtella* inhibited the growth of *Proteus vulgaris* (56%). Ethanolic crude extract of the stem bark of *C. laurinum* and *M. geminata* were particularly sensitive to *S. pyogenes*; moderate activity was also demonstrated by the stem bark of *C. laurinum* against *Escherichia coli*. MIC values indicated that the ethanolic extract showed significant microbiostatic action against *S. pyogenes* and *Staphylococcus aureus* (MIC 0.8 – 2 mg/ml), whereas the other strains were more resistant (MIC >2 mg/ml). Phytochemical evaluation revealed moderate to high contents of flavonoids, alkaloid and saponins in the ethanolic extract.

Key words: Antimicrobial activity, medicinal plants, phytochemical constituents.

INTRODUCTION

Recently, much attention has been directed toward extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants plays a vital role in covering the basic health needs in developing countries, particularly Africa (Munoz-Mingarro et al., 2003; Macfoy and Sama, 1983). In Sierra Leone were about 80% of the populace live with extremely low per capita income, the purchase of manufactured drugs becomes a big problem due to their high cost. This is compounded by their unavailability in hospitals and peripheral health centers when needed; hence the dependency on traditional medicine is high. Numerous publications abound on the traditional uses of medicinal plants as anti-inflammatory agents, antiplasmodial agents,

antimicrobial agents, anticholinergic agents, antihypertensive agents, etc. (Marshall et al., 2000; Manga et al., 2004; Okwu and Josiah, 2006; Okokon et al., 2006; Kastrup et al., 1999; Atindehou et al., 2002; Le Grand and Wondergem, 1990). Daziel (1937) identified Craterispermum laurinum as one of the medicinal plants in Sierra Leone. He reported that a decoction of the leaf or bark was used in the treatment of mild fever. Macfov and Samai (1983) reported that the aqueous extract of the dried stem bark of C. laurinum from the Eastern Province of Sierra Leone was used in the treatment of yellow fever. According to Vasieleva (1969), a decoction of the roots of Morinda geminata is used as a vomitive, laxative and an infusion of the leaves as a soothing and refreshing stomachic or externally as a lotion for fever and headache in Guinea. Abrew et al. (1999) reported the antimicrobial, antifungal and antiveast activity of methanol : water (9:1) extract of the dried leaves of M.

^{*}Corresponding author. E-mail: basil_ita@yahoo.com.

Comple	Plant	Ethanolic extract					Acetone extract				
Sample	organ	Alk	Fla	Sap	Ste/Ter	Tan	Alk	Fla	Sap	Ste/Ter	Tan
Alchornea hirtella	L	+	++	++	-	+	-	+	+	+	+
Craterispermum laurinum	RB	++	++	++	+	-	+	-	+	-	+
	SB	++	++	++	-	-	+	-	+	-	++
Morinda geminata	L	+	+	++	-	-	+	-	+	-	+
	SB	-	+	+	-	-	-	+	+	+	-

 Table 1. Phytochemical composition of the ethanolic and acetone extracts of plant samples.

L = Leaves; RB = root bark; SB = stem bark; Alk = alkaloids; Fla = flavonoids; Sap = saponins;

Ste/Ter = sterols/terpenes; Tan = tannins.

geminata. Le Grand et al. (1988) reported that the ethanolic extract of sun-dried leaves of *M. geminata* was active against *Staphylococcus aureus* and *Aspergillus niger* (a fungus). Adesogan (1973) investigated the antimetazoal activity of ethanolic (80%) extract of root bark of *M. geminata*; a positive result was indicated against *Acaris lumbricoides*. Reports by Samai and Barnish (1992) indicated that a hot water decoction of *Alchornea hirtella* is used in Sierra Leone by traditional healers to stop diarrhea.

Herbal folk medicines provide an interesting and still largely unexplored source for drug development with potential chemotherapeutic benefits. The validation of medicinal plant-based therapy is imperative for African developing countries as a greater percentage of the populace use such remedies (Poussett, 1994). The present work examines the antimicrobial activity and bioactive components in various plant organs of *Alchornea hirtella, Morinda geminata* and *C. laurinum* using various solvent systems.

MATERIALS AND METHODS

Materials

The experimental plant organs (leaves, stem bark, root bark) of *A. hirtella*, *M. geminata* and *C. laurinum* were collected from the Eastern Province of Sierra Leone and identified at the Department of Botany, Fourah Bay College, University of Sierra Leone, Freetown. A voucher specimen (No. 22312D) was deposited in the herbarium of the Department of Botany, Fourah Bay College. The various plant parts were sun-dried and ground into powder using a laboratory mill prior to analysis.

Cultures of the test microorganisms were obtained from Blue Shield Medical and Diagnostic laboratory in Freetown, Sierra Leone and sub-cultured in agar tubes to obtain pure isolates.

Extraction was first done with petroleum ether (60 - 80°C) in a soxhlet apparatus. The residue was dried, re-extracted with acetone and finally with ethanol. The obtained solutions were filtered using a Buchner funnel, and the filtrate evaporated to dryness in a rotary evaporator to give the crude petroleum ether, acetone and ethanolic extracts respectively. Aqueous extract was obtained by heating the sample with 500 ml of de-ionized water for 1 h. The mixture was filtered and the filtrate dried. All reagents were of analytical grade.

Phytochemical screening

Screening of the samples for bioactive components including alkaloids, tannins, sterols/terpenes, flavonoids and saponins was done using standard methods (Harborne, 1998; Trease and Evans, 1978).

Antimicrobial assay

Antimicrobial analysis was done on the extracts of the various plant organs using the disc diffusion method on two gram- positive and two gram – negative micro-organisms namely: *Staphylococcus aureus* (Sa), *Streptococcus pyogenes* (Sp), *Proteus vulgaris* (Pv) and *Escherichia coli* (Ec) respectively as described by the NCCLS (National Committee for Clinical Laboratory Standards, 1993), using the Muller-Hinton agar as culture medium. The minimum inhibitory concentration (MIC) was determined for the standard antibiotics and the most active extract in parallel experiments in order to control the sensitivity of the test microorganisms. All tests were performed in duplicate.

RESULTS AND DISCUSSION

The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bio-active compounds are alkaloids, flavonoids, tannins and phenolic compounds (Musyimi et al., 2008; Weimann and Heinrich, 1997; Atindehou et al., 2002; Edeoga et al., 2005).

Contents of phytochemical compounds in the plant extracts varied with the plant organ and solvent system used (Tables 1 and 2). Moderate to high levels of alkaloids, flavonoids and saponins were present in the ethanolic extract of Alchornea *hirtella*, *C. laurinum* and *Morinda geminata*. Tannins was only found in *A. hirtella* crude ethanolic extract, while sterols/terpenes was absent except in the ethanolic extract of the root bark of *C. laurinum*. The acetone extract had moderate content of tannins and saponins. Alkaloids were only found in the root bark and stem bark of *C. laurinum*. Flavonoids and sterols were found in the leaves of *A. hirtella* and stem bark of *M. geminata*. The petroleum ether extract had low to no contents of saponins. Flavonoids were present in

Comple	Plant	Petroleum ether extract						Aqueous extract					
Sample	organ	Alk	Fla	Sap	Ste/Ter	Tan	Alk	Fla	Sap	Ste/Ter	Tan		
Alchornea hirtella	L	-	+	+	-	-	++	+	++	+	+		
Craterispermum laurinum	RB	-	-	+	-	-	++	+	+	+	++		
	SB	-	-	+	-	+	+	++	++	++	+		
Morinda geminata	L	+	+	-	+	-	+	+	+	-	+		
	SB	-	-	+	+	+	+	+	+	+	-		

Table 2. Phytochemical composition of the petroleum ether and aqueous extracts of plant samples.

L = Leaves; RB = root bark; SB = stem bark; Alk = alkaloids; Fla = flavonoids; Sap = saponins; Ste/Ter = sterols/terpenes; Tan = tannins.

Table 3. Antimicrobial activity of petroleum ether and aqueous extracts.

Comple	Plant	Pe	etroleum et	her extract	*	Aqueous extract *				
Sample	organ Sp (%	Sp (%)	Sa (%)	Ec (%)	Pv(%)	Sp (%)	Sa (%)	Ec (%)	Pv (%)	
Alchornea hirtella	L	2 (6)	-	-	-	8 (27)	8 (28)	7 (33)	13 (56)	
Craterispermum	RB	-	2 (7)	-	-	8 (27)	6 (21)	10 (49)	3 (13)	
laurinum	SB	-	-	-	1(5)	11 (36)	-	8 (38)	5 (21)	
Morinda geminata	L	-	3 (11)	2 (9)	-	10 (33)	9 (29)	2 (11)	-	
	SB	1 (4)	2 (8)	-	-	18 (61)	15 (50)	2 (9)	8 (32)	
Solvent		-	-	-	-	-	-	-	-	
Ciprofloxacin	ref	30	30	21	23	30	30	21	23	

L = Leaves; RB = root bark; SB = stem bark; Sp = *Streptococcus pyogenes*; Sa = *Staphylococcus aureus*; Ec = *Escherichia coli* and Pv = Proteus *vulgaris*.

*Values are zones of inhibition in mm; Conc = 1 mg/ml; % = percentage efficacy relative to the standard drug, ciprofloxacin; - = not active.

Sample	Plant		Ethanoli	c extract*		Acetone extract*				
	organ	Sp (%)	Sa (%)	Ec (%)	Pv (%)	Sp (%)	Sa (%)	Ec (%)	Pv (%)	
Alchornea hirtella	L	17 (56)	8 (27)	3 (14)	8 (33)	2 (7)	-	3 (13)	3 (11)	
Craterispermum	RB	17 (56)	10 (33)	5 (23)	7 (32)	7 (22)	9 (31)	5 (22)	2 (8)	
laurinum	SB	25 (83)	17 (55)	11 (52)	6 (27)	12 (40)	5 (16)	3 (12)	-	
Morinda geminata	L	17 (56)	13 (43)	7 (33)	11 (48)	6 (20)	-	-	5 (21)	
	SB	18 (61)	11 (37)	2 (11)	10 (44)	5 (17)	6 (21)	3 (13)	-	
Solvent		-	-	-	-	-	-	-	-	
Ciprofloxacin	ref	30	30	21	23	30	30	21	23	

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L = Leaves; RB = root bark; SB = stem bark; Sp = *Streptococcus pyogenes*; Sa = *Staphylococcus aureus*; Ec = *Escherichia coli* and Pv = *Proteus vulgaris*.

*values are zones of inhibition in mm; Conc = 1 mg/ml; % = percentage efficacy relative to the standard drug, ciprofloxacin.

the leaves of *A. hirtella* and *M. geminata.* Sterols/ terpenes was present in *M. geminata* leaves, while alkaloids was absent except in the leaves of *M. geminata.* Like the alcoholic extract, the aqueous extract had moderate to high levels of phytochemical compounds; tannins and sterols/terpenes was absent in the stem bark and leaves of *M. geminata* and *A. hirtella* respectively.

The extracts showed variable degree of antimicrobial activities against one or more of the tested microorganisms. This was affected by the solvent system and plant organ under study. Generally, the tested organisms were resistant to the petroleum ether and the acetone extracts, except the acetone stem bark extract of *C. laurinum* which had an inhibition of 40% against *S. pyogenes* (Tables 3 and 4). This low antimicrobial activity may be attributed to the reduced ability of these solvents to extract phytochemical compounds or the presence of pigments or phenols, which are known to interfere with antimicrobial activity (Doughari, 2006).

The aqueous extract demonstrated low to moderate

Comple	Plant	MIC values							
Sample	organ	Sp	Sa	Ec	Pv				
Alchornea hirtella	L	1.9	-	-	-				
Craterispermum laurinum	RB	1.5	2.0	-	-				
	SB	0.8	1.8	2.2	-				
Morinda geminata	L	1.6	1.5	-	3.9				
	SB	1.0	1.9	-	2.7				
Ciprofloxacin	Ref	0.5	0.5	0.5	0.6				

 Table 5. MIC of ethanolic extract and reference drug*.

*MIC, Minimum inhibitory concentration; values given as mg/ml for ethanolic extract and as µg/ml for ciprofloxacin; - = not determined.

antimicrobial activity. The stem bark extract of M. geminata was sensitive to S. pyogenes (61% inhibition) and the leaf extract of A. hirtella inhibited the growth of P. vulgaris (56% inhibition). The roots bark extract of C. laurinum and the stem bark of M. geminata exhibited moderate activity against E. coli and Staph. aureus respectively. The ethanolic crude extract exhibited the highest degree of antimicrobial activity. The stem bark of C. laurinum and M. geminata were particularly sensitive to S. pvogenes, a gram positive haemolytic bacterium known to cause diseases such as pharyngitis, impetigo, scarlet fever, etc. Moderate activity was demonstrated by the stem bark of C. laurinum against E. coli, a microorganism noted for causing gastrointestinal infections, urinary tract infections, etc. Results from MIC values (Table 5) indicate that the ethanolic extracts of the tested plants showed significant microbiostatic action against S. pyogenes and Staph. aureus (MIC 0.8 - 2.0 mg/ml) whereas the other strains proved more resistant (MIC > 2 mg/ml). However, our MIC values were less than values obtained for the standard drug, ciprofloxacin.

The enhanced sensitivity of the ethanolic extract may be attributed to its high content of phytochemical compounds. Many alkaloids have pharmacological effects and could be associated with inhibition of nucleic acid, protein and membrane phospholipids biosynthesis (Shelton, 1991). Saponins have the property of precipitating and coagulating red blood cells (Okwu and Josiah, 2006). Flavonoids on the other hand are water soluble antioxidants and free radical scavengers, which are capable of preventing oxidative cell damage and have strong anticancer activity (Salah et al., 1995; Del-Rio et al., 1997; Okwu, 2004). Tannins have astringent properties, hasten healing of wounds and inflamed mucous membrane. A combination of these factors, together with the observed antimicrobial activity may explain some of the previous claims on the use of the plants in traditional medicine. Our results are in agreement with reports that many plant extracts have been as a source of medicinal plants to cure urinary tract infections, gastrointestinal disorders, enhance healing of wounds and stop bleeding, etc (Leite et. al., 2006; Okwu and Josiah, 2006).

From the present study, it can be seen that these plants possess compounds with antimicrobial activity. However, isolation and purification of the active components will be essential to give more insight into their modes of action.

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