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Full Length Research Paper

In vitro antimicrobial activity of crude leaf and stem bark extracts of *Gmelina arborea* (Roxb) against some pathogenic species of Enterobacteriaceae

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Phytochemical screening of the *Gmelina arborea* reveals the presence of carbohydrates, alkaloids, saponins, tannins, anthraquinones and cardiac glycosides. The presence of these bioactive compounds in plants is linked to biological activity. Determination of antimicrobial activity using the agar diffusion method showed that the crude extracts of the leave and stembark of the plant inhibited the growth of such recalcitrant pathogenic *Escherichia coli, Klebsiella pneumonieae, Proteus mirabilis, Shigella dysenteriea* and *Salmonella typhi* that frequently show above average resistance, the extent of which depended on the solvent and organism. Activity of the extracts was consistently less than the conventional antibiotic, tetracycline. The effectiveness of the extracts was more in the acidic than in alkaline conditions and also increased with increase in temperature. Results provided the scientific bases for the folkloric application of *G. arborea* as a medicinal plant and ways the plant can be used as source for newer antibiotic substances for the possible control of dysentery, diarrhea, typhoid fever and wound infections associated with these bacteria.

Key words: *Gmelina arborea,* phytochemicals, resistance, extracts, antibiotics, pathogenic, traditional medicine.

INTRODUCTION

Nigeria is covered with a large number of plant species, some of which have been used for centuries in folkloric medicines to diagnose, prevent and treat various ailments. More than 80% of the population, especially in the rural areas where infectious diseases are endemic and modern health care facilities are inadequate depend on traditional systems of medicine for their health problems (Kawahara et al., 1981). However, scientific investigations and information on the therapeutic potential of these medicinal plants are limited. This lack of scientific knowledge has restricted the use of traditional herbs as remedies to be used in conjunction with or as an alternative to orthodox medical treatment. As of now, only about 20% of the world medicinal plants have been screened for pharmacological and biological activities (Reynold and Lawson, 1978; Ndukwe et al., 2005, 2007). Natural products from both plants and microbial sources are used in pharmaceutical preparations either as pure or crude extracts (Parekh and Chanda, 2007). Presently, a lot of attention is focused on higher plants to determine their phytoconstituents with the aim of using them for the prevention and treatment of microbial infections and other diseases of non-microbial etiology as alternatives to synthetic drugs.

The ever increasing demand for safer and cheaper herbal recipes in the developed countries has led to the extraction and development of several drugs and chemotherapeutic agents from plants as well as from traditional herbal remedies (Falodun et al., 2006). The scientific literature is full of reports describing plants as the sleeping

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Abbreviations: MIC, minimum inhibitory concentration; **MBC,** minimum bactericidal concentration.

giant of the pharmaceutical industry (Smith, 1991; Michael, 2002), which when fully exploited will provide novel compounds to fight infectious diseases (Onyeagba et al., 2004; Muhsin and Amina, 2007; El-Mahmood and Ameh, 2007).

Gmelina arborea (Verbenaceae), a non-indigenous plant introduced to Africa from Asia, thrives very well in well-drained soils and grows naturally in the wild and is widely distributed in West Africa, from Senegal to Cameroon (Little, 1983; Michael, 2002). The root decoction is used in folk remedies for abdominal tumors in India. It is also a folk remedy for anasarca, anthrax, bilious disorder, bites, blood disorder, cholera, convulsions, delirium, diarrhea, dropsy, dyspepsia, epilepsy, fever, gout, headache, hemorrhage, intoxication, madness, rat bite, rheumatism, rinderpest, septicemia, small pox, snake bite, sores, sore throat, stomachic, swelling and urticaria (Duke and Wain, 1981).The fruit is astringent, diuretic and tonic. Ayurvedics prescribe them for alopecia, anemia, consumption, leprosy, strangury, thirst and vaginal discharges. The flower for blood disorders and leprosy, the root is antihelmintic, laxative, burning sensations, fever, hallucinations, piles and urinary discharges (Duke, 1984). The leaves are used for treatment of diarrhea, high blood pressure, malaria, scorpion and insect stings (Michael, 2002). Enteric infec-tions are common types bacterial infections accounting for reasonably high cost of health care expenditure and affect people of all races, ages and strata. Most of these bacteria are resistant to many antibiotics and non-antibiotic antimicrobial agents.

The prevalence of such infectious diseases, particularly diarrhea and dysentery, occasioned by poor living conditions, poverty and ignorance, makes the assay of this medicinal plant all the more important. The aim of this study was to examine the effects of the crude extracts of *G. arborea* leaves and stem bark on some pathogenic microorganisms associated with enteric infections. The potency of the crude extracts was compared to that of the standard antibiotic tetracycline.

MATERIALS AND METHODS

Collection and preparation of plant materials

Fresh leaves and bark of *G. arborea* were collected from Ussa town, Ussa local government area of Taraba State, Nigeria and were authenticated by Mr. O F Jatau of the Department of Forestry and Wildlife Management, Federal University of Technology, Yola, Nigeria. The freshly collected leaves and barks were air-dried under shade (room temperature) to constant weight and then pounded separately using mortar and pestle into smaller particles and later reduced to powder using electric blender (Kenwood). The powdered samples were stored in airtight containers and kept at room temperature until required.

Test organisms

Five clinical isolates of gram-negative enterobacteriaceae namely; *Escherichia coli, Klebsiella pneumoniea, Proteus mirabilis, Shigella dysenteriea* and *Salmonella typhi* were obtained from the Federal Medical Center, Yola Adamawa State, Nigeria. Identity of organisms was further confirmed using standard biochemical tests (Cheesbrough, 2002) in the Microbiology Laboratory of the Federal University of Technology, Yola and purity checked at regular intervals (El-Mahmood and Ameh, 2007).

Extraction procedure

The air-dried powdered plant samples (10.0 g of each) were soaked separately in 100 ml of distilled water, ethanol, acetone, chloroform and hexane respectively in a 500 ml sterile conical flask for 48 h at ambient temperature (35°C) with vigorous shaking at 3 h intervals. The crude extracts were then filtered first using muslin cloth and then using Whatman No. 1 filter paper. Each of the filtrates was evaporated to dryness and the dried substance stored in airtight bottles until required (Odebiyi and Sofowora, 1978).

Determination of antimicrobial susceptibility of the plant extracts

The agar diffusion method as by Lino and Deogracious (2006) was used for this purpose. Briefly, 1 ml of 18 h test culture adjusted to 0.5 McFarland (Baker and Thornsberg, 1983; NCCLS, 1990) was placed into a sterile plate and 19 ml molten agar at 45°C dispensed and the plate shaken for even spread and proper mixing of organisms and agar. The agar was allowed to solidify after which, wells (6 mm in diameter and 2.5 mm deep) were made on the surface of the agar medium using a sterile cork borer and various concentrations (5.0, 12.5, 25 and 50 mg/ml) of each of the extracts (0.5 ml) in distilled water were dispensed in each well. Chloramphenicol (12.5 mg/ml) in distilled water and sterile glycerol dispensed in two separate wells were used as positive and negative controls respectively. The culture plates were then incubated at 37°C for 24 h. Antimicrobial activity was determined by measurement of the diameter of zone of inhibition (mm).

Phytochemical screening of the plant extracts

Phytochemical screening of leaves and stem bark of *G. arborea* for bioactive components; saponins, alkaloids, phenolics, tannins, cardiac glycosides and anthraquinones was carried out as described by El-Mahmood and Ameh (2007).

Effects of temperature and pH on activity of plant extracts

The effect of temperature on the antimicrobial activity of the extracts was determined at 4 (refrigeration), 60,100°C and at ambient temperature (control) and the pH range of 2, 5 and 10 as described by Doughari et al. (2007). The untreated extract (pH 4.3) was used as control.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts

The minimum inhibitory concentration was determined by the macro broth dilution methods (NCCLS, 1993; Okeke et al., 2001) at various concentrations (6.5 - 500 mg/ml). Serial two-fold dilutions of each of the extracts was made in nutrient broth and duplicate tubes of each dilution were incubated with 1.0 ml (10^8 cfu/ml) of test organism and incubated at 37°C for 24 h. Test tube containing solutions of tetracycline (6.5 mg/ml) was used as positive control,

Table 1. Phytochemical components of extracts of *G. aborea*.

Chemical constituent	Leaves	Stem bark
Saponins	+	+
Alkaloids	+	+
Phenolics	+	-
Tannins	+	+
Cardiac glycoside	-	+
Anthraquinones	+	-

- = absent, + = present.

Table 2. Antimicrobial activity of *G. arborea* (Roxb) extracts (12.5 mg/ml) against the test bacteria.

Organisms	Zone of inhibition (mm)									
	Leaf extracts					Stem bark extracts				
	AE	EE	HX	CHL	Tet	AE	EE	HX	CHL	chlo
E. coli	11.0	13.0	3.0	18.0	24	6.0	9.0	4.0	10.0	24.0
K. pneumoniae	7.0	10.0	5.0	11.0	22	3.0	7.0	3.0	6.0	23.0
P. mirabilis	11.0	13.0	9.0	10.0	21	3.0	8.0	2.0	6.0	21.0
S. dysentriae	10.0	17.0	4.0	11.0	23	5.0	10.0	-	11.0	25.0
S. typhi	12.0	11.0	4.0	13.0	26	4.0	10.0	-	10.0	24.0

AE = aqueous extracts; EE = ethanol extracts; HX = hexane extracts; CHL = chloroform extracts; chlo = chloramphenicol (12.5 mg/ml).

while distilled water was used as negative control. The lowest concentration of extracts that did not permit any visible growth was considered as the MIC.

For the determination of the MBC, 2 loops full of test culture was taken from each of the broth tubes that showed no growth and inoculated into fresh agar plates and the plates were then incubated at 37°C for 24 h. The concentration of the extracts that showed no growth after incubation period was recorded as the MBC.

RESULTS

Phytochemical screening of the leave plant extracts revealed the presence of carbohydrates, alkaloids, phenols, tannins, saponins, anthracenes and cardiac glycosides (Table 1). Anthraquinone however was not detected in the stem bark extract. Results showed that both the leaf and stem bark extracts demonstrated antimicrobial activity against the test organisms (Table 2). *S. typhi* was the most susceptible of all the test bacteria (12.0 mm zone diameter of inhibition) while *K. pneumoneae* was the least susceptible (7.0 mm zone diameter of inhibition).

The activities of all the extracts was however less than those of the antibiotics. Results also revealed that for all the solvents used, chloroform extracts demonstrated the highest activity (17 mm zone diameter of inhibition) followed by the ethanol extracts (13 mm zone diameter of inhibition) and aqueous extracts (12 mm zone diameter of inhibition). The hexane extracts demonstrated the least activity (9 mm zone diameter of inhibition) against the test bacteria at the tested concentrations (50 mg/ml) (Table 2). Results showed that the activities of the extracts against the test organisms decreased as the pH was adjusted from acidic (pH 2) to alkaline (pH 10) (Table 3) but increased with rise in temperature (Table 4). At pH 2, the activity of the stem bark extracts against *E. coli* was 22 mm (zone diameter of inhibition) and at pH 10 the activity reduced to 12 mm. For *S. typhi,* at pH 2 and 4.2 (untreated), the activity of the extracts was 19 mm which also reduced to 16 mm at pH 10.

The antimicrobial activity followed a similar trend for all the other 4 test organisms (Table 3). For temperature effect, at 4°C and at ambient temperature (35°C) the activity against *E. coli* (for the leaf water extracts) increased from 9.0 (zone diameter of inhibition), to 11 and to 13.0 mm (stem bark water extracts) res-pectively at 100°C (40 mg/ml). For the ethanol extracts, the activity of the stem bark extract against *E. coli* was 14.0 mm at 4°C and at ambient temperature, but slightly increased to 19.0 mm at 100°C (Table 4).

The MIC and MBC values of the leaf extracts ranged between 100 - < 200 (aqueous extracts), 12.5 - 200 (ethanol extracts), 25 - 50 (chloro-form extracts) and < 200 for hexane extracts, while for the stem bark extracts the MIC and MBC values ranged between 100 – 200 (aqueous extracts), 12.5 -200 and 50 - < 200 for

Table 3. Effect of pH on antimicrobial activity of *G. arborea* extracts (12.5 mg/ml).

Organisms	Zone of inhibition (mm)															
	Leaf extracts															
	AE				EE				HX				CHL			
	pH 2	pH 5	pH 10	*pH 4.3	pH 2	pH 5	pH 10	*pH 4.3	pH 2	pH 5	pH 10	*pH 4.3	pH 2	pH 5	pH 10	*pH 4.3
E. coli	22.0	22.0	17.0	22.0	24.0	24.0	15.0	24.0	15.0	15.0	15.0	16.0	20.0	20.0	17.0	20.0
K. pneumoniae	21.0	21.0	19.0	21.0	24.0	24.0	14.0	24.0	16.0	17.0	14.0	17.0	20.0	21.0	18.0	22.0
P. mirabilis	20.0	20.0	17.0	20.0	23.0	23.0	17.0	23.0	18.0	18.0	17.0	16.0	20.0	20.0	17.0	23.0
S. dysenteriae	20.0	20.0	14.0	20.0	24.0	22.0	15.0	22.0	16.0	16.0	13.0	15.0	21.0	21.0	16.0	23.0
S. typhi	21.0	21.0	16.0	21.0	22.0	21.0	16.0	21.0	14.0	15.0	8.0	13.0	20.0	21.0	14.0	21.0
	Stem bark extracts															
	AE				EE				HX				CHL			
	pH 2	pH 5	pH 10	*pH 4.3	pH 2	pH 5	pH 10	*pH 4.3	pH 2	pH 5	pH 10	*pH 4.3	pH 2	pH 5	pH 10	*pH 4.3
E. coli	20.0	20.0	15.0	20.0	21.0	22.0	12.0	22.0	16.0	15.0	15.0	15.0	19.0	19.0	16.0	19.0
K. pneumoniae	19.0	19.0	16.0	18.0	21.0	20.0	14.0	21.0	14.0	16.0	12.0	16.0	20.0	21.0	17.0	21.0
P. mirabilis	20.0	20.0	16.0	18.0	24.0	22.0	15.0	23.0	14.0	15.0	16.0	14.0	21.0	21.0	15.0	21.0
S. dysenteriae	20.0	19.0	13.0	19.0	22.0	21.0	13.0	21.0	14.0	14.0	13.0	14.0	21.0	22.0	18.0	22.0
S. typhi	18.0	17.0	11.0	17.0	19.0	18.0	16.0	19.0	16.0	15.0	16.0	15.0	19.0	21.0	16.0	20.0

* = pH normal of untreated extract. AE = aqueous extracts; EE = ethanol extracts; HX = hexane extracts; CHL = chloroform extracts.

Table 4. Effect of temperature (°C) on antimicrobial activity of *G. arborea* extracts (12.5 mg/ml).

organisms	Zone of inhibition (mm)															
	Leaf extracts															
	AE				EE				HX				CHL			
	4	60	100	*35	4	60	100	*35	4	60	100	*35	4	60	100	*35
E. coli	9.0	10.0	13.0	9..0	14.0	16.0	19.0	14.0	8.0	10.0	13.0	8.0	14.0	16.0	18.0	14.0
K. pneumoniae	8.0	11.0	14.0	8.0	13.0	16.0	18.0	13.0	10.0	12.0	15.0	10.0	15.0	16.0	17.0	15.0
P. mirabilis	7.0	10.0	13.0	7.0	15.0	15.0	18.0	15.0	7.0	7.0	17.0	7.0	13.0	16.0	19.0	13.0
S. dysenteriae	12.0	12.0	15.0	12.0	12.0	14.0	17.0	12.0	-	10	14.0	-	13.0	15.0	20.0	13.0
S. typhi	12.0	12.0	14.0	12.0	14.0	16.0	19.0	14.0	-	10	16.0	-	13.0	16.0	18.0	13.0

Table 4. Cont'd.

	Stem bark extracts															
	AE				EE				HX				CHL			
	4	60	100	*35	4	60	100	*35	4	60	100	*35	4	60	100	*35
E.coli	7.0	9.0	12.0	7.0	15.0	16.0	19.0	15.0	7.0	7.0	13.0	7.0	13.0	15.0	19.0	13.0
K. pneumoniae	8.0	12.0	14.0	8.0	14.0	15.0	17.0	14.0	9.0	9.0	14.0	9.0	13.0	16.0	19.0	13.0
P. mirabilis	7.0	10.0	14.0	7.0	15.0	15.0	18.0	15.0	5.0	5.0	12.0	5.0	12.0	13.0	18.0	12.0
S. dysenteriae	8.0	9.0	13.0	8.0	15.0	15.0	17.0	15.0	8.0	8.0	13.0	8.0	13.0	15.0	17.0	13.0
S. typhi	9.0	10.0	15.0	9.0	14.0	15.0	17.0	14.0	6.0	6.0	15.0	6.0	12.0	13.0	17.0	12.0

*= ambient temperature; AE = aqueous extracts; EE = ethanol extracts; HX = hexane extracts; CHL = chloroform extracts

both chloroform and hexane extracts. The ethanol extracts of both plant parts demonstrated the lowest MIC and MBC values with the least values (MIC 12.5, MBC 50 mg/ml) demonstrated against *P. mirabilis* and *S. dysenteriae* by the leaf extracts and the stem bark extracts respectively, while tetracycline demonstrated the lowest values of MIC and MBC (6.5 mg/ml each) (Table 5).

DISCUSSION

The use of medicinal herbs in the treatment and prevention of infectious diseases has attracted the attention of scientists worldwide (Akachuku, 1981; Barry and Thornsberry, 1985; Adebahusi, 1993; Cimanga et al., 1998; Falodun et al., 2006). Phytochemical screening of the plant leaves and stem bark indicated the presence of some bioactive components. Results showed that plant leaves contained saponins, carbohydrates, alkaloids, phenolics, tannins and anthraquinones but no cardiac glycosides, while the stem bark possessed saponins, carbohydrates, alkaloids, tannins and anthraquinones but no phenolics and anthraquinones (Table 1). The presence of these phytochemical compounds is linked to biological activity, such as protection of the plant against infections (Damitoti et al., 2005; Samie et al., 2005). The presence of these glycoside moieties, some of which are known to be structurally and chemically related to sex hormones (oestrogens, gestrogens and androgens known to inhibit tumor growth) and flavonoids protects against gastric infections (Eberia et al., 1991; Ijeoma and Umar, 1997). The presence of alkaloids in this plant is also of great importance to humans because of their medicinal values as significant quantities are used as anti-malarial, analgesic and as stimulants. This therefore gives credence to some of the ethnomedical uses of the plant (Okeke et al., 2001, Michael, 2002). Many studies have established the usefulness of medicinal plants as a great source for the isolation of active principles for drug formulation (Falodun et al., 2006; Elujoba, 1996; Banso and Mann, 2006; El-Mahmood and Ameh, 2007). Emetine - a potent amoebicidal and quinine - a potent antimalarial agent, were isolated from plants. Physiostigmine, another potent drug isolated from Calabar bean is an effective drug in the management of glaucoma (Ndukwe et al., 2005, 2007). *G. arborea* plant extracts demonstrated antimicrobial activity against *E. coli, K. preumoniae, P. mirabilis*, *S. dysentriae* and *S. typhi* (Table 2). These are a group of enteric bacteria that cause diseases like diarrhea (Samie et al., 2005) dysentery and other stomach abnormalities (Jawetz, 2002). Kilani (2006) reported that methanol extracts of *Vitex doniana* (Verbeneceae) stem bark possessed antimicrobial activity against the most prevalent enteric pathogenic organisms (*S. typhi, S. dysentriae* and *E. coli*). *G. arborea* belong to the same family (Verbenanceae) as *V. doniana* and probably could be the reason why it showed antimicrobial activity against the same enteric pathogenic organisms as *V. doniana.* Results also showed that the leaf extracts had higher activity than the stem bark extracts (Table 2). This also agrees with the reports of Banso and Olatimeying (2001). They also reported that leaves tend to show higher activity than the bark and roots of most medicinal plants. The chloroform extracts demonstrated the highest activity while the hexane extracts demonstrated the lowest activity among other extraction solvents used. The hexane extract of stem bark had no activity on *S. dysentriae* and *S. typhi.* Extractability of phytoconstituents differs from solvent to solvent depending on their polarities. This accounts for the differences in activity of the various solvent extracts. Tetracycline (positive

Table 5. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (mg/ml) of extracts of *G. arborea* (Roxb).

Organisms	Zone of inhibition (mm)															
	Leaf extracts								Stem bark extracts							
	AE		EE		HX		CHL		AE		EE		HX		CHL	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. coli	100	200	50	100	< 200	< 200	25	50	200	< 200	50	50	100	200	200	<200
K. pneumoniae	50	50	100	50	< 200	< 200	25	50	100	200	25	25	100	200	100	200
P. mirabilis	100	100	50	100	< 200	< 200	25	50	200	200	12.5	50	50	100	100	200
S. dysentriae	100	100	50	50	< 200	< 200	25	25	100	200	12.5	50	100	200	50	200
S. typhi	100	200	50	50	< 200	< 200	50	50	200	200	100	200	100	<200	50	200
Tetracycline (control)	6.5 mg/l															

<200 = above 200 mg/ml; AE = aqueous extracts; EE = ethanol extracts; HX = hexane extracts; CHL = chloroform extracts.

control) demonstrated the highest activity against all the test bacteria (activity ranging between 21.0 and 26.0 mm zone diameter of inhibition, MIC and MBC values of 6.5 mg/ml respectively) compared to the plant extracts. Effect of pH on activity indicated that test organisms were more susceptible to acidic pH of the crude plant extracts than to the alkaline pH of the same crude extracts (Table 3). At acidic pH of 4 (normal pH of extract), aqueous, ethanol and chloroform leave extracts all showed higher activity ranging between 20.0 and 24.0 mm (zone diameter of inhibition) comparable to those of tetracycline for all test organisms. Results also showed that increase in temperature of the crude extracts increased the activity of the crude plant extracts (Table 4). This could suggest the reason why traditional healers often boil plant extracts before they are dispensed to sick persons. Some of the MIC and MBC values of the plant extracts are low (12.5 - 100 mg/ml), an indication that the constituents of the plant have therapeutic properties. The values also showed that the extracts demonstrated both static and cidal affects against the test bacteria (Table 5). For instance both the MIC and MBC values of the ethanol leave extracts against *K. preunnoniae* was 25 mg/ml indicating a bacteriostatic effect, while the respective values for the stem bark extracts against *S. dysenteriae* were 12.5 and 50 mg/ml respectively indicating a bactericidal effect. This is a strong indication that *G. arborea* is a medicinal plant and agrees with the literature review which suggested that the plant serves folk remedies for various ailments including those associated with gastro intestinal tract infections (Duke and Wain, 1981; Ndukwe et al., 2005, 2007; Doughari et al., 2007).

Conclusion

G. arborea (Roxb) demonstrated antimicrobial activity against some members of the entero-bacteriaceae associated with infectious diseases (Prescott et al., 2002; Nester and Pearsall, 2004), an indication of therapeutic potential against enteric gram-negative pathogenic bacteria and therefore can be used to treat infections such as gastroenteritis, dysentery, typhoid fever and shigellosis caused by these pathogens. It also suggests that the plant could be a source of antimicrobial and chemotherapeutic substances for drug development for the treatment of infectious diseases and supports its use by traditional medicine practitioners in the treatment of diarrhea and dysentery. Further studies on the plant alongside clinical trials to determine the potency of the plant as antimicrobial agents and studies on the efficacy of the plant against other forms of diseases like ulcer, high blood pressure and some viruses causing diseases like hepatitis B which are major killers in recent years should be carried out. Large production of the crude plant drug and more investments in research on the plant should be encouraged by the Federal Government.

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