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Screening of *Alafia multiflora* for Antibacterial, Antiradical Activity and LD_{50} Investigation

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Abstract: The purpose of this investigation deals with the antibacterial, antiradical activities and the toxicity (LD_{50} and haematological parameters) of *Alafia multiflora* stem barks. *A. multiflora* is a medicinal plant known for its curative effects on ulcerous wounds. Antibacterial activities of crude extracts were evaluated against *E. coli*, *S. aureus*, *E. agglomerans*, *P. aeruginosa*, *P. vulgaris*, *K. Pneumoniae* and the inhibitory zones as well as the Minimum Inhibition Concentrations (MIC) determined. Antiradical activity and total phenolic compounds of the extracts were evaluated. Methanol, methylene-chloride/methanol and aqueous extracts inhibited the growth of all the test microorganisms with MIC values ranged from 2.5 to 40 mg mL^{-1}. The best antiradical activity was obtained at 0.25 mg mL^{-1}. These results suggest that *A. multiflora* possess antibacterial and antiradical constituents. Aqueous and the methanol extracts administered in single oral doses of 0, 2.5, 5, 7.5 and 10 g kg^{-1} to groups of 10 rats (5 males, 5 females) yielded no mortality after 7 days observation. The LD_{50} values of the tested extracts were found to be above 5000 mg kg^{-1}, indicating that *A. multiflora* is rather poorly toxic. A significant increase of platelet count was observed in animals treated with aqueous and methanol extracts at 5 to 10 g kg^{-1}.

Key words: *Alafia multiflora*, antibacterial, antiradical, phenols, LD50 estimation

INTRODUCTION

Alafia multiflora Stapf is a large liana of Apocynaceae family. It is a multiple usage medicinal plant distributed in West and Central Africa. In Ghana, the latex diluted with water is taken orally to cure stubborn wounds (Tsala and Dimo, 2006; Abbiw, 1990). The latex mixed with bark scrapings is applied to wounds and leg ulcers and to ulcers caused by syphilis. The seeds are also used as an ingredient of arrow poison in the Democratic Republic of Congo (Tsala and Dimo, 2006; Neuwinger, 2000). In Cameroon the fresh latex, either alone or mixed with *Oncinotis glabrata* (Baill.) Stapf ex Hiern, is also applied to treat yaws. The stem bark or fruits decoction are taken to relieve abdominal pain. According to Farrell *et al.* (1991) latex and resin from *A. multiflora* may protect itself from pathogens by sanitizing and sealing wounds. Balansard *et al.* (1980) found that vanillic acid isolated from the latex possesses antibacterial properties, while Pais *et al.* (1971) isolated alafine (a pyrrolizidine alkaloid) from the seeds as an ester of syringic acid. There is a considerable evidence indicating association between reactive oxygen species and the physiopathology of chronic diseases such as diabetes, hypertension and cancer, aging, burns and chronic wounds (Beswick *et al.*, 2001; Tomasz, 2002; MacKay and Miller, 2003; Telgenhoff and Shroot, 2005). In order to protect themselves against free radicals, living organisms are endowed with defence systems implicating enzymes (catalase, superoxide dismutase, glutathione peroxidase/reductase) and exogenous bio-molecules (Vitamin C and E, β-carotene) and metabolite by-products (bilirubine, uric acid) (Llesuy *et al.*, 2001; Javanmardi *et al.*, 2003). The antioxidant potential of plants extracts is mainly related to the phenolic components, such as flavonoids, phenolic acids and phenolic diterpenes (Javanmardi *et al.*, 2003). Antioxidants inhibit or delay membrane peroxidation and can be expected to prevent cellular senescence in wound

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healing and chronic diseases (Telgenhoff and Shroot, 2005; Mahmood *et al*., 2005; Shivananda *et al*., 2006). Giving that *A. multiflora* is used for wound healing and demonstrated protective effect against CCl_4-induced oxidative stress in rats (Dimo *et al*., 2006). The aim of this study was to investigate the antibacterial activity, free radical scavenging effect and LD_{50} of various extracts of *A. multiflora*.

MATERIALS AND METHODS

Plant materials and preparation of extracts: Fresh stem barks were collected in May, in the village Nkolntara, near Yaoundé city (Central Province of Cameroon) and authenticated at the National Herbarium-Yaoundé, where the voucher specimen was conserved under the reference number 43196/HNC. The above plant parts were dried at room temperature, ground into a powder 5.5 kg was macerated in a mixture of methylene-chloride/methanol/ (1:1) (CM) for 48 h. The supernatant was evaporated at 80°C in a vacuum desicator to obtain 50 g of brown extract. For methylene-chloride (C) and Methanol (M) extracts, 15 g of CM extract was further fractionated in methanol to obtain 5.5 g methanol extract and 9.23 g methylene chloride extract. The hexane extract was prepared as follows: 5 g of the C were dissolved in hexane. This was mixed and filtered. The filtrate was evaporated and the Hexane (H) extract obtained (0.9 g) was used. The aqueous (W) extract was obtained by adding 5 L of distilled water to 1 kg of the fine powder. The mixture was allowed to stand and macerated at 50°C. The resulting infusion was filtered after 2 h and lyophilised to obtain 21, 17 g of a brown extract.

Antibacterial activity

Test organisms: Six species of microorganisms namely *Staphylococcus aureus*, *Proteus vulgaris* (Gram positive bacteria), *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus agglomerans* (Gram negative bacteria) were used in this study. They were all clinical isolates obtained from Centre Pasteur du Cameroun (Yaoundé). The identity of the strains was confirmed using the standard biochemical methods (Cheesbrough, 2000). The test organisms were maintained on agar slant at 4°C and were subcultured on the fresh appropriate agar plate 24 h prior to any antimicrobial test.

Culture media: Muller Hinton Agar (MHA) was used for the diffusion assays and Nutrient broth containing 0.05% phenol red and supplemented with 10% glucose (NBGP) was used for MIC determination.

The agar-well diffusion test: The antimicrobial diffusion test was carried out as described by Candan *et al*. (2003) using a cell suspension of about $1.5\ 10^6$ cfu mL^{-1} obtained from a McFarland turbidity standard N° 0.5. The suspension was standardised by adjusting the optical density to 0.1 at 600 nm (SHIMADZU UV-120-01 spectrophotometer) (Tereschuck *et al*., 1997). This was used to inoculate by flooding the surface of MHA plates (90 mm ø). Excess liquid was air-dried under a sterile hood. Wells of 6 mm were bored into the agar plate using the bold end of a pipette tip and then 75 µL of extract of desire concentration was dropped into the wells. A well prepared with only the corresponding volume of nutrient broth was used as negative control. The plates were incubated at 37°C for 24 h. Antimicrobial activity was evaluated by measuring the diameter of the Inhibition Zone (IZ), around the well. Extracts with IZ > 7 mm was considered.

Tube dilution test: MICs of test samples found active by the diffusion test as well as the MICs of the reference antibiotic (gentamycin) were determined as follows: extract containing NBGP prepared at the concentration of 160 mg mL^{-1} was serially diluted two-fold to obtain concentrations range of 1.25 to 160 mg mL^{-1} in a total volume of 5 mL. Gentamycin containing NBGP (156.25 µg mL^{-1}) was also diluted the same way to obtain concentrations range of 0.0625 to 8 mg mL^{-1}. Each tube, as well as the negative control (extract-free) was inoculated with 50 µL of standard inoculum of test organism (approximately 5×10^6 cells) (Camporese *et al*., 2003) and incubated at 37°C for 24 h. Microbial growth was determined by observing the change of colour in the tubes which change to yellow when there is growth. The lowest concentration showing no colour change was considered as the MIC. The results recorded for each bioassay was the average of a simultaneous duplicated test.

Free radical-scavenging activity determination: The free radical scavenging activity of the extracts was measured by DPPH, according to the previously described technique (Brand-Williams *et al*., 1995). The calculation of the DPPH inhibition was done by Qian and Nohinbere (2004) method.

Total soluble phenolic compounds: The total soluble phenolic compounds present in the different plant extracts was determined according to the method described by Singleton *et al*. (1999). The results recorded for each bioassay was the average of simultaneous triplicate test.

LD_{50} investigation and haematological function: Toxicological assessment. The test was carried out following the methods described by WHO (1992). Five groups of 10 inbred Wistar rats weighing between 70 and 120 g (5 males and 5 females), were constituted based on the administered doses, i.e. 0, 2.5, 5, 7.5 and 10 g kg^{-1}. The animals were maintained on a 12 h light/dark cycle with water *ad libitum* and fed with laboratory baked food made of maize (50%), soybeans (25%) and wheat flour (25%), supplemented with table salt, palm oil, fish and bone powder. They were deprived of food 18 h prior to administration of the extracts. Prior authorization for the use of laboratory animals was obtained from the Cameroon National Ethics Committee (Reg. N° FWA-IRB00001954). The test animal received intragastrically single oral doses of aqueous and methanol extracts of *A. multiflora* and were under close observation for 72 h after dosing. The weight, food and water intake was also recorded for 7 days. At the end of this period, animals were sacrificed by decapitation and the blood was collected for the determination of haematological parameters. The relative weights of the liver, the kidneys, the heart and the lung were also determined.

Haematological analysis: Haematological parameters were determined using an HUMACOUNT HUMAN GmbH haematological analyser. They included haemoglobin concentration, red blood cells and white blood cells count, differential white blood cells count, haematocrit, mean cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and platelet count.

Statistical analysis: The one way Analysis of Variance (ANOVA) and Student-Neuman-Keuls multiple comparison tests of the SPSS program was used to determine statistical differences between treatments. $p<0.05$ was considered to be statistically significant. Data are expressed as mean±SEM.

RESULTS

Antibacterial activity: Out of six plant extracts screened, three (CM, M and W extracts) inhibited the growth of test organisms in the Petri dishes (agar-well diffusion test) the (Table 1). The diameter of the IZ ranged from 10 to 23 mm. M extract showed a greater inhibiting activity compared to CM extract. The lowest IZ was obtained with W extract on *S. aureus*, while the highest IZ value was obtained with the M extract on *K. pneumoniae*. The C extract was found to be not active against *S. aureus*, *P. vulgaris*, *P. aeruginosa*, while the hexane extract was not inhibitory to any of the test organisms (Table 1). Three extracts (CM, M and W) inhibited like gentamycin, the growth of all the six tested bacteria. The results indicated a stronger activity of the M extract against all the bacteria tested, with Minimum Inhibitory Concentrations (MICs) ranging from 2.5 to 10 mg mL^{-1} (Table 2).

Antiradical activity and total soluble phenolic compounds: The results obtained showed that DPPH solution was bleached with all the samples tested. However, differences could be observed between the different antiradicals used depending to their concentrations. As indicated on

Table 1: Inhibition zones of various extracts of *Alafia multiflora*

	Inhibition zones (mm)[a]						
Microorganisms	W_{20}	$C\text{-}M_{20}$	$C\text{-}M_{40}$	C_{20}	M_{20}	H_{20}	$G_{0.0125}$
Escherichia coli	12	11	15	13	16	na	26
Staphylococcus aureus	10	na	15	na	16	na	18
Enterococcus agglomerans	16	15	17	12	15	na	20
Pseudomonas aeruginosa	17	na	20	na	21	na	26
Proteus vulgaris	19	17	13	na	21	na	26
Klepsiella pneumoniae	14	12	17	17	23	na	27

W = Aqueous extract; C-M = Methylene-chloride-methanolic extract; C = Methylene-chloride extract; M = Methanolic extract; H = Hexane extract; G = Gentamycin; na = Not active [a]. Diameter of inhibition zone including diameter of well. The number is the concentration at which the extracts have been tested (mg mL^{-1})

Table 2: Minimum inhibitory concentration of various extracts of *Alafia multiflora*

	Minimum inhibitory concentration (mg mL^{-1})					
Microorganisms	W	C-M	C	M	H	G
Escherichia coli	20	5	na	2.5	na	≤0.0625
Staphylococcus aureus	40	40	20	10.0	na	≤0.0625
Enterococcus agglomerans	10	10	20	2.5	na	≤0.0625
Pseudomonas aeruginosa	10	20	10	2.5	na	≤0.0625
Proteus vulgaris	20	20	na	2.5	na	≤0.0625
Klepsiella pneumoniae	20	20	20	2.5	na	≤0.0625

W = Aqueous extract; C-M = Methylene-chloride-methanolic extract tested; C = Methylene-chloride extract; M = Methanolic extract; H = Hexane extract tested at 20 mg; G = Gentamycin (na = Not active[a])

Table 3: Antiradical activity of gallic acid and *Alafia multiflora* extracts (% inhibition of DPPH) and total soluble phenols, using gallic acid as standard)

	Inhibition of DPPH (%)					
Concentration (mg mL^{-1})	GA	W	C-M	C	M	H
0.05	96.52	59.76	73.12	44.51	45.58	23.52
0.1	96.96	69.25	75.80	46.65	61.49	25.93
0.20	96.52	73.66	80.48	70.32	86.76	32.48
0.25	94.58	73.26	82.21	70.85	80.74	39.43
Total soluble phenols (GAE mg^{-1})	47.14±0.01	54.17±0.00	60.67±0.00	69.83±0.00	22.47±0.01	47.14±0.01

W = Aqueous extract; C-M = Methylene-chloride-methanolic extract; C = Methylene-chloride extract; M = Methanolic extract; H = Hexane extract

Table 4: Mean organs relative weight (mg g^{-1} body weight) of rats treated with various acute doses (2.5-10 g kg^{-}) of aqueous extract of *Alafia multiflora*

Dose of extract ($g\ kg^{-1}$)	Sex	Liver	Kidneys	Heart	Lungs	Testicles/ovaries)
0	M	36.70±1.52	8.60±0.23	4.29±0.09	9.81±0.64	12.01±0.76
	F	43.54±1.45	9.04±0.23	5.09±0.16	8.34±0.17	0.56±0.04
2.5	M	39.51±1.85	7.71±0.20	3.79±0.05	10.09±0.46	18.17±1.01
	F	37.98±0.89[ad]	7.55±0.29[a]	5.27±1.45	12.07±0.22[d]	1.75±1.14
5	M	39.91±1.07	8.26±0.36	4.15±0.18	10.16±0.35	17.82±0.53
	F	38.50±1.30[ad]	7.65±0.23[a]	3.76±0.08	9.40±0.78[d]	0.81±0.03
7.5	M	35.29±1.36	7.81±0.33	3.73±0.14	11.09±0.89	20.30±0.98[a]
	F	42.80±1.36[d]	8.01±0.26[a]	3.89±0.21	7.88±0.27	0.53±0.05
10	M	34.95±0.53	8.37±0.51	3.95±0.14	10.61±0.20	21.66±0.56[a]
	F	37.43±0.41[ad]	7.44±0.27[a]	4.11±0.33	9.67±0.17[d]	0.60±0.04

[a]: Indicates a statistically significant difference (p = 0.05) as compared to dose 0 g kg^{-1} (n = 5). [d]: Indicates a statistically significant difference (p = 0.05) as compared to dose 7.5 g kg (n = 5)

Table 5 : Mean organs relative weight (mg g^{-1} body weight) of rats treated with various acute doses (2.5-10 g kg^{-1}) of methanol extract of *Alafia multiflora*

Dose of extract ($g\ kg^{-1}$)	Sex	Liver	Kidneys	Heart	Lungs	Testicles/ovaries
0	M	36.70±1.52	8.60±0.23	4.29±0.09	9.81±0.64	12.01±0.76[c]
	F	43.52±43.54	9.01±9.04	5.09±0.16	8.30±0.34	0.56±0.56
2.5	M	47.71±3.20[a]	8.74±0.27	4.99±0.31	8.13±0.74	11.69±1.33[c]
	F	42.79±1.13	8.61±0.26	4.40±0.09	9.22±0.13	0.46±0.03
5	M	42.05±0.75	6.95±0.22[ab]	4.22±0.14	8.61±1.05	12.99±0.80[c]
	F	42.69±0.43	7.14±0.06[ab]	4.17±0.20	9.95±0.76	0.64±0.03
7.5	M	44.44±1.22	7.28±0.13[ab]	4.58±0.32	8.74±0.49	14.65±0.70[c]
	F	46.34±1.56	7.74±0.30[ab]	4.67±0.24	8.88±0.32	0.68±0.05
10	M	49.15±3.01[a]	9.90±0.64[bcd]	4.89±0.22	9.57±0.26	24.47±2.11
	F	48.35±5.32	7.66±0.20[ab]	3.83±0.3	8.08±0.17	0.46±0.09

[a]: Indicates a statistically significant difference (p = 0.05) as compared to dose 0 g kg^{-1}; [b]: Indicates a statistically significant difference (p = 0.05) as compared to dose 0 and 2.5 g kg^{-1}; [c]: Indicates a statistically significant difference (p = 0.05) as compared to dose and 10 g kg^{-1} (n = 5)

Table 3, DPPH inhibition was greater for the gallic acid compared to that of *A. multiflora* extracts. All the extractives exhibited a range of antiradical activity above 60% for W, CM, C and M extracts, except the H extract. Total phenolic content values were evaluated in term of gallic acid equivalent mg^{-1} (GAE mg^{-1}) of extract used. The total amount of phenolic groups was higher in the M extract (69.83) and the C extract (60.67), the CM extract (54.17), the W extract (47,14) and the H extract (22.47) (Table 3).

Toxicological assessment: Oral administration of 0, 2.5, 5, 7.5 and 10 g kg^{-1} of W and M extracts did not result in animal mortality or important morbidity. Therefore the approximate LD_{50} values estimated using Yamanaka *et al.* (1990) method should be above 5 g kg^{-1}.

Animal weight was not reduced by single dose administration of the extracts within a period of 7 days observation. No significant food and water intake were observed between the treated groups and the control.

Above 2.5 g kg^{-1}, W extract led to a significant ($p<0.05$) decrease in liver and kidney relative weight in females and had no effect on the same organs in males (Table 4). M extract increased liver relative weight of males treated only at the dose of 10 g kg^{-1}. A significant ($p<0.05$) decrease of kidney relative weight was obtained with animals treated with 5, 7.5 and 10 g kg^{-1} of the M extract (Table 5). There was no effect on heart relative weight of rats treated with both extracts. Rats treated with 10 g kg^{-1} of M extract had a lung relative weight higher than those of the other treated groups (including the control group). Animal treated with W extract at 7.5 and 10 g kg^{-1} showed a significantly increased ($p<0.05$) testis relative weight. This was also observed with rats treated with 10 g kg^{-1} of M extract.

Haematological parameters recorded in this study are shown in Table 6 (for W extract) and Table 7 (for M extract). There was no significant change in the white blood cells and red blood cells count, haemoglobin concentration and haematocrit. However, both W and M

Table 6: Effect of acute oral doses of *Alafia multiflora* aqueous extract on haematological parameters in rats

Doses (g kg^{-1})	WBC (× 10^9 L^{-1})	LY (%)	MI (%)	GR (%)	RBC (×10^{12}L^{-1})	HGB (g dL^{-1})
0	15.82±2.40	56.08±8.25	2.60±0.63	41.30±7.78	6.880±0.22	10.65±0.315
2.5	11.13±0.80	65.00±3.92	1.40±0.35	29.82±2.78	7.570±0.34	10.94±0.42
5	8.93±1.03	60.02±3.06	1.50±0.32	27.97±2.70	7.150±0.27	10.41±0.40
7.5	7.78±1.64	67.68±0.27	3.72±1.06	28.59±0.84	6.840±0.61	9.30±1.17
10	8.93±0.60	63.28±3.72	1.88±0.54	30.79±3.95	8.022±0.14	11.78±0.19

Doses (g kg^{-1})	HCT (%)	MCH (fL)	TCHC (pg)	MCHC (g dL^{-1})	PLT (10^9 L^{-1})	PCT (×10^9 L^{-1})	MPV (fL)
0	38.42±2.05	55.76±1.46	15.52±0.39	27.90±0.99	312.20±59.95	0.192±0.03	6.85±0.09
2.5	40.12±1.60	55.26±1.27	15.10±0.16	28.44±0.50	1501.50±453.74	1.112±0.19	6.34±0.24
5	36.76±1.27	48.06±0.37	13.64±0.34	26.44±0.97	3998.96±1257.18	1.820±1.11	5.53±0.29
7.5	33.46±3.33	49.20±0.58	13.36±0.56	27.40±0.95	4422.00±1478.48	2.400±0.78	5.42±0.28
10	39.75±0.86	48.80±0.80	14.60±0.18	29.25±0.45	3998.96±1345.18	3.530±1.00	5.53±0.09

Values are mean±SEM (n = 4). WBC = White Blood Cells; LY = Lymphocytes; MI = Monocytes; GR = Granulocytes; RBC = Red Blood Cells; HGB = Haemoglobin; HCT = Haematocrit; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration; PLT = Platelet; MPV = Mean Platelet Volume; PCT = Thrombocrit

Table 7: Effect of acute oral doses of *Alafia multiflora* methanol extract on haematological parameters in rats

Doses (g kg^{-1})	WBC (× 10^9 L^{-1})	LY (%)	MI (%)	GR (%)	RBC (×10^{12} L^{-1})	HGB (g dL^{-1})
0	15.82±2.40	56.08±8.25	2.60±0.63	41.30±7.78	6.88±0.22	10.65±0.31
2.5	12.70±2.17	65.85±4.19	2.45±0.33	31.72±4.52	7.38±0.36	11.74±0.10
5	11.38±1.45	53.17±2.24	3.23±1.06	43.64±3.00	6.77±0.36	10.87±0.37
7.5	9.49±1.29	66.26±5.01	2.83±0.94	30.92±5.84	7.15±0.30	11.29±0.27
10	9.95±0.39	64.76±5.94	1.71±0.62	33.51±5.88	7.75±0.45	11.74±0.44

Doses (g kg^{-1})	HCT (%)	MCH (fL)	TCHC (pg)	MCHC (g dL^{-1})	PLT (10^9 L^{-1})	PCT (X10^9 L^{-1})	MPV (fL)
0	38.42±2.05	55.76±1.46	15.52±0.39	27.90±0.99	312.2±59.95	0.192±0.03	6.85±0.09
2.5	40.28±0.69	51.60±0.60^a	15.00±0.32	27.76±0.37	469.6±49.89^d	2.070±0.19^{ac}	6.72±0.33
5	35.46±1.99	52.76±0.43^{ae}	16.19±0.44	30.95±0.93	682.6±112.99	0.600±0.19	6.86±0.12
7.5	36.06±1.49	50.62±0.25^a	15.31±0.26	30.27±0.61	4450.5±532.11^{abc}	1.790±0.07^{ac}	5.74±0.38^{abc}
10	38.27±2.04	49.60±0.44^a	14.98±0.24	30.31±0.51	4516.0±557.56^{abc}	2.080±0.27^{ac}	5.38±0.11^{abc}

Values are mean±SEM (n = 4). WBC = White Blood Cells; LY = Lymphocytes; MI = Monocytes; GR = Granulocytes; RBC = Red Blood Cells; HGB = Haemoglobin; HCT = Haematocrit; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration; RDW = Red Blood Cells Distribution Width; PLT = Platelet; MPV = Mean Platelet Volume; PCT = Thrombocrit. ^{a}: p<0.05 as compare to dose 0 g kg^{-1}; ^{b}: p<0.05 as compare to dose 2.5 g kg^{-1}, ^{c}: p<0.05 as compare to dose 5 g kg^{-1}, ^{d}: p<0.05 as compare to dose 7.5 g kg^{-1}; ^{e}: p<0.05 as compare to dose 10 g kg^{-1} (n = 4)

extract induced a relative low eosinophils count, an increase lymphocytes and platelets count. Moreover, all the animals treated with methanol M extract showed a significantly increase of platelet count. The mean platelet volume was significantly (p<0.05) increased with animals treated at 7.5 and 10 g kg^{-1}.

DISCUSSION

CM, M and W extracts inhibited the growth of all the pathogens tested. Based on the values of the antimicrobial parameters (IZ, MICs), one could speculate that the stem bark extracts from *Alafia multiflora* would be a useful source for treating ailments caused by the bacteria tested. Since these fractions are not pure, it would be difficult to speculate about the mechanisms of the antimicrobial activities observed. The Present results confirmed the evidence of previous studies reporting that methanol is a better solvent for more consistent extraction of antimicrobial substances from medicinal plants compared to other solvents, such as water, ethanol and hexane (Karaman *et al*., 2003).

It is known that phenolic compounds exercise their antiradical propertied by providing a neutralising OH group that bind to the free radical compound. Therefore the higher the OH group of a given phenolic compound the higher its scavenging potential (Brand-Williams *et al*., 1995). The antiradical activity of extracts from *A. multiflora* was not strictly correlated with the phenol content indicating that other factors playing major roles as antiradicals are involved as suggest by many authors (Llesuy *et al*., 2001; Ilhami *et al*., 2004). In fact the typical phenolics that possess antioxidant activity are known to be mainly phenolics acid (gallic acid, vanillic acid, syringic acid, ascorbic acid, ferrulic acid, coumaric acid, etc.) and flavonoids (Brand-Williams *et al*., 1995; Qian and Nihorimbere, 2004). Phytochemical screening of the extracts used showed the presence of phenols, tannins, flavonoids, anthraquinones and alkaloids in all extracts, except for the lipid rich H extract (unpublished data).

The difficulty of healing observed with ulcerous wound is not only due to the presence of pathogenic bacteria resistant to the host defence mechanisms, but also to excess production of free radicals by the host defence cells which promote cell death and thus the persistence of the wound (Cheesbrought, 2000; Telgenhoff and Shroot, 2005). Gallic acid is known to possess both antibacterial and antiradical properties (Fernandez *et al*., 1996). Furthermore, vanillic acid (Balansard *et al*., 1980) and an ester of syringic acid (Pais *et al*., 1971) were isolated from stem and seeds of *A. multiflora* respectively. Balansard *et al.* (1980) found that the isolated vanillic acid was also highly antibacterial. This justify the more the healing success obtained with the sap and the bark of this plant in the treatment of ulcerous wounds and skin problems.

Giving that the toxicity assays revealed no remarkably behaviour change, no gross pathological change in animals treated with the aqueous and the methanol extracts at doses up to 10 g kg^{-1} and considering the relative low toxicity (LD_{50} > 5 g kg^{-1}, OCED, 2000) the use of these extracts at the therapeutic doses will be safe. A chemical with such low toxicity does not need further testing for lethal effects (Yamanaka *et al*., 1990). The extracts led to a decreased liver and kidney relative weight at higher doses, the methanol extract significantly increased ($p<0.05$) platelets count at doses 7.5 and 10 g kg^{-1}. The mechanism of action of these observed abnormalities was not established. We did not determine if renal injury may develop independently of hepatic events. However, agranulocytosis and hepatorenal toxicity of the *Alafia multiflora* extracts at higher dose represent some adverse effects usually attributed to topical medicines when given orally (Harms and Saurat, 1992). Despite the liver and kidney hypotrophy, no mortality was observed after 7 days in animals treated with up to 10 g kg^{-1}. It is therefore not excluded that these abnormal observations were due solely to the quantity of extract that was given to the animals, since below 5 g kg^{-1} no such abnormalities were obtained. Moreover, according to OCED (2000), LD_{50} of plant extract greater or equal to 5000 mg kg^{-1} is indicative of poor toxicity. This goes further to confirm our assertion that the security margin of *A. multiflora* (toxicity wise) is quite large.

The fact that at 2.5 g kg^{-1} of the aqueous extract generated decrease of toxicity signs (liver and kidney relative weight) in female animals but not in males suggest that the female animals are more sensitive to the eventual toxic principle present in this extract. Further studies are needed to elucidate this observed sex-related side effect. With methanol extract the same abnormal signs were observed in both male and female animals. This suggest that this same toxic agent may be more concentrated in the methanol extract and thus affect both males and females, or it may be here a question of a different principle acting on the same organs.

CONCLUSIONS

The known antimicrobial mechanisms associated to each group of chemical to which the isolated compounds belong and/or others may explain the antibacterial properties of the extracts used in this study. The total phenolic content was higher in the methanol extract which confirms its free radical-scavenging activity. The results of the present study provide an important basis for the use of methanol extract from the stem bark of *Alafia multiflora* for the treatment of infections associated to the microorganisms tested and the management of oxidative stress. Aqueous and methanol extracts of *A. multiflora* showed a wide margin of safety at acute dosage. The induction of liver and kidneys relative weight abnormalities in male and females substantiates an apparent sex difference in tolerance of *A. multiflora* extracts. However, further pharmacological and toxicological studies currently going on in our laboratory may confirm this hypothesis. More data are also needed to determine the active antibacterial and antiradical components of the extracts.

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