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Full Length Research Paper

Evaluation of antibacterial activity of *Synclisa scabrida* for the treatment of bacterial gastroenteritis and other diseases

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Ethanolic, cold water and hot water extracts of the root of *Synclisia scabrida* were tested, by the agar-well diffusion and macro-broth methods, for activity against clinical and typed strains of *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus* sp. and *Bacillus subtilis*. The ethanolic extract was bactericidal at the minimum inhibitory concentration (MIC) against 8 of the 10 test organisms (MIC, 3.125 to 12.50 mg/ml and MBC, 3.125 to 25.00 mg/ml). The cold water extract was bactericidal against *E. coli* (*clin*) and *B. subtilis* ATCC 6051 strains and bacteriostatic against 5 others while the hot water extract was bacteriostatic against 2 of the organisms at MIC. Three column chromatographic fractions of the crude ethanolic extract, F_{ss-1}, F_{ss-2}, and F_{ss-3}, were active against 5 to 8 of the 10 test bacterial strains. F_{ss-3} was bacteriostatic against the *Proteus* sp. (*clin*), which was not susceptible to the crude ethanolic extract. Conversely, the crude ethanolic extract was bactericidal against *P. aeruginosa* ATCC 10145 to which none of the fractions showed activity. Thus, the results justify the folklore application of extracts of *S. scabrida* in treatment of bacterial gastroenteritis among other diseases.

Key words: *Synclisia scabrida*, root extracts, antibacterial activity, Nigeria.

INTRODUCTION

Synclisia scabrida (Meirs) of the family, Menispermaceae, is a common shrub of tropical Africa present in southern Nigeria, Cameroon, Gabon, Democratic Republic of Congo and Angola (Hutchison and Dalziel, 1954). It is commonly used as fodder for domestic animals but also has a folklore reputation as herbal remedy for lower abdominal pains, listlessness, mental strain and certain sexually transmitted diseases. Aqueous decoctions of the leaves, stem bark and root have been prescribed in ethnomedicine in cases of gastroenteritis. Sokomba et al. (1986) detected two alkaloids in the water extract and five in the ethanolic extract of the leaves; and reported hot ethanol extracts of the leaves to exhibit some antimicrobial activity. With these observations in view, the study reported here set out to validate the folklore use of whole root extracts of the plant (in water or alcohol) among the *Igbos* of southeastern Nigeria to treat gastrointestinal disorders. Thus, ethanolic and water (hot and cold) extracts of whole root preparations of *S. scabrida* were evaluated for antibacterial activity against isolates of locally diagnosed aetiologic agents of gastroenteritis and typed bacterial strains used as controls.

MATERIALS AND METHODS

Plant material

Whole roots of the plant, taxonomically authenticated by A. O. Ozioko of Botany Department, University of Nigeria, Nsukka as *Synclisia scabrida* Meirs (Igbo: *Uzi*), was obtained from the herbal garden of Chief Nwaenyi Ossai, a traditional healer from Orba, Udenu Local Government Area of Enugu State. Voucher specimen

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was deposited in the herbarium of the Botany Department, University of Nigeria, Nsukka.

Preparation and phytochemical analysis of plant extracts

The whole root samples, dried in the dark at room temperature, were pulverized with a mechanical grinder. A 50 g weight of the powder was extracted in cold water, hot water, or ethanol (BDH) and the extract filtered, dried and sterilized as described by Okeke et al. (2001). Phytochemical screening of the reconstituted extract was done using standard phytochemical methods (Harbone, 1973; Evans, 1989).The concentrations of the metabolites detected were expressed as low (+), moderate (++) or high (+++) depending on the intensity of the colour reaction.

Test bacterial strains

Local bacterial isolates from cases of gastrointestinal disorder, namely, *Escherichia coli* (*clin*), *Salmonella sp* (*clin*), *Proteus sp* (*clin*), *Staphylococcus aureus* (*clin*) and *Pseudomonas aeruginosa* (*clin*) were obtained from the Medical Diagnostic Laboratory, Department of Microbiology, University of Nigeria, Nsukka. The Department of Veterinary Medicine and Pathology of the above University provided *Salmonella kintambo* SSRL 113, a typed local clinical isolate. The Bioresources Development and Conservation Project (BDCP), Nsukka supplied the remaining typed strains – *E. coli* ATCC 11775, *S. aureus* ATCC 12600, *P. aeruginosa* ATCC 10145 and *Bacillus subtilis* ATCC 6051. All test bacterial strains were purified by streaking and re-isolating three successive times on Mueller Hinton agar, MHA (Oxoid). Identity of each strain was confirmed by standard bacteriological methods (Cheesbrough, 1984).

Testing extracts for antibacterial activity

The inoculum size of each test strain was standardised at 5 x 10^5 cfu/ml using McFarland Nephelometer according to the National Committee for Clinical Laboratory Standards (NCCLS, 1993, Okeke et al., 2001). A 1.0 ml volume of the standardized bacterial suspension was evenly spread on MHA with a sterile glass spreader. After allowing the inoculum to dry at room temperature, 6 mm-diameter wells were bored in the agar with sterile cork-borer. Each extract was checked for antibacterial activity by introducing 100 µl of a 25 mg/ml concentration into triplicate wells. The plates were allowed to stand at room temperature for 1 h for extract to diffuse into the agar and then incubated at 37°C for 18 h in a humidified incubator. Subsequently, the plates were examined for bacterial growth inhibition and the inhibition zone diameter (IZD) measured to the nearest mm. Only extracts showing measurable IZD in this preliminary screening were further studied.

Determination of MIC and MBC

The minimum inhibitory concentration (MIC) was determined by both agar well diffusion (Okeke et al., 2001) and macro-broth dilution (NCCLS, 1993) methods. A 100 µl volume of two-fold serial dilutions of the extracts reconstituted in distilled water (cold and hot water extracts) or 5% DMSO (ethanolic extract) was introduced into triplicate wells in MHA plates pre- inoculated with test bacterial strain. The extracts were allowed to diffuse into the MHA at room temperature before incubation at 37°C for 18 h.

For macro-broth dilution assay, the reconstituted extract was serially diluted two-fold in Mueller Hinton broth (MHB, Oxoid) medium. Duplicate tubes of each dilution were inoculated with 5 x 10^5 cells (cfu) of the test bacterial strain and cultures incubated in a water bath at 37°C for 18 h. Two-fold serial dilutions of ciprofloxacin (µg/ml concentrations) were included in each experiment as controls. MIC was taken as the lowest concentration of extract or drug showing clear zone of inhibition in the agar-well diffusion technique and as the highest dilution (least concentration) of extract or drug showing no detectable growth in the macro-broth assay.

For determination of minimum bactericidal concentration (MBC), 0.1 ml of culture medium was aspirated from each macro-broth assay tube showing no apparent growth and sub-cultured in fresh MHA. After incubation at 37°C for 24 h, the least concentration showing no visible growth on subculture was taken as the MBC. MIC index was computed from the values of MIC/MBC determined by the agar-well diffusion and macro-broth assays, respectively.

Determination of rate of kill

Rate of kill was determined by assay of bacterial cell-death time and was done for ethanolic extract against *E. coli* (*clin*) only. Approximately 5 x 10^5 cfu of test bacterial strain was introduced into 10 ml of MHB containing 3.125, 6.25, 12.5 or 25.0 mg/ml (i.e. ½MIC, 1MIC, 2MIC or 4MIC) of extract. The controls consisted of two sets of duplicate cultures, one without extract or drug (negative control) and the other supplemented with 0.5 µg/ml of ciprofloxacin (positive control). All experiments were incubated in a shaker water bath at 37°C. At 30 min intervals, 0.2 ml of sample was withdrawn, diluted in ten-fold series and 0.2 ml of each dilution plated in triplicate on MHA. After 24 h incubation at 37°C, emergent bacterial colonies were counted and compared with the count of the culture control without extract or drug. The results were expressed as negative or positive $\log_{10}$ values according to Batch et al. (1988).

Cytotoxicity assay

The brine shrimps used were obtained by hatching 5 mg of eggs of *Artemia salina* (Interpet Ltd., England) in natural seawater after incubation at about 29°C for 48 h. The larvae (nauplii) were allowed another 48 h in seawater to ensure survival and maturity before use.

Three doses of plant extract (2, 20, and 200 µg/ml), in 5% DMSO and/or seawater, were tested. Each extract preparation was dispensed into clean test tubes in 10-ml volumes and tested in triplicates. To each tube was added 10 brine shrimps and the experiment incubated at 29°C for 24 h in a water bath, after which each tube was examined and the surviving brine shrimps counted and recorded. The control consisted of 10 brine shrimps per tube in 5% DMSO and or seawater without plant extract. The 50% lethal concentration, LC_{50}, was computed using the Finney Probit Analysis Computer programme.

RESULTS

The yield of dry extracts from 50 g powdered plant material and the pH of the reconstituted preparations are shown in Table 1. More extracts were obtained by weight with hot water (24.7%) and cold water (11.27%) than with ethanol (4.53%). Whereas the water extracts had acid pH (4.6 and 4.7, respectively) the ethanolic extract had a

Table 1. Yield of *S. scabrida* constituents extracted by ethanol, cold- and hot-water maceration, respectively.

Extract	Yield (g)	Yield (%)	pH[c]
Cold water	5.63	11.27[a]	4.7
Hot water	12.35	24.70[a]	4.6
Ethanol	2.27	4.53[a]	7.2
Fractions of ethanolic extract			
F_{ss-1}	1.12	32.00[b]	4.5
F_{ss-2}	0.36	10.30[b]	4.7
F_{ss-3}	0.28	8.00[b]	3.8

[a]Yield as percentage of 50 g of plant material.
[b]Yield as percentage of 3.5 g of ethanolic extract.
[c]pH of wet extract before drying.

Table 2. Phytochemical components of whole root extracts of *S. scabrida*.

Plant constituent	Extracts			Fractions of ethanol extract		
	Cold water	Hot water	Ethanol	F_{ss-1}	F_{ss-2}	F_{ss-3}
Alkaloid	+++	+++	+++	++	-	-
Flavonoid	-	-	++	+	+	++
Tannin	+++	++	++	+	-	-
Saponin	+++	+++	+	++	++	++
Glycosides	-	-	++	-	-	++
Anthroquinone	-	-	-	ND	ND	ND
Carbohydrate	+++	++	+++	+	+	+
Protein	+	++	+	ND	ND	ND

-,Not detectable; +, low concentration; ++, medium concentration; +++, high concentration; ND, not determined.

neutral pH of 7.2. However, column chromatographic fractions of the ethanolic extract, F_{ss-1}, F_{ss-2}, and F_{ss-3} had acid pH (3.8-4.7) and 32.0, 10.3 and 8.0% yields, respectively.

Alkaloids, flavonoid, tannin, saponin, glycosides, carbohydrate and protein were the phytochemicals detected in varying proportions in the extracts as well as in the fractions of the ethanolic extract. Protein, detected at low concentration (+) in the crude ethanolic extract was not detected at all in any of the three fractions. Anthroquinone was not detected in any sample (Table 2).

Overall, 8 out of the 10 (80%) test strains were susceptible to the ethanolic extract, 7 (70%) to the cold water extract and only 2 (20%) to the hot water extract. The highest inhibition zone diameter, IZD (29.00±0.20 mm) was observed also with ethanolic extract against *B. subtilis* ATCC 6051 followed by 27.25±1.50 mm activity of the same extract against *S. aureus* (*clin*). Cold water extract ranked second with IZD of 27.0±1.0 mm against *B. subtilis* 6051. Both the cold water and ethanolic extracts each produced IZD of 24.5±1.50 mm against *Salmonella sp* (*clin*). The least IZD (5.5±0.2 mm) was achieved with hot water extract against *E. coli* (*clin*). *P. aeruginosa* (*clin*) and *Proteus sp* (*clin*) were not susceptible to activity of any of the extracts while *P. aeruginosa* ATCC 10145 was sensitive to the ethanolic extract only (IZD, 5.90 ± 0.10 mm; MIC, 12.50; MBC, 12.50 and MIC/MBC index 1.0) . Ciprofloxacin was active against 9 of the 10 (90%) test bacterial strains with IZD ranging from 7.05±0.35 mm against *P. aeruginosa* (*clin*) to 31.85± 0.25 mm against *E. coli* ATCC 11775 (Table 3). Thus, for the bacterial strains which showed susceptibility to the extracts, *P. aeruginosa* was sensitive to 1 out of 3 (33.33%) of the extracts, *E. coli* (*clin*) and *B. subtilis* to all (100%) and the rest, each sensitive to 2 (66.67%) of the extracts (Table 3).

The MIC and MBC values of the cold water extracts (0.195-6.250 mg/ml and 0.390- 3.125 mg/ml, respectively) were lower than those of the ethanolic extract (3.125-12.50 mg/ml, 3.125-25.00 mg/ml) The MIC and MBC values of ciprofloxacin were 0.625-80.00 μg/ml and 1.250-80 μg/ml, respectively (Table 3).

Table 4 shows the antibacterial activity of the column chromatographic fractions of the crude ethanolic extract. The fractions, F_{ss-1}, F_{ss-2} and F_{ss-3} produced IZD ranges of 3.50±0.50 to 29.60±0.50 mm, 7.20±0.01 to 11.50±1.00

Table 3. Susceptibility of test bacterial strains to whole root extracts of *S. scabrida*.

Test Bacterial strains	Antibacterial Activity																Proportion showing activity per strain (%)
	Cold water extract				Hot water extract				Ethanolic extract				Ciprofloxacin				
	IZD (mm)	MIC (mg/ml)	MBC (mg/ml)	MIC-MBC Index	IZD (mm)	MIC (mg/ml)	MBC (mg/ml)	MIC-MBC Index	IZD (mm)	MIC (mg/ml)	MBC (mg/ml)	MIC-MBC Index	IZD (mm)	MIC (µg/ml)	MBC (µg/ml)	MIC - MBC Index	
Escherichia coli(*clin*)	16.5±0.55	3.125	3.125	1.00	5.5±0.20	3.125	0.00	0.00	17.5±0.50	12.50	25.00	0.5	24±1.0	10.00	10.00	1.0	3/3 (100.00)
E. coli ATCC 11775	20.25±1.25	3.125	0.00	0.00	0.00	0.00	0.00	0.00	10.90±0.90	12.50	12.50	1.0	31.85±0.25	10.00	20.00	0.5	2/3 (66.67)
Salmmella sp. (*clin*)	24.5±1.50	6.250	0.00	0.00	0.00	0.00	0.00	0.00	24.5±1.50	6.250	6.250	1.0	31.50±0.5	2.50	5.00	0.5	2/3 (66.67)
S. kintambo SSRL113	17.10±0.70	3.125	0.00	0.00	0.00	0.00	0.00	0.00	19.±1.40	3.125	3.125	1.0	21.80±0.00	1.250	1.250	1.0	2/3 (66.67)
Staphylococcus aureus (*clin*)	16.0±1.0	1.560	0.00	0.00	0.00	0.00	0.00	0.00	27.25±0.25	6.25	12.50	0.5	21.25±0.25	10.00	10.00	1.0	2/3 (66.67)
S. acureus ATCC 12600	9.0±0.4	0.780	0.00	0.00	0.00	0.00	0.00	0.00	22.50±0.50	12.50	12.50	1.0	26.85±0.45	2.50	5.00	0.5	2/3 (66.67)
Pseudomonas aeruginosa(*clin*)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	7.05±0.35	80.00	0.00	0.00	0/3 (0.00)
Ps.aeruginosa ATCC 10145	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.90±0.10	12.50	12.50	1.0	0.00	0.00	0.00	0.00	1/3 (33.33)
Protens sp (*clin*)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.0±1.0	0.625	1.250	0.5	0/3 (0.00)
Bacithus subtilis ATCC 6051	27.0±1.0	0.195	0.390	0.50	13.6∀0.01	3.125	0.00	0.00	29.00±1.00	3.125	6.250	0.5	9.0±1.0	80.00	80.00	1.0	3/3 (100.00)
Proportion Susceptible (%)	7/10 (70.00)				2/10 (20.00)				8/10 (80.00)				9/10 (90.00)				-

MIC, Minimum inhibitory concentration; MBC, minimum bactericidal concentration; and IZD, inhibition zone diameter.

Table 4. Antibacterial activity of column chromatographic fractions of whole root ethanolic extract of *S. scabrida*.

Test Bacterial strains	Antibacterial Activity												
	Fraction 1 (F_{ss-1})				***Fraction 2 (F_{ss-2})***				***Fraction 3 (F_{ss-3})***				**Proportion showing activity per strain (%)**
	IZD (mm)	**MIC (mg/ml)**	**MBC (mg/ml)**	**MIC - MBC Index**	**IZD (mm)**	**MIC (mg/ml)**	**MBC (mg/ml)**	**MIC - MBC Index**	**IZD (mm)**	**MIC (mg/ml)**	**MBC (mg/ml)**	**MIC - MBC Index**	
Escherichia coli (*clin*)	12.50±0.50	25.00	0.00	0.00	0.00±0.00	0.00	0.00	0.00	12.50±0.00	3.125	6.250	0.50	2/3 (66.67)
Escherichia coli ATCC 1175	8.70±0.55	50.00	0.00	0.00	7.20±0.10	3.125	0.00	0.00	10.00±1.00	6.250	6.250	1.00	3/3 (100.00)
Salmmella sp. (*clin*)	15.00±0.00	3.125	12.50	0.25	9.50±0.00	6.250	0.00	0.00	6.00±0.55	3.125	0.00	0.00	3/3 (100.00)
S.almonella kintambo SSRL113	3.50±0.50	6.250	0.00	0.00	11.50±1.00	12.50	0.00	0.00	18.00±1.00	3.125	0.00	0.00	3/3(100.00)
S.taphylococcus aureus (*clin*)	5.00±1.00	50.250	0.00	0.00	0.00	0.00	0.00	0.00	5.00±0.50	25.00	0.00	0.00	2/3 (66.67)
Staphylococcus acureus ATCC 12600	17.50±0.00	3.125	6.250	0.50	0.00	0.00	0.00	0.00	7.00±0.50	3.125	0.00	0.00	2/3 (66.67)
Pseudomonas aeruginosa (*clin*)	ND	ND	ND	ND	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (0.00)
Pseudomonas aeruginosa ATCC 10145	0.00±0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (0.00)
Protens sp (*clin*)	0.00±0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.20±1.00	6.250	0.00	0.00	1/3 (33.33)
Bacithus subtilis ATCC 6051	29.60±0.50	3.125	0.00	0.00	8.00±1.00	6.250	0.00	0.00	12.50±0.50	6.250	0.00	0.00	3/3 (100.00)
Proportion Susceptible (%)	7/9 (77.80)				4/10 (40.00)				7/10 (70.00)				-

Table 5. Rate of kill of *E. coli* (*clin*) at different concentrations (multiples of MIC) of the *S. scabrida* ethanol extract.

Concentration of Extract (mg/ml)	Viable cell reduction per exposure time (log_{10})			
	30 min	60 min	90 min	120 min
0.5MIC (3.125)	+0.10	+0.37	+0.41	+0.43
1.0MIC (6.25)	-0.40	0.63	-0.70	0.73
2.0MIC (12.50)	-0.37	-0.85	-3.70	-3.70
4.0MIC (25.6)	-3.70	-3.70	-3.70	-3.70
0.0MIC (Negative control)	+0.23	+0.38	+0.41	+0.43
Ciprofloxacin (0.8 μg/ml)	>-5.80	>-5.80	>-5.80	>-5.80

Table 6. Evaluation of *S. scabrida* whole root extracts for cytotoxicity by the Brine Shrimp lethality assay method.

Extract	Percent Brine Shrimps killed per extract concentration			50% lethal concentration (LC_{50}, mg/ml)
	200 ppm	20 ppm	2 ppm	
Cold water	100.00	60.00	46.67	114.169
Hot water	75.00	40.00	0.00	202.068
Ethanol	100.00	56.67	16.67	36.57

mm and 3.20±1.00 to 18.00±1.00 mm, respectively. Although the crude ethanolic extract was active against *P. aeruginosa* ATCC 10145 (IZD, 5.9±0.10; MIC, 12.50 and MIC/MBC index, 1.0), no activity was observed with any of the partially purified fractions (F_{ss-1}, F_{ss-2} or F_{ss-3}) against any of the two *Pseudomonas* strains. Conversely, the crude ethanolic extract showed no activity against *Proteus sp* (*clin*) but F_{ss-3} exhibited activity against this strain (IZD, 3.20±1.00; MIC, 6.250).

Table 5 shows net log_{10} increase (+) or reduction (-) in viable *E. coli* (clin) cell count (cfu/ml) following exposure to varying concentrations of extracts/drug for different periods of time (min). There was net growth of the test bacterial strain following exposure to 3.125 mg/ml concentration (1/2 MIC) of the ethanolic extract for 30-120min. However, 6.25 mg/ml (1 MIC), 12.5 mg/ml (2MIC) and 25.0 mg/ml (4MIC) produced the reductions in bacterial cell counts at the various exposure time shown in the table. The 0.8 μg/ml concentration of ciprofloxacin killed all the bacterial cells within 30 min of exposure. The 50 percent lethal concentration, LC_{50}, calculated for each extract (against the brine shrimps), is given in Table 6.

DISCUSSION

The low yield of plant extracts may reflect the low efficiency of the cold maceration method compared with the alternative, the soxhlet extraction (Ibrahim et al., 1977) . Cold maceration was adopted in order to conform to the traditional method of medicinal plant preparation. It is conceivable that the low yield inherent in this extraction method necessitates the large volumes (250 – 400 ml of aqueous or 20-50 ml of ethanol preparations, sometimes taken thrice daily) prescribed in the traditional herbal treatments. Such large volumes may be required to deliver the effective dose of the active principle(s).

Alkaloid, tannin, saponin, carbohydrate and protein were present in all extracts but in varying proportions while authroquinone was not detected in any extract. Antimicrobial properties of several plant extracts have been attributed to secondary metabolites such as some of these (Watt and Breyer-Brandwijk, 1967; Leven et al., 1979). The significance of the levels and types of metabolite present *vis-à-vis* the antibacterial activity detected in each extract is not known. The disparity in antibacterial activity between the cold and hot water extracts in spite of the similarity in the type of secondary metabolites they contained may suggest heat-inactivation of the components in the hot water extract.

The spectra of antibacterial activity exhibited by the cold-water and ethanolic extracts (70% and 80%, respectively) are ethnomedicinally significant because these two are the traditional forms in which *S. scabrida* is prepared and dispensed. The cold water extract, which apparently exhibits a lower MIC value, is the form preferred in treatment of gastrointestinal disorders while the ethanolic preparation of the root, particularly, is applied in treatment of sexually transmitted diseases (STD).

Column chromatographic separation would, at least, achieve a partial purification. Thus, appearance of antibacterial activity in the three fractions (F_{ss-1}, F_{ss-2} and

F_{SS-3}) of the ethanolic extract may suggest presence of more than one antibacterial constituent in each extract. However, the metabolites detected in the crude extract were either present in one fraction of the partially purified sample (e.g. alkaloid in F_{SS-1} and glycoside in F_{SS-2}) or they were redistributed in the three fractions (e.g. flavonoid and saponin). It is not possible at this stage to know whether the putative active components act synergistically, additively or antagonistically. The observation that the crude ethanolic extract had no antibacterial activity against *Proteus sp* (*clin*), which was susceptible to F_{SS-3} activity (IZD, 3.20±1.00mm; MIC, 6.250mg/ml), is remarkable. This observation was reproduced in three repeat experiments and may indicate antagonism between certain components of the crude extract. Therefore, purification may be required to potentate such constituents. Conversely, the antibacterial activity of the crude ethanolic extract against *P. aeruginosa* ATCC 10145 was apparently lost following partial purification by column chromatography, thus, supporting the suggestion of Kafaru (1994) that crude preparations could, sometimes, exhibit higher efficacy than pure plant substances. Both results considered together, would suggest that the effect of purification on anti-bacterial activity of plant constituents would depend on the type of interactions (antagonism, synergy or additivity) occurring between the plant constituents.

The rate of kill of test bacterial cells varied with concentration of extract, duration of exposure and even the bacterial strain tested (the latter result not shown). That half the MIC (3.125 mg/ml) did not inhibit growth of *E. coli* (*clin*) while the MIC (6.25 mg/ml) and its multiples (12.50 mg/ml and 25.0 mg/ml) did, confirm the reliability of the MIC determination. Overall, the results indicate that the extracts were potent antibacterial preparations, at least, *in vitro*. *In vivo* evaluation may be required to ascertain that active but non-toxic concentrations of extracts may be absorbed and may remain bioactive for the time duration required to completely kill the pathogen.

The brine shrimps lethality assay showed the ethanolic extract to be more cytotoxic or more bioactive, killing the shrimps at lower concentration (LC_{50}=36.57 μg/ml) than the cold water extract (LC_{50}=114.169 μg/ml) or the hot water extract (LC_{50} =>202.068 μg/ml). This assay method is used to screen plant extracts generally for bioactive compounds (McLaughlin et al., 1991). Bioactive compounds are pharmacologically toxic at higher concentrations, hence the application of the brine shrimp method in testing for cytotoxicity (Meyer et al., 1982). However, brine shrimp toxicity may not be extrapolated to mean toxicity to intact laboratory animals or man because the latter have more efficient mechanisms for breaking down similar substances found in foods of plant origin.

In summary, the *in vitro* activity of *S. scabrida* cold water and ethanolic extracts against recognized enteropathogens, *E. coli* and *Salmonella sp*, as well as other bacterial strains often associated with non-gonococcal urethritis may provide a scientific justification for the traditional (folklore) use of the plant in treatment of gastroenteritis and sexually transmitted diseases.

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