Phytochemical investigation, toxicity and antimicrobial screening of essential oil and extracts from leaves and stem bark of *Hura crepitans* (Euphorbiaceae)

Ganiyat K. Oloyede[1]*, Mutairu B. Olatinwo[1]

1. Natural products/Medicinal Chemistry Unit, Department of Chemistry, University of Ibadan, Nigeria.
oloyedegk @ gmail.com Telephone: +234 803 562 2238
*Author to whom correspondence should be addressed.

Abstract: The crude methanol extract of *Hura crepitans* was obtained and screened for the presence of secondary plant metabolites. The leaf essential oil was also obtained by hydro-distillation and GC and GC/MS analysis of the colourless essential oil showed the presence of seven compounds with ethyl propionate (39.6%) and isopentyl alcohol (10.6%) being the major constituents. The LC_{50} obtained from brine shrimp lethality test indicated that all the extracts were toxic at varied degrees. The hexane extract of the bark of *H. crepitans* was the most toxic with LC_{50} 0.001 µg/ml. The partitioned extracts and essential oil were subjected to antimicrobial screening against 10 microorganisms; 6 bacteria and 4 fungi at 6.25 - 200 mg/ml and 3.125 - 200 mg/ml respectively. Appreciable *in vitro* activity was observed in the antimicrobial tests. The ethylacetate extract of the stem bark was the most active in the antimicrobial screening as it inhibited *A. niger* and *C. albicans* at all concentrations suggestive of a selective antifungal activity, when compared to standards; gentamicin for bacteria and tioconazole for fungi. Alkaloids, steroids and phenolic compounds were found in the methanol extracts of *H. crepitans* leaves and stem bark but flavonoids and tannins were only present in the bark. Lastly, the presence of ethyl propionate and isopentyl alcohol in this plant oil showed that it can be a useful source of preservative or flavoring agent in feed and food industry. This study apparently highlights the biochemical basis for possible use of the *H. crepitans* in ethno-medicine.
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1. Introduction

Medicinal plants have been used traditionally in the treatment of numerous human diseases and are the primary source of medicine in rural areas of the developing countries. Natural products derived from medicinal plants are also sources of biologically active compounds, many of which have been the basis for the development of new lead chemicals for pharmaceuticals (Michael, 1990, Hamburger and Hostettmann, 1991, Negi, et al, 1993, Baker et al., 1995, Yue-Zhong Shu, 1998, Pamploma-Roger, 1999, Chitme et al., 2003, Ayuveda and Verpoorte, 2005).

The screening of plant extracts for antimicrobial activities is of significant importance because of the urgent need for compounds that would be added to or replace the current antimicrobial agents to which microbes have become largely resistant (Tichy and Novak, 1998, El-seedi et al, 2002). Microorganisms have developed resistance to many antibiotics as a result of the indiscriminate use of antimicrobial drugs in the treatment of infectious diseases (Cowan, 1999, Ates and Erdogrul, 2003). Plants must also be investigated for any inherent toxicity before being consumed. A rapid and inexpensive test, Brine shrimp (*Artemia salina*) toxicity test (BST) has been used for screening of biological and toxic activities (Meyer et al, 1982, De Rosa et al, 1994, Sahpaz et al, 1994, Colman-Saizarbitoria et al, 1995, Siqueira et al, 1998).

Hura crepitans (Sandbox tree) of the family Euphorbiaceae has enjoyed many ethno-medicinal applications as emetic, purgative, antimicrobial, anti-inflammatory and used in the treatment of leprosy. Phytochemical, antibacterial and toxicology studies have also been investigated (Burkill, 2000, Lasisi et al, 2001, Oderinde et al., 2009). The juice from the plant contains two lectins which have haema-glutinating activity that inhibits protein synthesis. Huratoxin**,** a piscicidal constituent (widely used to catch fish in different parts of the world) was isolated from the milky sap of *H. crepitans* along with hexahydrohuratoxin and keto-enal. A proteolytic enzyme, hurain and crepitin (a toxic toxalbumin) were isolated from the sap of *H. crepitans* (Jaffe, 1943, Kawazu, 1972). There is not much reported work on the leaves and stem bark of *H. crepitans.* Recently, Oloyede and Olatinwo (2011) investigated the i*n vitro* antioxidant activity of extracts from the leaves of *H. crepitans* using two assay methods and the extracts were found to possess free radical scavenging activity.

In this paper, we aimed at obtaining *Hura crepitans* crude extract and carry out phytochemical screening. The essential oil from the leaf was obtained by hydrodistillation and analyzed using Gas chromatography/Gas chromatography-Mass spectrometry. The toxicity of the leaves and stem bark extracts of *H. crepitans* was determined by Brine shrimp lethality assay (Meyer et al, 1982, Keddy et al, 1995, Dvorack et al, 1999). Antimicrobial activity of the extracts and essential oil were also determined by agar well diffusion method and the activity was compared with gentamicin and tioconazole which are antibacterial and antifungal standards respectively.The secondary plant metabolites responsible for the observed activities were also reported.

2. Materials and methods

2.1. Plant collection and identification

The leaves and stem bark of *H. crepitans* were collected at the Botanical Gardens, University of Ibadan, Oyo State in September, 2009 and identified at the Herbarium unit of Botany and Microbiology Department, University of Ibadan by Dr Ayodele.

2.2. Chemicals and Reagents:

Methanol, hexane, ethyl acetate (EtOAc), butanol (BuOH), chloroform, hydrochloric acid, ammonia solution, naphthol, bismuth nitrate, potassium iodide, sodium hydroxide, copper acetate, NaOH, sodium chloride, copper sulphate pentahydrate, ferric chloride, conc. tetraoxosulphate (VI) acid, conc. HCl, ammonia solution, sodium potassium tartarate, potassium chloride, glacial acetic acid, disodium hydrogen phosphate and dihydrogen potassium phosphate were all BDH chemicals and solvents (solvents were distilled before use). Dimethylsulphoxide (DMSO) (M&B, England), hydrogen peroxide (Merck, Germany) and Brine shrimp larvae eggs were obtained from Ocean Star International, Inc. Company, USA.

2.3. Equipment and Apparatus:

Improvised Soxhlet Apparatus for extraction, Mettler analytical balance H80 (UK), Water Bath (Gallenkamp), Rotavapor RII0 (Buchi, England), Gas Chromatograph (Clarus 500; Type: PE AutoSystem), GC-Mass spectrophotometer (Agilent Technologies) and Hydro distiller - Clavenger apparatus.

2.4. Test Organisms:

Staphylococcus aureus, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonellae typhi*, *Bacillus subtilis* (bacteria); *Aspergillus niger, Candida albicans*, *Candida tropicalis* and *Candida glabrata* (fungi) were used (microorganisms were collected from the stock of the Department of Pharmaceutical Microbiology, Faculty of Pharmacy of University of Ibadan). The test organisms were maintained on nutrient agar slopes and kept in a refrigerator at 4 ^{o}C. 100 ml aliquots of nutrient broth were inoculated with the culture of test micro-organisms using a loop and then incubated at 37 ^{o}C for 24 hrs. Also brine shrimp larvae eggs used for toxicity studies already in sealed container was kept in a refrigerator below 0^{o}C.

2.5. Reference standards:

Gentamicin (5 mg/ml) for bacteria and tioconazole (70%) for fungi for antimicrobial studies. The drugs were obtained from the authorized University Medical Hospital, Jaja Clinic Pharmacy, University of Ibadan, Nigeria. DMSO was used for toxicity studies.

2.6. Sample preparation

The leaves and stem bark of *H. crepitans* were weighed separately and air-dried for 4 weeks until the weight was constant and then pulverized using mill machine. The pulverized samples were weighed and kept for further analysis. Essential oil was however obtained from fresh leaf samples.

2.7. Extraction/ partitioning procedure

Pulverized dried leaves and stem bark (2 kg each) of *H. crepitans* was extracted respectively with 5 Lt of methanol. The extracts were collected and concentrated with the aid of a Bucchi rotavapor and stored in a desiccator prior to further analysis. The crude methanol extract was partitioned in hexane, ethylacetate and butanol and screened for the presence of alkaloids, flavonoids, steroids, tannins, saponins, glycosides, phenolics, anthraquinones, reducing sugars and carbohydrates (Sofowora, 1982, Finar, 1983, Harborne, 1998). Thereafter, toxicity test and antimicrobial screening were carried out.

2.8. Isolation of Essential Oil

The oil was obtained by hydro distillation from freshly collected leaf samples (300 g) of *H. crepitans* in accordance with the European Pharmacopoeia, 1996, using a Clavenger type apparatus. The essential oil was collected after 3 hours and stored at 4 ^{0}C until analysis. The oil yield was calculated relative to the dry matter.

Analysis of the Essential Oils

Gas chromatography:

Essential oil (1 ml) was injected neat into a Clarus 500 gas chromatograph GC system coupled to a 5975C VLMSD mass spectrometer with an injector 7683B series device and equipped with a flame ionization detector (FID). An Agilent (9091)-413:325

^{0}C HP-5 columns (30 m x 320 µm x 0.25µm) was used, Helium was used as carrier gas, and the flow through the column was 1.2 mL min^{-1}. The GC oven temperature was initially programmed at 40 ^{0}C (hold for 1 min) and finally at 220 ^{0}C (hold for 5 min) at a rate of 80 ^{0}C/min while the trial temperature was 37.25 ^{0}C. The column heater was set at 270 ^{0}C and was a split less mode while the pressure was 10.153 psi with an average velocity of 66.45 cm/sec and a hold-up time of 0.75245 min was recorded. Mass spectrometry was run in the electron impact mode (EI) at 70eV. The percentage compositions were obtained from electronic integration measurements using flame ionization detector (FID), set at 250^{o}C. The peak numbers and relative percentages of the characterized components are given in Table 1.

Gas chromatography–mass spectrometry:

The essential oil was analysed by GC-MS on an Agilent Technologies 7890A GC system coupled to a 5975C VLMSD mass spectrometer with an injector 7683B series device. An Agilent (9091)-413:325 ^{0}C HP-5 column (30 m x 320 µm x 0.25 µm) was used with helium as carrier gas at a flow rate of 1.2 ml/min. GC oven temperature and conditions were as described above. Mass spectra were recorded at 70eV with the injector temperature set at 250^{o}C.

Identification of Components:

The retention indices determined with reference to homologous series of *n*-alkanes and comparison of the mass spectral fragmentation patterns were used to identify individual constituents of the oil (NIST 08.L database/chemstation data system) with data previously reported in literature (Sur, 1991, Adams 2001, Alali and Al-Lafi, 2003).

2.9. Toxicity analysis: Brine shrimp lethality test

The toxicity of the extracts was determined by brine shrimp lethality test (BST). The shrimp's eggs were hatched in sea water for 48 h at room temperature. The nauplii (harvested shrimps) were attrated to one side of the vials with a light source. Solutions of the extracts were made in DMSO, at varying concentrations (10000, 1000 and 100 µg/ml) and incubated in triplicate vials with the brine shrimp larvae. Ten brine shrimp larvae were placed in each of the triplicate vials. Control brine shrimp larvae were placed in a mixture of sea water and DMSO only. After 24 h the vials were examined against a lighted background and the average number of larvae that survived in each vial was determined. The concentration at fifty percent mortality of the larvae (LC_{50}) was determined using the Finney computer programme (Meyer et al, 1982, Falope et al, 1993, Oloyede et al, 2010).

2.10. Antimicrobial activity

Bacterial and Fungal strains and Growth conditions

The cultures of these micro-organisms, *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. typhi*, *B. subtilis* (bacteria); *A. niger*, *C. albicans*, *C. tropicalis* and *C. glabrata* (fungi) were collected from the National Collection of Industrial Microorganism (NCIM) by Pharmaceutical Microbiology Department, University of Ibadan, Nigeria. The cultures were propagated three times in the appropriate media under aseptic condition to make them physiologically active prior to use and were grown in nutrient broth at 37^{0}C and 27^{0}C for bacteria and fungi respectively and maintained in nutrient agar slants at 11^{0}C for 8 hours.

Preparation of antibiotics and plant extracts for the experiment

The antibiotics and dried plant extracts were weighed and dissolved in the respective solvent of extraction to prepare appropriate dilution to give the following concentrations of 6.25- 200 mg/ml while 3.125-200 mg/ml was used for the oil.

Pour plate method for bacteria

The required amount of agar was prepared according to Manufacturer's specification. Culture of each organism was prepared by pouring 0.1 ml into 9.9 ml of Sterile Distilled Water (SDW) to give 10 ml of solution at 1:100 (10^{-2}) and 0.2 ml was poured into the prepared molten Nutrient Agar (NA) at 45^{0}C. This was poured aseptically into sterile plates and allowed to set on the bench for 45 minutes. The stock was maintained on nutrient agar slant and sub-cultured in nutrient broth for incubation at 37°C prior to each antimicrobial testing. The test organisms were inoculated on prepared discs. Thereafter the cups (9 mm diameter) were aseptically bored into the solid nutrient agar using a sterile cork borer. The test solutions of extracts/oil were then introduced into each of the designated cups on each plate ensuring that no spillage occurred. The same amount of the standard antimicrobial agent and solvents were introduced using syringes into the remaining cups on each plate to act as positive and negative controls respectively. The plates were left at room temperature for 2 hours, allowed to diffuse into the medium, turned upside-down and thereafter incubated at 37^{0}C for 24 h in an incubator. Clear zones of inhibition were observed. Activity or inactivity of each extract was tested in triplicate and the diameters of zones of inhibition were measured in millimetre (mm) using a transparent well-calibrated ruler. The positive control for bacteria was gentamicin at 5 mg/ml (Bayer et al., 1986, Kalemba and Kunicka, 2003, Cushine and Lamb

2005, Duraipndiyan et al., 2006).

Surface plate method for fungi

Sabouraud Dextrose Agar (SDA) was melted, sterilized and poured aseptically into the sterile plates and allowed to cool down for 45 minutes. A 0.2 ml of 1:100 dilution of the organism was spread on the surface using a sterile spreader and a sterile cork-borer was used to create wells inside the set plates. The same procedure described for anti-bacterial activity above was adopted from this stage. The positive control for fungi was tioconazole (70%). All the plates for fungi test were incubated at 28^0C for 48 hours and clear zones of inhibition were observed and recorded using the same method described above for bacteria (Bayer et al., 1986, Lis-Balchin and Deans, 1997, Koo et al., 2000).

3. Results and discussion

Secondary plant metabolites found in the leaves and stem bark of the crude methanol extracts of *H. crepitans* include alkaloids, steroids and phenolics. Flavonoids, cardiac-active glycosides and tannins though present in the bark were however absent in the leaves. Saponins and carbohydrates were beyond detectable limit. Freshly collected leaf samples (300 g) of *H. crepitans* were subjected to hydro distillation to give 1.20% (w/w) essential oil. The essential oil, colorless with characteristic smell was analyzed by GC and GC/MS systems using a polar column. Seven constituents representing 66.1% of the total essential oil was identified in the plant. The result of the analysis is presented in Table 1.

Table 1: Chemical constituents of the volatile oil from the leaves of *Hura crepitans**

Peak no.	Compound	RRI	% composition
1.	Ethyl butyrate	0172	4.3
2.	Ethyl propionate	0126	39.6
3.	Isopentyl acetate	0238	0.5
4.	Isopentyl alcohol	0134	10.6
5.	Isopentyl butyrate	0204	2.4
6.	Methyl butyrate	0130	0.5
7.	n-Octene	0166	8.2
Total			66.1%

*****Percentages calculated from the flame ionization detection data. RRI, relative retention indices calculated against n-alkanes.

(A) **(B)**

Figure 1: Ethyl propionate (A) and isopentyl alcohol (B)

The leaf essential oil was dominated by esters. Ethyl propionate (39.6%), an ethyl ester of propionic acid ($C_5H_{10}O_2$) and isopentyl alcohol (10.6%), also known as isoamyl alcohol ($C_5H_{12}O$) (Fig 1) were the major constituents of *H. crepitans* oil and occurred as clear, colorless oils with characteristic smell.

Brine Shrimp Lethality Test

Brine shrimp toxicity test was carried out on the different extracts of *H. crepitans* leaves and stem bark (Table 2-3). The LC_{50} (concentration that killed 50% of the *Artemia salina* nauplii) obtained indicated that all the extracts were toxic at varied degrees. Samples with LC_{50} values less that 1000 µg/ml are toxic while values greater than 1000 µg/ml are non-toxic. The hexane fraction of the bark of *H. crepitans* was the most toxic at 0.001µg/ml while the ethylacetate fraction of the leaves was the least toxic at 1.423 µg/ml. The order of toxicity of *H. crepitans* stem bark extract is Hexane extract > EtOAc extract > BuOH extract > crude methanol extract while that of the leaves is crude methanol extract > BuOH extract > Hexane extract > EtOAc extract. *H. crepitans* can therefore be beneficial in the therapy of some ailments involving cell or tumour growth because of this inherent toxicity.

Table 2: Results of Brine shrimp lethality test of *H. crepitans* leaves extracts*

Conc./	10000 µg/ml		1000 µg/ml		100 µg/ml		Control		
Extract	**S**	**D**	**S**	**D**	**S**	**D**	**S**	**D**	**LC_{50} (µg/ml)**
Methanol	0	30	8	22	14	16	30	0	0.379
Hexane	0	30	3	27	11	19	30	0	1.033
EtOAc	0	30	1	29	10	20	30	0	1.423
Butanol	0	30	20	10	27	3	30	0	0.394

*LC_{50} < 1000 µg/ml =Toxic, LC_{50} > 1000 µg/ml = Not Toxic S- Survivor, D-Death

Table 3: Results of Brine shrimp lethality test of *H. crepitans* stem bark extracts*

Conc./	10000 µg/ml		1000 µg/ml		100 µg/ml		Control		
Extract	**S**	**D**	**S**	**D**	**S**	**D**	**S**	**D**	**LC_{50} (µg/ml)**
Methanol	0	30	5	25	8	22	30	0	1.263
Hexane	0	30	0	30	0	30	30	0	0.001
EtOAc	0	30	13	17	20	10	30	0	0.142
Butanol	10	20	16	14	21	7	30	0	0.440

*LC_{50} < 1000 µg/ml =Toxic, LC_{50} > 1000 µg/ml = Not Toxic S- Survivor, D-Death

Antimicrobial screening

Zones of inhibition (mm) measured for *H. crepitans* leaves extracts, showed that, the ethylacetate extract inhibited only *A. niger* at 200, 100 and 50 mg/ml (24, 16 and 10 mm). The crude methanol extract was generally not active except on *K. pneumonae* and *A. niger* at 100 and 200 mg/ml (14 and 10 mm) respectively while the butanol extract was active on *A. niger* at 100 and 200 mg/ml (18 and 12 mm) and *S. aureus* at 200 mg/ml (16 mm). *H. crepitans* stem bark extracts were generally better in activity than the leaves' extracts and results are presented in Tables 4-7. The crude methanol extract inhibited *S. aureus*, *E. coli*, *B. subtilis*, *K. pneumonae* and *A. niger* at 50-200 mg/ml but no activity was observed on *P. aeruginosa*, *S. typhi*, *A. niger*, *C. albicans*, *C. tropicalis* and *C. glabrata* (Table 4). The hexane extract was active on *E. coli*, *A. niger*, *C. albicans* and *C. glabrata* at 50-200 mg/ml (Table 5). The ethylacetate extract however showed better activity on two fungi; *A. niger* and *C. albicans* at all concentrations than tioconazole (Table 6) and was also active on *B. subtilis* and *P. aeruginosa* at 200 mg/ml. The butanol extract too (Table 7), showed appreciable activity as it inhibited the growth of *B. subtilis*, *A. niger* and *C. albicans* at 100-200 mg/ml. Overall, it was observed that the extracts showed selective inhibition of the bacterial and fungal strains used in this study and that the stem bark extracts were more effective as antimicrobial agents than the leaves' extracts which may have been as a result of the presence of flavonoid in the bark which was absent in the leaves. Polarity of the solvents used may have also played a significant role in the effectiveness of the plant extracts as antimicrobial agents (Rojas et al, 2003). The activity was however beyond detectable limit at 3.125 - 200 mg/ml for the essential oil as no inhibition was observed for the micro-organism strains used in this study.

Table 4: Antimicrobial activity of Crude methanol extract (bark) *

	Zones of inhibition (mm)									
	S.a	*E.c*	*B. s*	*P.a*	*Kleb.*	*Sal.*	*A.n*	*C.a*	*C.t*	*C.g*
1.	18	24	26	-	20	-	30	-	-	-
2.	16	18	20	-	14	-	24	-	-	-
3.	12	12	18	-	-	-	16	-	-	-
4.	10	-	12	-	-	-	12	-	-	-
5.	-	-	10	-	-	-	10	-	-	-
6.	-	-	-	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	36	24	30	36	36	26	22	20	24	20

*Integers 1-6 represent crude methanol extract at various concentrations {200 (1), 100 (2), 50 (3), 25 (4), 12.5 (5) and 6.25 mg/ml (6)}. -ve = negative control (methanol); +ve = positive control (gentamicin at 5 mg/ml)/tioconazole (70%)); - = no inhibition; S.a = *Staphylococcus aureus*; E.c = *Escherichia coli*; B.a = *Bacillus subtilis*; P.a = *Pseudomonas aeruginosa*; Kleb. = *Klebsiellae pneumonae*; Sal. = *Salmonellae typhi*; A.n = *Aspergillus niger*; C.a = *Candida albicans*; C.t = *Candida tropicalis* and C.g = *Candida glabrata.*

Table 5: Antimicrobial activity of Hexane extract (bark) *

	Zones of inhibition (mm)									
	S.a	*E.c*	*B. s*	*P.a*	*Kleb.*	*Sal.*	*A.n*	*C.a*	*C.t*	*C.g*
1.	-	14	-	-	-	-	24	20	-	14
2.	-	12	-	-	-	-	18	16	-	12
3.	-	10	-	-	-	-	10	14	-	10
4.	-	-	-	-	-	-	-	10	-	-
5.	-	-	-	-	-	-	-	-	-	-
6.	-	-	-	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	36	28	32	36	26	28	24	20	22	20

*Integers 1-6 represent hexane extract at various concentrations {200 (1), 100 (2), 50 (3), 25 (4), 12.5 (5) and 6.25 mg/ml (6)}. -ve = negative control (hexane); +ve = positive control (gentamicin at 5 mg/ml)/tioconazole (70%)); - = no inhibition; S.a = *Staphylococcus aureus*; E.c = *Escherichia coli*; B.a = *Bacillus subtilis*; P.a = *Pseudomonas aeruginosa*; Kleb. = *Klebsiellae pneumonae*; Sal. = *Salmonellae typhi*; A.n = *Aspergillus niger*; C.a = *Candida albicans*; C.t = *Candida tropicalis* and C.g = *Candida glabrata.*

Table 6: Antimicrobial activity of Ethylacetate extract (bark) *

	Zones of inhibition (mm)									
	S.a	*E.c*	*B. s*	*P.a*	*Kleb.*	*Sal.*	*A.n*	*C.a*	*C.t*	*C.g*
1.	-	-	16	16	-	-	30	36	-	-
2.	-	-	10	12	-	-	24	24	-	-
3.	-	-	-	-	-	-	16	16	-	-
4.	-	-	-	-	-	-	14	14	-	-
5.	-	-	-	-	-	-	12	12	-	-
6.	-	-	-	-	-	-	10	10	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	26	30	36	38	26	24	22	20	24

*Integers 1-6 represent ethylacetate extract at various concentrations {200 (1), 100 (2), 50 (3), 25 (4), 12.5 (5) and 6.25bmg/ml (6)}. -ve = negative control (ethylacetate); +ve = positive control (gentamicin at 5mg/ml)/tioconazole (70%)); - = no inhibition; S.a = *Staphylococcus aureus*; E.c = *Escherichia coli*; B.a = *Bacillus subtilis*; P.a = *Pseudomonas aeruginosa*; Kleb. = *Klebsiellae pneumonae*; Sal. = *Salmonellae typhi*; A.n = *Aspergillus niger*; C.a = *Candida albicans*; C.t = *Candida tropicalis* and C.g = *Candida glabrata.*

Table 7: Antimicrobial activity of Butanol extract (bark) *

	Zones of inhibition (mm)									
	S.a	*E.c*	*B. s*	*P.a*	*Kleb.*	*Sal.*	*A.n*	*C.a*	*C.t*	*C.g*
1.	-	-	14	-	-	-	28	34	-	-
2.	-	-	12	-	-	-	26	26	-	-
3.	-	-	-	-	-	-	-	-	-	-
4.	-	-	-	-	-	-	-	-	-	-
5.	-	-	-	-	-	-	-	-	-	-
6.	-	-	-	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-	-
+ve	38	24	30	36	38	26	24	22	20	24

*Integers 1-6 represent butanol extract at various concentrations {200 (1), 100 (2), 50 (3), 25 (4), 12.5 (5) and 6.25 mg/ml (6)}. -ve = negative control (methanol); +ve = positive control (gentamicin at 5 mg/ml)/tioconazole (70%)); - = no inhibition; S.a = *Staphylococcus aureus*; E.c = *Escherichia coli*; B.a = *Bacillus subtilis*; P.a = *Pseudomonas aeruginosa*; Kleb. = *Klebsiellae pneumonae*; Sal. = *Salmonellae typhi*; A.n = *Aspergillus niger*; C.a = *Candida albicans*; C.t = *Candida tropicalis* and C.g = *Candida glabrata.*

According to Jalsenjak *et al*., (1987), Sivropoulou *et al*., (1997) and Sur *et al*., (1991), camphor and 1,8-Cineole are mainly responsible for the antimicrobial activity of
Plant's oil. In our analysis, these compounds were not found in the oil justifying the oil's in-activity towards the bacterial and fungal strains used. But the presence of ethyl propionate and isopentyl alcohol in large amount in this plant showed that it may be a useful source of preservative or flavoring agent in feed and food industry.

4. Conclusion

Alkaloids, steroids and phenols were present in the methanol extract of leaves of *H. crepitans* while the bark showed the presence of alkaloids, flavonoids and tannins. The extracts from the bark were effective antimicrobial agents and the ethylacetate fraction was the most active as it inhibited *Aspergillus niger* and *Candida albicans* at 6.25-200 mg/ml indicating a selective antimicrobial activity especially on the fungi strains used in this analysis. The LC_{50} values obtained from toxicity test were part of the evidences that supported the traditional uses of *H. crepitans* in curing eczema and leprosy. The extracts from the plant were all toxic at varied degrees which suggest diverse biological activity especially in the therapy of diseases involving cell or tumor growth. GC/GC-MS analysis of *H. crepitans* essential oil revealed the presence of seven components constituting 66.1% of the total percentage. Ethyl propionate and isopentyl alcohol were the main constituents found in the essential oil. *H. crepitans* may therefore be a source of medicinally important plant drugs.

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Correspondence to:
Ganiyat K. Oloyede (PhD).
Natural products/Medicinal Chemistry Unit, Department of Chemistry, University of Ibadan, Nigeria.
Telephone: +234 803 562 2238
Emails:oloyedegk@gmail.com;
gk.oloyede@mail.ui.edu.ng

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