Fjrs.futa.edu.ng
FUTA Journal of Research in Sciences
ISSN: 2315 – 8239 (Print); E-ISSN: 2489 - 0413

FUTA Journal of Research in Sciences, Vol. 15(1), April, 2019: 75-84

Chemical Composition and Bioactivity Studies of the Essential oils from *Thevetia peruviana* and *Hura crepitans*

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ABSTRACT

Volatile constituents of plants also known as essential oils have been known and used by man through ages. Essential oils were extracted from air-dried leaves of *Thevetia peruviana* (TP) and *Hura crepitans* (HC) through hydro-distillation using Clevenger-type apparatus. The extracted oils were analysed using Fourier Transformed Infrared (FTIR) and Gas chromatography/Flame ionization Detection (GC/FID) spectrometry techniques. The bioactivity of the oils was tested against five (5) bacteria isolates: *Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Xanthomonas axonopodis* and *Streptococcus bovis* and three (3) fungi species: *Fusarium oxysporum, Colletotrichum gloeosporioides* and *Cercosporazeae-maydis*. GC-MS results revealed that forty-four (44) compounds were present in each of the samples. The prominent compounds present in HC essential oil were beta-pinene (30.78 %) and nerol (31.29 %) while 1, 8-cineole (38.62 %) and linalool (32.89 %) were the major compositions of TP essential oil. Activity against bacteria and fungi species range between 6 mm to 14 mm and 5 % to 26 % respectively which was confirmed theoretically using Spatan-14 software. Thus essential oils from TP and HC could be potential sources of pharmaceutical.

Key words: *Thevetia peruviana*, *Hura crepitans*, essential oil, bioactivity.

INTRODUCTION

Essential oils are natural products obtained from plants. They are formed as varied and complex volatile mixtures of hydrocarbons aldehyde, alcohols, and ketone which can be found deposited in all plant organs (flower, leaves, stem, seed, bark and root) (Linares*et al.*, 2005).

Essential oils are usuallyobtained through distillation because of their high volatility. Other processes of extraction are: expression, solvent extraction, absolute oil extraction, resin taping and cold pressing. They contain about 20-60 compounds at different concentrations, characterized by two to three major compounds at high concentration (between 20 and 70 %) compared to other compounds present in trace amounts. Generally, the prominent constituents determine the biological activity of the essential oil (Reed, 2000).

Essential oils and their components are becoming more popular because of their relatively safe status, wide acceptance and their exploitation for many functional uses (Sawamura, 2000). The production of essential o ils by plants is believed to be primarily as a defe nce mechanism against pathogens and pests (Ox enham, 2003). Apart from being defence mecha nism for the plants they have also been reported to be use ful in aromatherapy, food preservation and as

fragrance (Afolayan and Ashafa, 2009).

Plant terpenoids are used extensively for their aromatic characteristic. They are useful in traditional medicine and therefore are under investigation for antibacterial, antineoplast ic and other pharmaceutical functions (Nita, *et al.*, 2014). Some essential oils, including many of the *citrus* peel oils, are photosensitizers, increasing the skin's vul- nerability to sunlight (Kaddu, *et al.*, 2001). The use of essential oils in pregnancy is not recommended due to inadequate published e vidence to demonstrate evidence of safety (Bona, 2001). Pregnant women often report abnormal sensitivities to smells and taste and essential oils could cause such irritations and nausea (Nordin, *et al.*, 2007).

TP plant thrives very well in Nigeria, it is readily found in every part of the country. *Thevetia* plant remains a plant of little economic value because its potentials are yet to be investigated and exploited.

The seeds, leaves, fruits and roots of *T. peruviana* are considered as potential sources of biologicallyactive compounds with insecticidal activity (Ambang, *et al*., 2005), as rodenticides (Oji and Okafor, 2000), anti- fungi (Ambang, *et al.*, 2010) and anti-viralagents (Tewtrakul, *et al.*, 2002). Also *T. peruviana* has been shown to be effective in reducing the inoculum pressure as well as the incidence of brown rot in soft wood (Ambang, *et al.*, 2010).

H. crepitans also known as Sandbox tree is a tropical plant belonging to the family of *Euphorbiaceae*. It is commonly planted in the cities and villages of the south-western part of Nigeria to provide shades and it is locally called "Odan Mecca" or "Aroyin" (Fowomola and Akindahunsi, 2007). In some climes, the leaves are used for medicinal purposes (Singh, *et al.*, 2012). Studies on the essential oils from *T. peruviana* and *H. crepitans* have not been widely reported in literature. Thus in this work, we investigated the compositions and bioactivity of the essential oils from the two plants.

Plate 1: TP plant and its dried leaves

Plate 2: HC plant and its dried leaves

Materials and Methods

The samples: *c* leaves sample (Plate 1) and *H.creptians* leaves (Plate 2) were gathered within Akure, Ondo State Nigeria and identified at Crop, Soil and Pest Management Laboratory of the Federal University of Technology, Akure. The samples were air dried and pulverize prior to hydro-distillation.

Extraction of Essential oils

The samples above were subjected to hydro disti llation for 3 hrs using an all glass Clevenger-type apparatus, according to British pharmacopoeia, (1980) specification.

Gas chromatography/ Flame Ionization Detection (GC/FID) Analysis

The essential oils obtained from TP and HC were analysed through a Perkin-Elmer Auto System (HP 6890/ HP Chem Station Rev. A09.01 (1206) Software) GC, integrated with a dual Flame Ionization Detection (FID) system. The initial oven temperature was 40^{o}C raised to 200^{o}C for 2 minutes. The detector temperature was set at 300^{o}C for the duration of the analysis. The injection was of the split mode system and hydrogen was used as the carrier gas with flow rate set at 1.0 mL/minute.

IR Analysis

The infrared analysis was carried out using Shimadu Fourier Transformed Infrared (FTIR) Spectrometer between 500 to 4000 cm^{-1} with KBr disc.

INHIBITORY TEST

Bacteria and Fungi

The bacteria used for this experiment were *Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Xanthomonas axonopodis* and *Streptococcus bovis.* They were all clinical isolates and were cultured aerobically at 37^{o}C for 24hours on peptone water. Agar well diffusion methods as described by Norrel and Messely, (1997) was used with little modification. The microbes were seeded on the sterile nutrient agar (NA) plate containing 8 mm wells. About 0.5ml (0.02 g/ml) of each samples were then introduced into the bored well and incubated for 24hours at 37^{o}C.

Control plates were also set up using Dimethylformamide (D.M.F.), methanol and standard antibiotics, Streptomycin Sulphate. Zones of inhibition around the wells were measured and the results were quoted as the radii (mm) of the zone of inhibition. The fungi species used for this experiment were *Fusarium oxysporum, Colletotrichum gloeosporioides and Cercosporazeae-maydis*. Samples (0.02g/ml) were prepared and 0.5ml of each samples were aseptically mixed with 15ml of sterile molten potato dextrose agar (P.D. A).The fungi were inoculated at the centre of the plate with the aid of 4mm cork borer, sterile needle and syringe. Benhite, a standard antifungal agent was used as a control at 2.5g/ml, (D.M.F.) and methanol impregnated plates were equally prepared as control. All the plates were incubated at 27^{o}Cfor 72 hours while mycelia growths were measured at 24hours interval. Mycelia growth inhibition was measured and calculated in percentage using the equation:

$$\% \text{ growth inhibition} = \frac{NTR-TR}{NTR} \times 100$$

Where: NTR = Inhibition diameter of untreated plates; TR = Inhibition diameter of Treated plates.

Chemical-activity Relationship

To further confirm the chemical activities of 1, 8-Cineole, Beta-pinene, Linalool and Nerol the quantum chemical computations of the ground state molecular geometries; polarizabilities, energies and frontier orbital energies (E_H and E_L) were carried out using semi-empirical parameterized methods 3 (SE-PM3) molecular orbital theory in vacuum with Spartan 14. The energy optimizations of the compounds leading to the energy minima were carried out (Kosar *et al.,* 2012). Each of the molecules was allowed to relax to enable all the calculations converge to the optimized geometries of each compound and to correspond to an energy minimum (Kosar *et al.,* 2012). The optimized structures of those compounds were used to obtain their ground state molecular geometry parameters; polarizabilities, dipole moment, energies and the frontier molecular orbital energies at the same level of theory (Anbarasan *et al.*, 2010)

RESULTS AND DISCUSSION

Characterization of Essential Oil

The FTIR spectra (Figs. 1 and 2) for the two samples had peaks between 3439 and 3441 cm^{-1} which indicate the presence of either OH (hydroxyl) or (amine) NH. Intense peaks of $–CH_2$ and $–CH_3$double and single bonds appeared at 2924 and 2854 cm^{-1}respectively. There were no significant peaks at the window regions of

the two samples. There are carbonyl functional groups appearing at 1732 and 1712 cm^{-1}. These observations are evidence in the structures of most of the compounds present in the samples e. g. Nerol, Neral, Camphor, Limonene, Linalool, 1, 8-cineole and Beta-pinene etc. The peaks between 1631 and 1633 cm^{-1} confirmed that –OH observed between 3439 and 3441 cm^{-1} belong to alcohol component of the oil and not moisture.

Essential oils Constituents

The chemical constituents of the essential oils are reported in Tables 1 and 2. The most prominent compounds in the *Thevetia peruviana* leaves' essential oil are 1, 8-Cineole (38.62 %) and linalool (32.89 %) while Beta pinene (30.76 %) and nerol (31.29 %) are the major constituents in *Hura crepitan's* essential oil. 1,8-Cineole (8.840 %) and Linalool (12.70 %) are available in moderate quantities in *Hura crepitans*. It has been reported that 1,8,-cineole is a broadly distributed natural odorant with an eucalyptus-like smell. It belongs to the class of monoterpenes and is present in many herbs used in everyday cooking and in commercial foods, such as basil, rosemary, sage, cardamom, ginger, and peppermint (de Vincenzi *et al*., 2002).

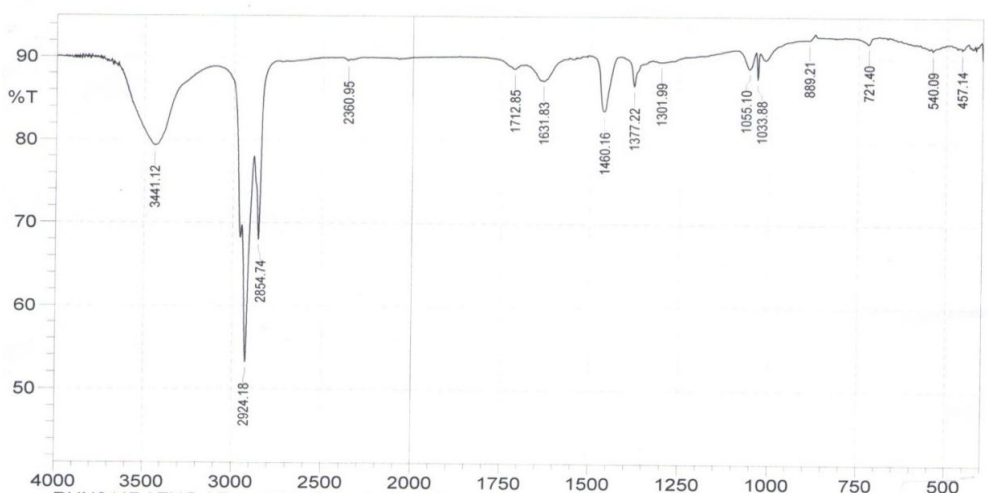


Figure 1: IR Spectrum of *Thevetia peruviana* leave essential oil

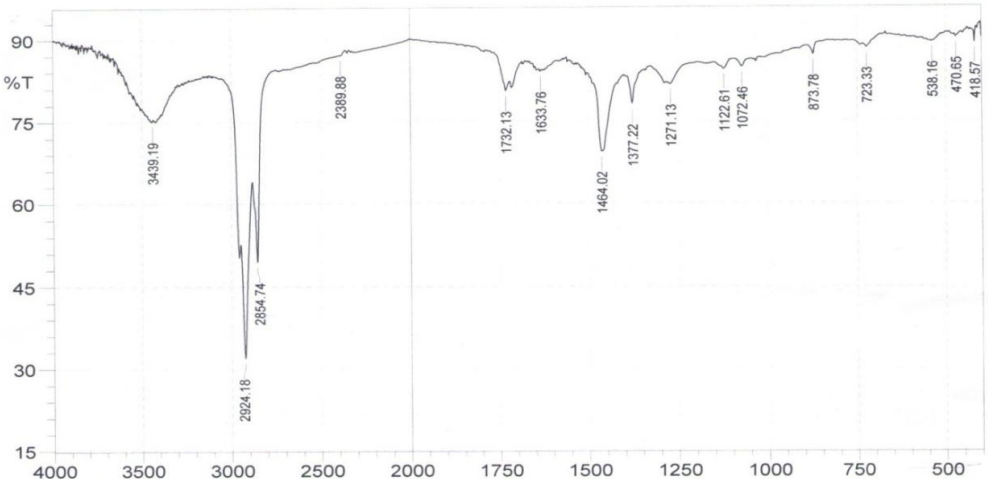


Figure 2: IR Spectrum of *Hura crepitans* leave essential oil

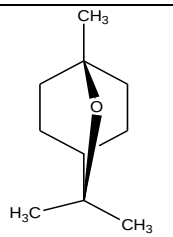
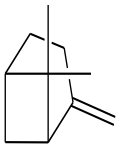
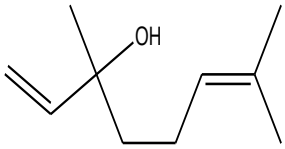
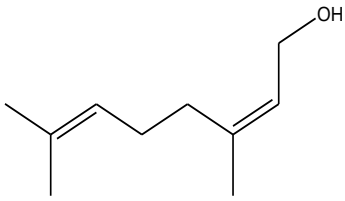
Table 1: *Thevetia peruviana* Constituents and their percentages

S/N	Retention time (min)	Names	Percentage (%)
1	7.753	alpha-Cymene	0.005
2	8.331	Sabinene	0.079
3	9.043	Camphene	0.004
4	9.600	alpha-Pinene	0.001
5	10.447	beta- Pinene	0.001
6	11.374	Limonene	0.007
7	11.832	Thymol	0.006
8	12.296	cis- Ocimene	0.005
9	12.841	beta Myrcene	1.520
10	13.204	Allo ocimine	0.003
11	13.721	Alpha thujene	0.003
12	14.362	Alpha terpinene	0.006
13	14.930	1,8-Cineole	38.629
14	15.923	Neral	0.004
15	16.612	Geijerene	0.005
16	17.088	Camphor	7.802
17	17.441	Nerol	0.003
18	17.709	Linalool	32.890
19	18.199	Borneol	0.005
20	18.551	Alpha terpineol	0.004
21	18.790	Limonene oxide	0.004
22	19.119	terpinen-4-ol	0.005
23	19.619	thymyl methl ether	0.003
24	20.037	Ascaridole	0.003
25	20.251	Linalyl acetate	3.666
26	20.593	Dihydrocarveol	7.881
27	20.818	Alpha terpinenyl acetate	0.004
28	21.229	Ethyl cinnamate	0.005
29	21.637	Borneol acetate	0.004
30	21.724	Neryl acetate	0.002
31	21.929	Geranyl acetate	0.005
32	22.598	Beta caryophyllene	0.014
33	22.931	trans-Alpha-bergamotene	0.005
34	23.250	Gama cardinene	0.004
35	23.356	Beta-elemene	0.002
36	24.061	Bicyclogermacrene	0.007
37	24.467	Phytol	0.002
38	25.020	Alpha copane	0.001
39	25.781	Germacrene D	0.007
40	26.280	Acetyleugenol	0.002
41	26.653	Elemicin	0.001
42	27.254	Benzyl benzoate	0.004
43	28.053	Alpha humulene	0.004
44	28.540	1-allyl-4-methoxybenzene	7.380
	TOTAL		100

Table 2: *Hura crepitans* Constituents and their percentages

S/N	Retention time (min)	Names	Percentage (%)
1	7.825	Alpha-cymene	0.021
2	8.308	Sabinene	1.514
3	8.979	Camphene	0.017
4	9.698	Alpha-pinene	5.824
5	11.317	Beta- pinene	30.762
6	11.809	Limonene	1.187
7	12.022	Thymol	0.020
8	12.453	Cis- ocimene	0.024
9	12.777	Beta myrcene	1.188
10	13.201	Allo ocimine	0.011
11	13.704	Alpha thujene	1.168
12	14.399	Alpha terpinene	0.023
13	14.941	1,8-Cineole	8.840
14	15.981	Neral	1.915
15	16.552	Geijerene	0.022
16	17.100	Camphor	1.917
17	17.714	Nerol	31.294
18	17.966	Linalool	12.702
19	18.206	Borneol	1.203
20	18.593	Alpha terpineol	0.013
21	18.788	Limonene oxide	0.014
22	19.121	Terpinen-4-OL	0.017
23	19.608	Thymyl methl ether	0.012
24	20.041	Ascaridole	0.012
25	20.245	Linalyl acetate	0.010
26	20.581	Dihydrocarveol	0.025
27	20.811	Alpha terpinenyl acetate	0.016
28	21.220	Ethyl cinnamate	0.021
29	21.623	Borneol acetate	0.018
30	21.724	Neryl acetate	0.009
31	21.873	Geranyl acetate	0.019
32	22.585	Beta caryophyllene	0.018
33	22.920	trans-Alpha-bergamotene	0.018
34	23.241	gama Cardinene	0.013
35	23.361	Beta-elemene	0.005
36	24.082	Bicyclogermacrene	0.024
37	24.388	Phytol	0.008
38	25.008	Alpha copane	0.006
39	25.731	Germacrene D	0.014
40	26.260	Acetyleugenol	0.007
41	26.638	Elemicin	0.004
42	27.299	Benzyl benzoate	0.014
43	28.037	alpha Humulene	0.014
44	28.511	1-allyl-4-methoxybenzene	0.016
	TOTAL		100

Table 3: Structures of the major compounds in the samples' Essential oils

S/N	Names	Chemical Structures	*Thevetia peruviana* (%)	*Hura crepitans* (%)
	MAJOR COMPOUNDS			
1	1,8-Cineole	 1,3,3-trimethyl-2-oxa-bicyclo[2.2.2]octane	38.629	8.840
2	Beta- pinene	 6,6-dimethyl-2-methylenebicyclo[3.1.1]heptane (beta-pinene)	0.001	30.762
3	Linalool	 3,7-dimethylocta-1,6-dien-3-ol (Linalool)	32.890	12.702
4	Nerol	 (Z)-3,7-dimethylocta-2,6-dien-1-ol (Nerol)	0.003	31.294
	TOTAL		**71.523**	**83.598**

The most important natural source is eucalyptus essential oil, more than 80 % of which is 1, 8-cineole (Iqbal *et al.,* 2011). Alpha Pinene/ Beta pinene has been reported to be medicines for the treatment of inflammation, microbial infections, Gastro-intestinal problems and other conditions (Iqbal *et al.*, 2011).

INHIBITORY TEST

The results of the antimicrobial activity studies of the two plants are presented in Tables 4 and 5. It was observed that *H. crepitans* eaves essential oil was better antimicrobial agent than TP leaves essential oil on the five (5) bacteria species used. The two essential oils were moderately potent but inferior to *Streptomycin sulphate* used as standard. The results revealed that the samples may be potential sources of antibacterial agents. In contrast to the antibacterial activity, *T. peruviana* leaves essential oil was more active as antifungal agent than the *H. crepitans* leaves essential oil.

However, *C. glocosporioides* showed better susc eptibility to *H. crepitans* essential oil. Both oils inhibited *C. zaea-maydis* at the same percentage (5.76 %). In comparison with the standard used as control, the samples performances were below the average, but may be more effective if concentrations were increased.

Table 4: Inhibitory effects on some selected bacteria after 24 hours incubation in (mm)

Samples	*S. typhi*	*S. aureus*	*P. aeruginosa*	*X. axonopodis*	*S. bovis*
Hura creptians	14.00	7.00	8.20	6.00	9.00
Thevetia peruviana	11.00	7.00	6.80	7.50	8.00
Streptomycin sulphate (standard)	17.00	18.00	14.00	28.00	15.00

Table 5: Inhibitory Effects on Three Selected Pathogens after 72 hours of Incubation in (%)

Samples	*Fusarium oxysporum*	*Colletotrichum gloeosporioides*	*Cercospora Zeae-maydis*
Hura creptians oil	23.07	25.32	5.76
Thevetia peruviana oil	26.15	20.78	5.76
Mancozeb (standard)	87.00	85.00	70.00

It has been reported that major constituents are responsible for a substance action (Mattys *et al.*, 2000). Thus, Beta-pinene, nerol, linalool and 1, 8-cineole may be considered as the active ingredients responsible for the observed bioactivity. The global activity descriptors of the major compounds in the essential oils are shown in Table 6.This theoretical finding corroborates the experimental bio-activity of the prominent compounds. Nerol with the smallest HOMO-LUMO energy-gap (10.25 eV) has the highest reactivity, followed by Linalool (10.42 eV), then beta-pinene (10.81 eV) and the least was 1, 8-Cineole (13.22 eV). The large energy gap (13.22 eV) implies the high stability of the compound while small energy gap (10.25 eV) as in figure 3 above indicates the low stability of the compound and hence low and high biological activity respectively (Alabi *et al*., 2018).

Table 6: Global reactivity descriptors of the major constituents

Properties	Formulae	1,8-Cineole	Beta-pinene	Linalool	Nerol
Energy gap (eV)	$E_L - E_H$	13.22	10.81	10.42	10.25
Chemical potential(K)	$½(E_H-E_L)$	-6.61	-5.41	-5.21	-5.13
Hardness (η)	$½(E_L-E_H)$	6.61	5.41	5.21	5.13
Softness (S)	$\frac{1}{2\eta}$	0.08	0.09	0.10	0.11
Electrophilicity	$\frac{K^2}{2\eta}$	-3.31	-2.71	-2.61	-2.57
Electronegativity	$\frac{I+EA}{2}$	3.77	4.30	4.31	4.67
Ionization potential, I (eV)	$-E_H$	10.38	9.70	9.52	9.43
Electron affinity, EA (eV)	$-E_L$	-2.84	-1.11	-0.90	-0.82
Dipole moment, μ (D)		1.35	0.35	1.72	1.73
Polarizability (α)		52.95	52.64	54.79	
E_{HOMO}(eV)		-10.38	-9.70	-9.52	-9.43
E_{LUMO}(eV)		2.84	1.11	0.90	0.82

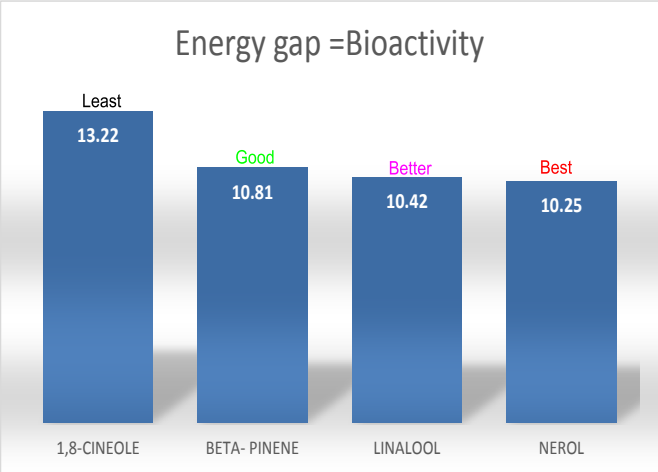


Figure 3: Shows the decrease or increase in activity of the samples

Other parameters followed the same pattern, Nerol that is the softest (0.11) has the least value for the hardness (5.13) and 1, 8-Cineole that is hardest (6.61) also has the least value for the softness (0.08) Table 6. The global softness and hardness of compounds indicate increases and decreases the movement of the system towards a more or less stable configuration (Alabi *et al.*, 2018). These results signify that Nerol was the most reactive while 1, 8-Cineole was the least (figure 3). The electrophilicity value follows the same trend, the value calculated for the Nerol is the greatest (-2.57), followed by Linalool (-2.61), the Beta-pinene (-2.71) and 1,8- cineole (-3.31). Also, Nerol with the highest electron affinity (EA) (-0.82 eV) has the least ionization potential (9.42 eV), followed by Linalool with (-0.90 eV) and (9.52 eV), then Beta-pinene (-1.11 eV) and (9.70 eV) and the least is still 1, 8-cineole (-2.84 eV) and (10.38 eV) respectively. These findings therefore support/confirm the experimental results that nerol plus beta-pinene are slightly more bioactive than1, 8-cineole plus linalool.

CONCLUSION

The chemical constituents and bioactivity of the essential oils from *H. crepitans* and *T. peruviana* have been investigated. Beta-pinene (30.78 %) and Nerol (31.29 %) were the prominent compounds present in *H. crepitans* essential oil while 1,8-cineole (38.62 %) and linlalool (32.89 %) were the major constituents in *T. peruviana* essential oil. The oils inhibited both bacteria and fungi isolate used in this research, but they were better inhibitors of bacteria than fungi. The two essential oils could therefore be potential sources of antibacterial agents.

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