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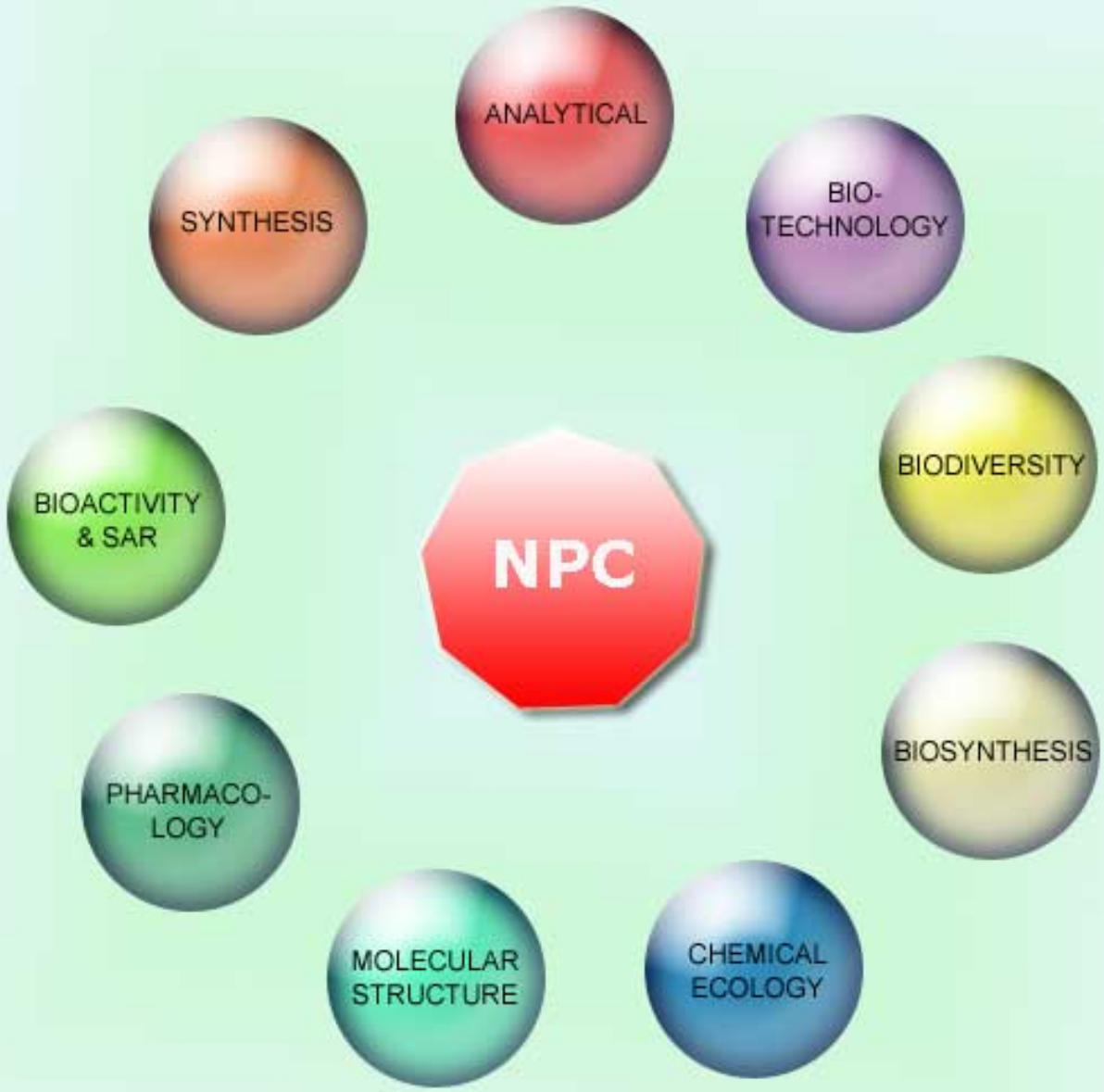

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GC-MS Analysis of the Leaf Essential Oil of *Ipomea pes-caprae*, a Traditional Herbal Medicine in Mauritius

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The chemical compositions of the essential oils of the fresh and dried leaves of *Ipomea pes-caprae* from Mauritius were studied for the first time by gas chromatography-mass spectrometry and 70 compounds were identified. The major components were found to be 8-cedren-13-ol (13.0%), (*E*)-nerolidol (7.0%), guaiol (6.2%), α-cadinol (6.2%) and limonene (6.1%) in fresh leaves and β-caryophyllene (36.6%), α-copaene (8.0%), germacrene D (7.3%), phytol (5.8%), δ-cadinene (5.7%), and α-humulene (5.4%) in the dried leaf samples. The relationship between the anti-hemorrhoidal activity of *Ipomea pes-caprae*, one of its traditional uses in Mauritius, and the chemical composition of the essential oil samples is also discussed.

Keywords: *Ipomea pes-caprae*, essential oil, monoterpenes, sesquiterpenes.

The genus *Ipomea* (Convolvulaceae) consists of more than 200 species widely distributed in tropical and subtropical countries. Some of them are frequently used in folk medicine for the treatment of several diseases [1]**.** *I. pes-caprae*, commonly known in Mauritius as "Liane batatran", has been traditionally used to cure stone fish stings and alleviate people suffering from hemorrhoids (personal communication with the fishermen of Mauritius). Pre-clinical and clinical investigations validated some of the ethnopharmacological properties of the plant. A light petroleum extract was shown to inhibit the contraction of the guinea-pig ileum stimulated by four different spasmogens in a dose-dependant manner [2]**.** β-Damascenone and (*E*)-phytol were later isolated and proved to be responsible for the antispasmodic activity exhibited by the plant [3]. Pongprayoon and colleagues [4] additionally isolated 2-hydroxy-4,4,7-trimethyl-1(4H)naphthalenone, (–)-mellein, eugenol, and 4-vinylguaiacol from the same fraction. These compounds were shown to exhibit anti-inflammatory properties *via* the inhibition of prostaglandin activity in a dose-dependant manner. The extract of *I. pes-caprae* also demonstrated ability to neutralize crude jellyfish venoms [5]. The extract of the leaves also exhibited antinociceptive activities [6]. In Mauritius, people suffering from hemorrhoids usually either take a bath with a decoction of the plant or sit on a recipient containing the hot decoction in order that the vapor reaches the hemorrhoids. It was hence deduced that the anti-hemorrhoid activity of the plant might reside, at least in part, in the constituents of the plant essential oil. The only available information regarding the essential oil of *I. pes-caprae* is its physical properties [7]. Therefore, in this paper, in an attempt to validate the use of *I. pes-caprae* in the treatment of hemorrhoids, we report for the first time the separation and identification of the components of its essential oils using GC-MS.

Separate hydro-distillation of fresh and dried aerial parts of *I. pes-caprae* yielded clear oils, the yields being 0.005 and 0.019 %, respectively. The oils were separately subjected to GC-MS analysis. The retention times, retention indices calculated according to [8], and percentages of the compounds identified in the essential oils from the fresh and dried leaves are detailed in Table 1. The components are listed in elution order on the DB-XLB column.

Table 1: Percentage composition of the essential oil from the fresh and dried leaves of *I. pes-caprae* (L.) R. Br.

Compounds	Rt	Retention Indices (FAME)	Fresh plant Area %	Dried plant Area %
Tricyclene	3.51	666.8	0.03	ND
α-Thujene	3.64	677.1	0.2	0.05
α-Pinene	3.70	681.9	3.2	0.88
Camphene	3.95	701.5	0.4	0.12
β-Pinene	4.40	731.9	1.4	0.38
β-Myrcene	4.67	750.1	0.8	0.25
δ-3-Carene	4.95	769.1	0.3	0.08
α-Terpinene	5.13	781.2	0.1	0.03
p-Cymene	5.30	792.7	4.6	1.61
Limonene	5.35	796.1	6.1	1.74
(*Z*)-β-Ocimene	5.42	800.8	0.1	0.05
(*E*)-β-Ocimene	5.58	811.4	ND	0.03
γ-Terpinene	5.80	826.0	0.6	0.24
(*Z*)-Linalool oxide (furanoid)	6.00	839.2	0.0	0.01
Terpinolene	6.19	851.8	0.05	0.07
Fenchone	6.31	859.7	0.05	0.09
Linalool	6.45	869.0	3.7	1.82
(*Z*)-Thujone (α-thujone)	6.68	884.2	0.1	0.24
Methyl octanoate	6.85	895.5	0.1	0.07
(*E*)-Pinocarveol	7.06	909.7	0.4	ND
(*E*)-Verbenol	7.19	918.5	0.4	0.03
Camphor	7.25	922.6	0.1	0.13
Menthone	7.54	942.3	0.3	0.08
Ethyl benzoate	7.64	949.1	0.07	0.06
Terpinen-4-ol	7.76	957.3	0.5	0.14
p-Cymen-8-ol	7.92	968.2	0.4	0.03
α-Terpineol	8.02	975.0	3.3	0.38
Estragol	8.08	979.0	ND	0.06
Safranal	8.14	983.1	0.64	0.17
Decanal	8.20	987.2	ND	0.05
Verbenone	8.31	994.7	1.01	0.05
(*E*)-Carveol	8.42	1002.3	0.2	0.4
(*Z*)-Carveol	8.46	1005.1	0.1	ND
Nerol	8.72	1023.7	0.5	0.36
Carvone	8.94	1039.4	0.2	0.02
2-(*E*)-Decenal	9.09	1050.1	0.5	0.29
Citral	9.18	1056.6	0.08	0.02
Thymol	9.24	1060.9	ND	1.28
Carvacrol	9.46	1076.6	0.2	ND
(*E,E*)-2,4-Decadienal	9.56	1083.7	0.1	0.12
δ-Elemene	9.80	1100.9	0.3	0.07
α-Cubebene	9.96	1112.9	ND	0.84
α-Terpinyl acetate	10.07	1121.2	0.2	0.12
Neryl acetate	10.20	1131.0	0.9	0.1
Eugenol	10.29	1137.7	2.7	0.05
α-Copaene	10.37	1143.8	0.2	7.97
Geranyl acetate	10.48	1152.0	1.7	4.21
(*E*)-β-Damascenone	10.51	1154.3	0.5	ND
β-Elemene	10.60	1161.1	0.4	0.41
β-Caryophyllene	10.95	1187.4	1.4	36.57
α-Humulene	11.45	1226.3	0.4	5.43
Geranyl acetone	11.52	1231.9	ND	0.03
γ-Muurolene	11.74	1249.4	0.4	0.13
Germacrene-D	11.83	1256.5	0.4	7.35
β-Ionone	11.91	1262.9	0.4	1.51
Cuparene	12.00	1270.0	ND	0.15
Tridecanal	12.06	1274.8	0.3	0.58
δ-Cadinene	12.26	1290.6	0.5	5.7
(*Z*)-Calamenene	12.38	1300.2	ND	0.09
(*E*)-Nerolidol	12.71	1327.7	7.0	0.12
Dodecanoic acid	12.79	1334.3	ND	0.22
Caryophyllene oxide	13.18	1366.8	2.0	3.94
Guaiol	13.36	1381.8	6.2	0.08
Cedrol	13.54	1396.8	0.2	0.41
α-Muurolol	13.97	1434.1	1.6	0.75
α-Cadinol	14.05	1441.0	6.2	0.3
α-Bisabolol	14.40	1471.5	2.2	0.02
8-Cedren-13-ol	15.12	1535.6	13.0	0.03
Hexahydrofarnesyl acetone	15.88	1605.0	0.2	3.02
Phytol	18.47	1864.8	0.3	5.84
Total			81.5	97.45

Rt, retention times on DB-XLB column
ND, not dedected.

It is to be noted that FAME (fatty acid methyl esters) have been used for indices calculation instead of *n*-alkanes since the DB-XLB is more polar than the ones normally used for Kovats and related indices calculation; so, indices based on FAME give higher specificity [8].

A total of 60 and 65 compounds, representing 81% and 97 % of the volatiles from the fresh and dried leaves respectively, were identified by means of their retention times and mass spectral fragmentation patterns. Unidentified components were present in such low amounts that either no mass spectrum could be recorded or the spectrum was too poor for interpretation. Some high boiling compounds were also identified from the essential oils due to the temperature gradient (up to 310°C) and the stationary phase (DB-XLB, extremely low bleeding) used.

From Table 1 it is evident that there are high quantitative differences in the compositions of both oils, albeit distilled from the same plant sample (fresh and dried). This stresses the importance of analysis of those oils and could explain differences in biological properties.

From Table 1 it is also clear that monoterpenoids and sesquiterpenoids constitute the main groups of compounds detected in both the fresh and dried leaves essential oils: they contain respectively 30.2% and 10.8% monoterpenoids and 42.5% and 70.4% sesquiterpenoids. This shows that drying of the leaves induces a loss of monoterpenoids, usually more volatile than sesquiterpenoids. Relative proportions are also very different, indicating that, during the drying process, not only evaporation but also transformations occur, which might be enzymatic or not. The major components (> 3%) of the fresh leaves essential oil were α-pinene (3.2%), *p*-cymene (4.6%), limonene (6.1%), linalool (3.7%), α-terpineol (3.3%), (*E*)-nerolidol (7.0%), guaiol (6.2%), α-cadinol (6.2%) and 8-cedren-13-ol (13.0%), while those of the dried leaves include α-copaene (8.0%), geranyl acetate (4.2%), β-caryophyllene (36.6%), α-humulene (5.4%), germacrene D (7.3%), δ-cadinene (5.7%), caryophyllene oxide (3.9%), hexahydrofarnesyl acetone (3.0%) and phytol (5.8%). The presence of some of these components can partially explain one of its traditional uses in Mauritius.

The three main signs and symptoms of hemorrhoids are severe pain, bleeding and inflammation [9]. The anti-inflammatory and antinociceptive activities of the oil could be imputed to the presence of the following compounds in quantitative amounts in the oil of the fresh leaves: α-pinene [10], limonene [11,12], linalool [13,14], α-terpineol [15], eugenol [2,16] and caryophyllene oxide [17]; compounds known to possess analgesic and/or anti-inflammatory properties on different models. For the essential oil from the dry leaves, β-caryophyllene [18], phytol [3,19] and caryophyllene oxide [18] are the main anti-inflammatory constituents.

Furthermore, the oil obtained from the fresh leaves of *I. pes-caprae* contains compounds which could help the permeation of the anti-inflammatory and antinociceptive agents through the skin. In fact limonene [20] is reported to promote percutaneous absorption of nonsteroidal anti-inflammatory drugs in rats while nerolidol has been shown to increase the skin permeation of naproxen® [21].

In light of the present study, the traditional usage of *I. pes-caprae* by Mauritian folks for its anti-hemorrhoidal activity is fully justified. As discussed above, oils obtained from both the dried and fresh leaves of the plant have been shown to possess several compounds that can synergistically reduce the symptoms of hemorrhoids and alleviate people suffering from the affliction. Additionally, this study emphasized that the use of fresh leaves of *I. pes-caprae* is expected to be more effective in the treatment of hemorrhoids than the dried leaves since the former retains most of its monoterpenes, which other studies have previously shown to possess biological activities relevant to the cure of hemorrhoids.

Experimental

Plant material: The leaves of *Ipomea pes-caprae* (L.) R. Br. (Convolvulaceae) were collected along the seashore of Grand Gaube, a small fishermen's village at the north-northeast part of the Island of Mauritius during January 2003 (summer). A voucher specimen of the plant, bearing No. MAU 23727, has been deposited at the National Herbarium at the Mauritius Sugar Industry Research Institute (MSIRI).

Preparation of extracts: Half of the collected leaf sample was immediately investigated and the other part was dried in shade at room temperature for two days and then analyzed (as dried plant material). The essential oils from the fresh and dried leaves of *I. pes-caprae* were obtained by hydro-distillation in a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia [22] with *n*-hexane. The essential oil was collected in *n*-hexane and stored at 4°C in the dark. Essential oil yields from fresh and air dried plant material were 0.005 and 0.019% respectively (based on fresh and dried mass of samples).

Gas chromatography-mass spectrometry: GC-MS analyses were carried out on a Thermo Quest Trace GC 2000 coupled to a Trace MS mass spectrometer, equipped with PTV split-splitless injector, fused silica capillary column (DB-XLB, 15m x 0.25 mm) and electron impact detector. Samples were injected (1 µL of the 10% solution of essential oils in *n*-hexane) in split mode (1:40). Injector temperature was 220°C. Column temperature was programmed as follows: isothermal at 40°C for 1 min, then increased to 250°C, at a rate of 10°C min^{-1}, and subsequently at a rate of 15°C min^{-1} to 310°C. This temperature was held isothermally for 15 min. Helium was used as carrier gas (flow rate: 1 mL/min). Mass spectra were recorded in the scan mode at 70 eV (40-415 U). The ion source temperature was 230°C.

Qualitative and quantitative determination: Triplicate analyses of each oil sample were performed and quantitative results are presented as a mean of data derived from GC–MS analyses. Identification of individual constituents was made by comparing their mass spectra with the NIST library of mass spectra and literature [23], as well as by comparison of their retention indices to those of authentic samples, when available. Quantitative analysis (in % of the total peak areas) was performed by peak area measurement (TIC).

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