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Chemical constituents of the traditional skin care and fragrance nut, *Cyperus esculentus* (Tigernut)

Tesfaw Gugsa and Estifanos Ele Yaya

Abstract
In this study major components of the smoke obtained from burned *Cyperus esculentus* L. (Tigernut) were investigated. The rhizome part of the plant material was burnt and the smoke was captured in methanol and n-hexane using an inverted funnel that is connected to two suction flasks. *p*-Vinylguaiacol, *p*-Vanillin and Cyprotundone were isolated from the smoke trapped by methanol. Using GC-MS about 40 compounds were identified from the smoke trapped by methanol and hexane. Cyperene (35.94 %), *p*-Vinylguaiacol (11.57 %), α-Copaene (9.49 %), Limonene (8.98 %), Coumaran (7.84 %), Cymene (7.73 %) and β-Pinene (6.70 %) were the major components of the hexane and methanol condensates. Retention index was calculated for all the identified compounds.

Keywords: traditional medicine, *Cyperus esculentus* L. (Tigernut), medicinal smoke, rhizome

1. Introduction

1.1 Plant derived medicinal smokes

From time immemorial, smokes from the medicinal plants have been in use by humans for the treatment of different diseases. It was a common practice in many cultures and among famous ancient physicians. The records, written on clay tablets (from Mesopotamia dated about 2600 BC) are still in use as references for the treatment different ailments ranging from coughs and colds to parasitic infections and inflammations [1]. Plant derived smoke has multiple uses, including air purification, flavoring, medicinal, seed germination, pest control, preservation, religious and veterinary among other historical and modern applications [2].

1.2 *Cyperus esculentus* (Tiger nut)

Tiger nut, botanically called *Cyperus esculentus* is a perennial herb of both the tropic and temperate regions of the world. Naturally it grows as a weed on wastelands and farming areas [3]. The underground part, the sweet tubers, is valued for its nutritional and health benefit [4]. *Cyperus esculentus* belongs to the division Magnoliophyta, class-Lioliopsida, order-Cyperales and family-Cyperaceae [5]. It is also known as nut sedge, earth nuts, earth almond, rush nut, and chufas [6]. The sedge family or cyperaceae, which has 5000 species under 90 genera, is one of the largest families of the flowering plants and is the third largest of monocotyledons (monocots) after orchidaceae and poaceae [7]. It is an erect annual or perennial herb that produces tubers with grayish orange to dark brown color with the length of about 3cm.

Volatile constituents of *Cyperus esculentus* such as *p*-Vinylguaiacol (2-methoxy-4-vinylphenol), a phenolic compound, can be used as flavoring agent, anti-inflammatory, antimicrobial, analgesic and antioxidant [8]. Vanillin (4-hydroxy-3-methoxy benzaldehyde) displays antioxidant and antimicrobial properties and it is active against food spoilage bacteria. It is also used as a tracer component in many dairy products, pastry products, in cosmetics and drug preparations [9]. Cyprotundone is an antimicrobial, analgesic, anti-inflammatory, antibacterial, antimutagenic, antioxidant, antifungal and antiallergic compound [10].

The nut is widely used as a skincare and a fragrance among the tribes living in Borena region located in northern part of Ethiopia, in Oromia regional state. The local women use smoke generated from the nut as a deodorant and an additive to prepare the traditional hair oils. The smoke is generated by chopping the rhizomes into pieces and by placing it in a small clay-pot of about 200-300 mL with few grains of burning charcoals. By sitting on a small stool and covering their whole body with a blanket, women put the pot underneath their legs that can last from 30 min to an hour. That will allow the whole body to be cover by the smoke from the burnt rhizomes. Aroma from the smoke is attractive and positively taken and recommended by

the local men. It is considered as sign of beauty and ways of culturally protecting unnecessary odor. The smoke is also allowed to pass over fresh cow butter and can be used for hair and skin care. Similarly, towels with which new born babies wrapped up with are also treated with the same smoke on a daily bases to keep it uncontaminated so as to protect skin from rash and itching.

The physical observation and as it has been witnessed by local users, there is no sign of fungal infections and skin disease among children and women of the locality. We believe that the smoke from *Cyperus esculentus* rhizome is responsible for the clean skin of people in remote rural area of Borana, Ethiopia. That is why the researchers are interested to investigate the chemical composition of the smoke of *Cyperus esculentus* rhizome.

2. Material and Methods

Rhizomes of *Cyperus esculentus* (Qundhii or Engicha, local names) were collected from Yabelo the capital town of Borena Zone, Oromia regional state, Ethiopia. One kg chipped and air dried rhizomes of *Cyperus esculentus* was burned using an electrical stove (Figure 1). The smoke produced was collected using inverted funnel fitted with a heat resistance rubber tube. The smoke was allowed to pass thorough a 250 mL suction flask containing 100 ml of methanol which in turn connected to another 250 mL suction flask that contains hexane. The hexane containing suction flask was connected to a water aspirator in order to assist a continuous flow of smoke from the smoker into the solvents.

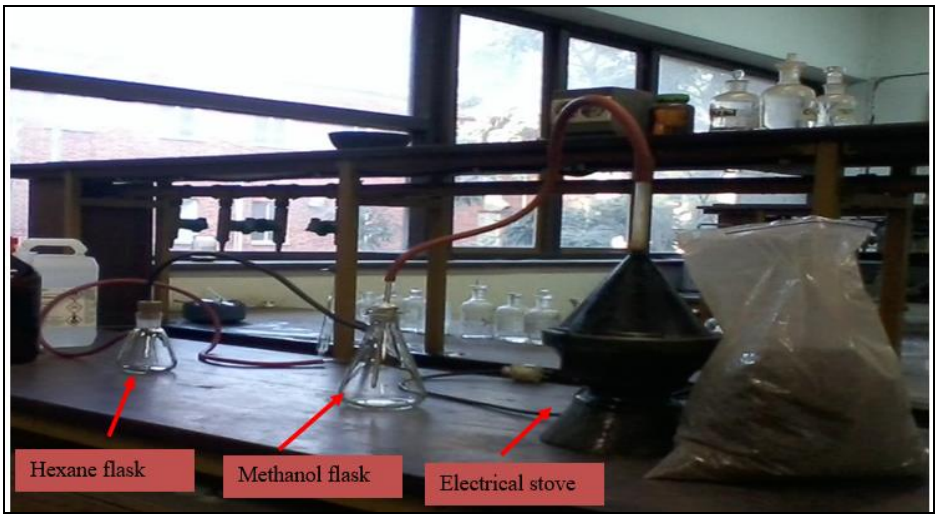


Fig 1: Smoke collection apparatus set-up

The GCMS analysis was carried out using Agilent technologies 7820A GC and 5977E MSD system equipped with auto sampler. Chromatographic separations were carried out using DB-1701 column of 30 m length, 0.25 mm internal diameter and 0.25 µm column phase thickness. Injection mode was split-less, helium was a carrier gas and 1µl volume of the sample was injected to the inlet heated to 275˚C. Initial oven temperature was 65˚C with 1 min hold time then heated to 240˚C with ramp 15˚C/min and 3 min hold time. For the GC-MS analysis, 10 ppm methanol and hexane extracts were injected separately and analyzed (Table 1 and 2). Library search was based on NIST-14 library. The NMR spectra were recorded on Bruker Avance 400 MHz spectrometer. The chemical shifts (δ) are reported in parts per million (ppm) relative to TMS. The δ values are referenced to $CHCl_3$ in $CDCl_3$ at 7.27 ppm. In order to elucidate the structures of the isolated compounds, one dimensional proton (^1H) and carbon (^{13}C) NMR, and IR and UV experiments were conducted.

3. Results and Discussion

The smoke constituents of *Cyperus esculentus* rhizome trapped by n-hexane and methanol, are listed in tables 1 and 2. For the identifications of individual components in the extracts, the mass (GCMS library; NIST-14) and the RI data [11, 12] were used. The calculated RI values were compared with the data reported using the non-polar column (in most cases DB-5). Surprisingly, the RI values for the identified compounds fall between the values reported using non-polar (DB-5, DB-1 etc.) and polar (wax) columns. The trend is consistent for most of the compounds. This could be explained from the point of view that the column type that was used (DB-1701) has characteristic feature of both non polar and polar columns (low/mid-polarity). Except few, most of the data for identified compounds were well matched with the NIST library. For those compounds with low correlation and whose RIs are not available or somehow suspicious, the mass spectra are attached as footnotes of the tables for all interested readers (Figure 2).

Table 1: The smoke constituents of *Cyperus esculentus* rhizome trapped by hexane.

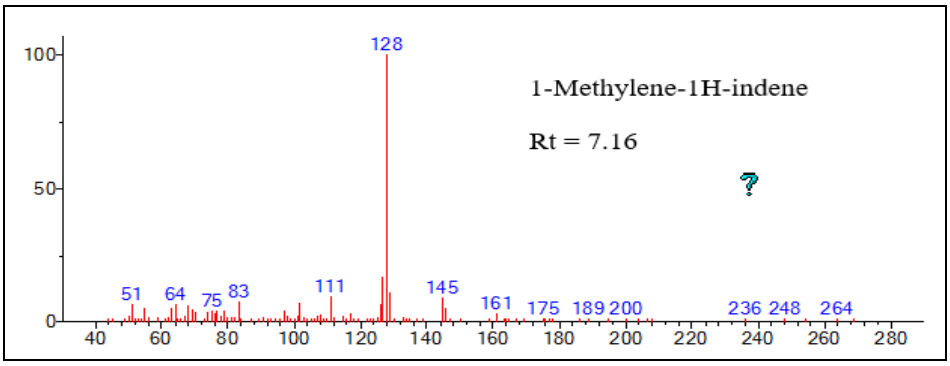
PK#	Name	Formula	MW	t_R	RI Calculated	Ref- RI [11, 12]	Area %	Qual.%
1	β-Pinene	$C_{10}H_{16}$	136	4.24	1015.60	0980	6.70	94
2	Limonene	$C_{10}H_{16}$	136	4.77	1061.32	1024	8.98	99
3	Cymene	$C_{10}H_{13}$	133	4.95	1081.38	1068	7.73	97
4	*p*- α -Dimethylstyrene	$C_{10}H_{12}$	132	5.77	1162.79	1089	1.96	96
5	1-Methylene-1H-indene	$C_{10}H_8$	128	7.16	1304.93	1098	2.54	78
6	Cyprotene	$C_{14}H_{24}$	192	7.71	1365.17	1345	1.68	98
7	1,2,3,4-tetrahydro-1,4,6-trimethylnaphthalene	$C_{13}H_{18}$	174	7.80	1375.03	N/A	1.50	45
8	Eudesma-1,4(15),11-triene	$C_{15}H_{22}$	202	8.12	1410.67	1472	1.47	60

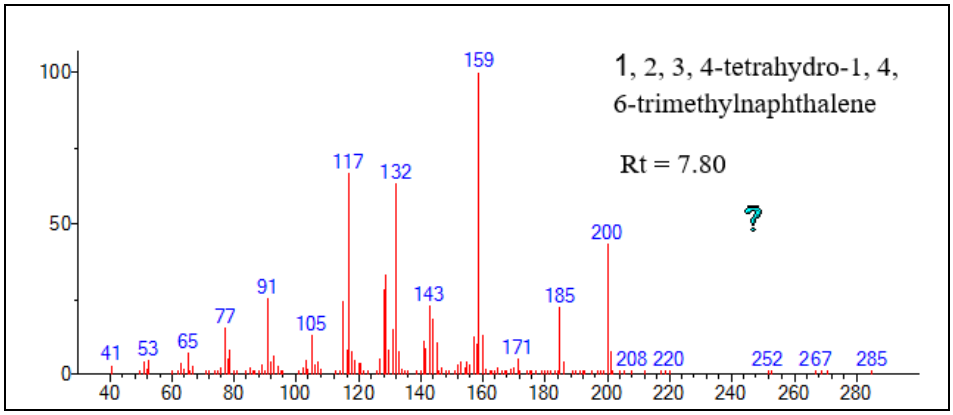
9	α-Copaene	$C_{15}H_{24}$	203	8.17	1416.47	1374	9.49	98
10	Dehydroaromadendrene	$C_{15}H_{22}$	202	8.24	1424.59	1450	2.62	93
11	Cyperene	$C_{15}H_{24}$	204	8.47	1451.28	1398	30.25	99
12	Cyperadiene	$C_{15}H_{22}$	202	8.59	1465.19	1442	2.15	90
13	Rotundene	$C_{15}H_{24}$	204	9.03	1517.07	1469	5.85	98
14	δ-Guaiene	$C_{15}H_{24}$	204	9.40	1562.19	1502	1.12	98
15	2,4-Quinolinediol	$C_9H_7NO_2$	161	9.71	1602.69	N/A	1.06	76
16	Calamenene	$C_{15}H_{22}$	202	9.74	1603.86	1537	1.16	96
17	α-Calacorene	$C_{15}H_{20}$	200	9.99	1636.04	1544	1.12	98
18	Humulene-1,2-epoxide	$C_{15}H_{24}$	204	10.98	1765.65	1601	1.83	46
19	Cadalene	$C_{15}H_{18}$	198	11.27	1803.85	1676	2.46	98
20	Mustakone	$C_{15}H_{22}O$	218	11.94	1884.38	1676	1.58	27
21	Rotundone	$C_{15}H_{22}O$	218	12.02	1893.99	1703	0.89	25
22	Cyperotundone	$C_{15}H_{22}O$	218	12.06	1898.80	1695	2.71	99
23	5-Eicosene	$C_{20}H_{40}$	280	12.64	2003.75	1988	1.57	95

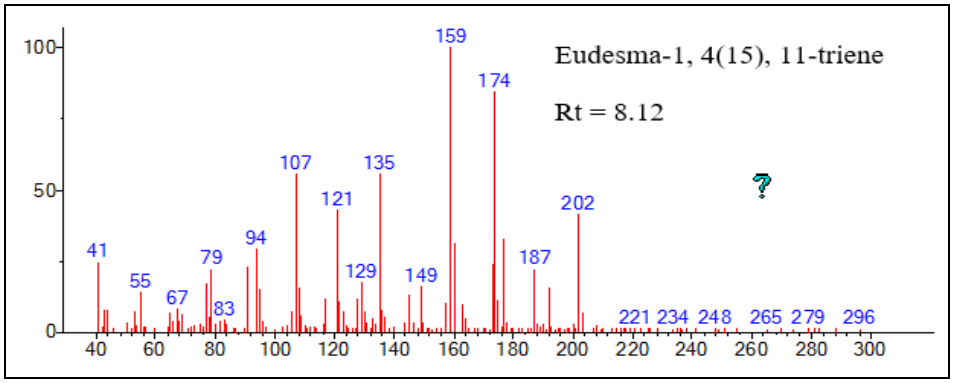
Table 2: The smoke constituents of *Cyperus esculentus* rhizome trapped by methanol.

PK#	Name	Formula	MW	t_R	RI calculated	Ref- RI [11, 12]	Area %	Qual.%
1	Phenol	C_6H_6O	94	6.31	1215.97	979	4.57	90
2	2-Methoxy phenol	$C_7H_8O_2$	124	6.56	1242.07	1089	5.12	94
3	3-methylphenol	C_7H_8O	108	7.16	1304.93	1076	5.57	96
4	4-Ethylphenol	$C_8H_{10}O$	122	7.99	1395.84	1166	3.86	94
5	*p*-Ethylguaiacol	$C_9H_{12}O_2$	152	8.34	1436.19	1287	4.20	90
6	Cyperene	$C_{15}H_{24}$	204	8.47	1451.28	1398	5.67	99
7	Coumaran	C_8H_8O	120	8.78	1477.96	1237	7.84	74
8	*p*-Vinylguaiacol	$C_9H_{10}O_2$	150	8.86	1496.51	1309	11.57	95
9	Syringol	$C_8H_{10}O_3$	154	9.32	1552.44	1362	3.41	97
10	Caryophyllene oxide	$C_{15}H_{24}O$	220	11.24	1800.24	1583	5.13	51
11	Caryophylla-4(12),8(13)-dien-5α-ol	$C_{15}H_{24}O$	220	11.27	1803.85	1627	4.46	62
12	Alloaromadendrene oxide	$C_{15}H_{24}O$	220	11.41	1820.67	1646	5.15	90
13	Mustakone	$C_{15}H_{22}O$	218	11.95	1885.58	1676	3.43	87
14	Rotundone	$C_{15}H_{22}O$	218	12.02	1893.99	1703	3.08	55
15	Cyperotundone	$C_{15}H_{22}O$	218	12.07	1920.84	1718	5.63	99
16	5-Eicosene	$C_{20}H_{40}$	280	12.64	2003.75	1988	6.67	96
17	Isoaromadendrene epoxide	$C_{15}H_{24}O$	220	13.09	2071.21	N/A	4.28	47
18	Clovanediol	C15H26O2	238	13.72	2154.14	N/A	5.62	27
19	Corymbolone	C15H24O2	236	14.02	2191.22	N/A	4.74	38

N/A = Not available in reference materials



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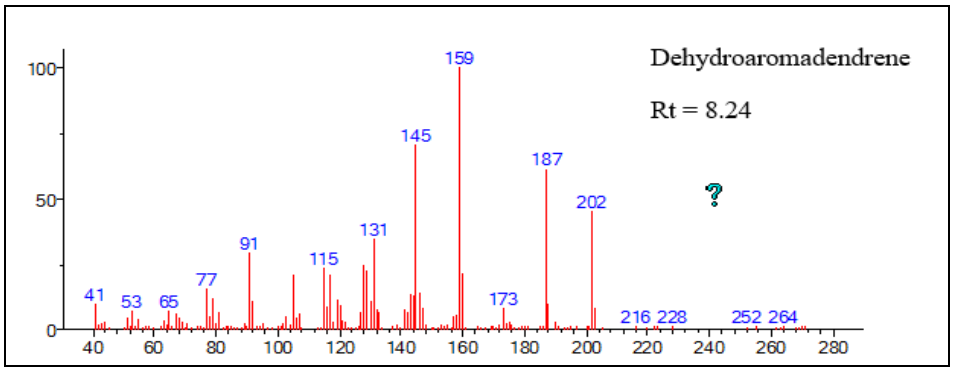
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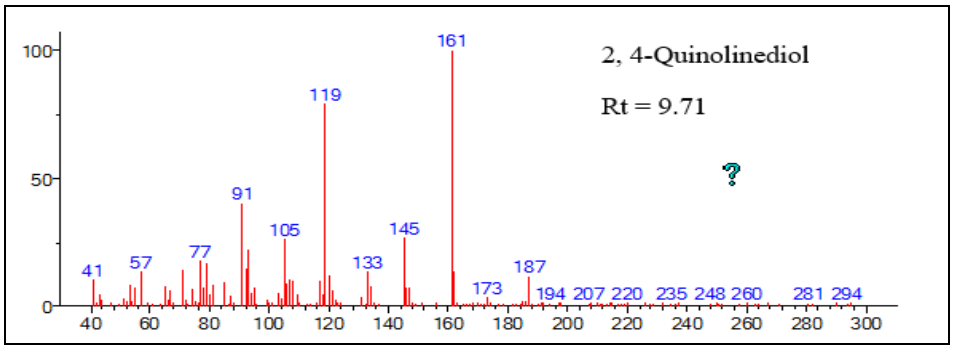
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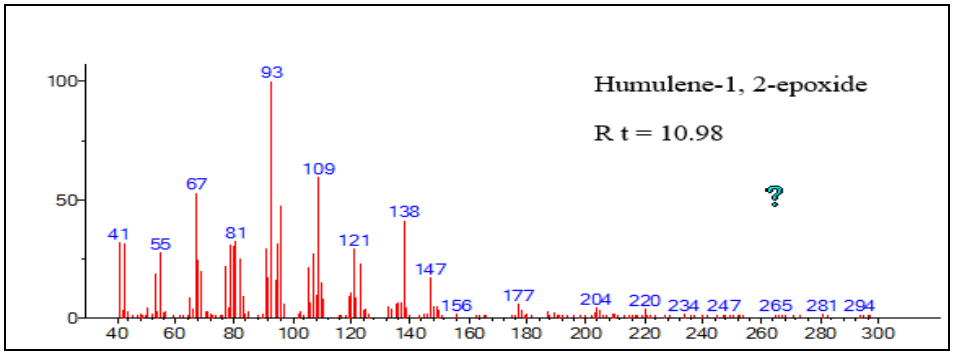
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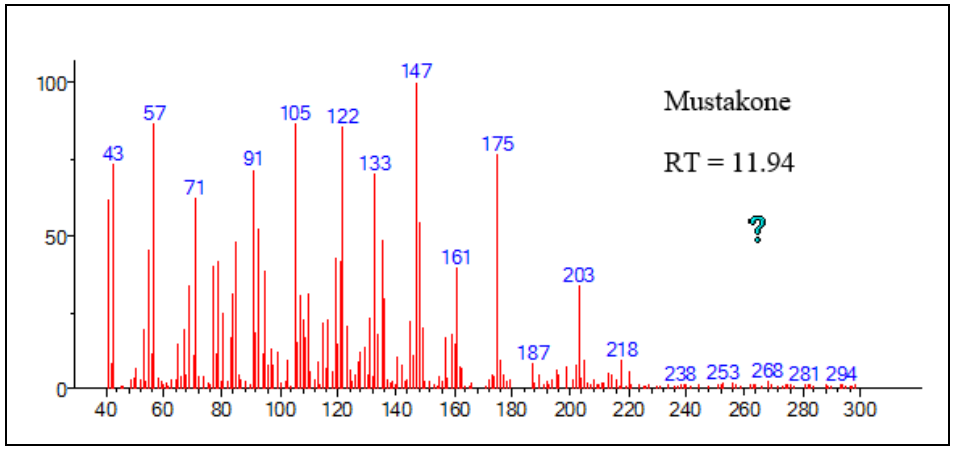
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RT = 11.94
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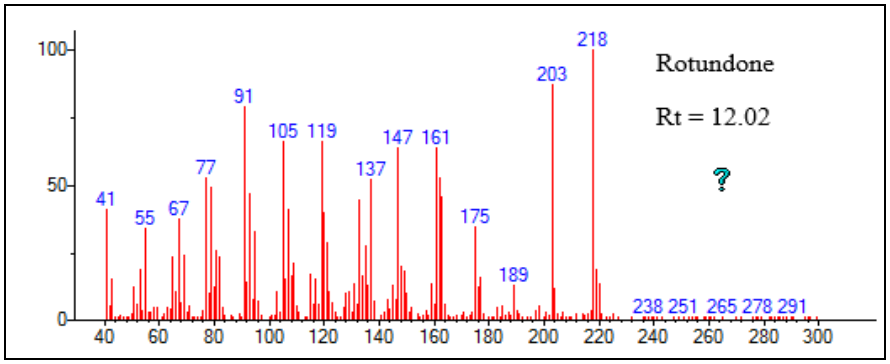
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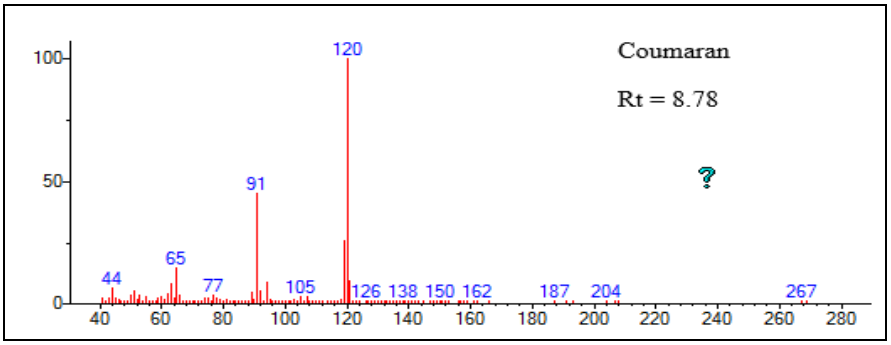
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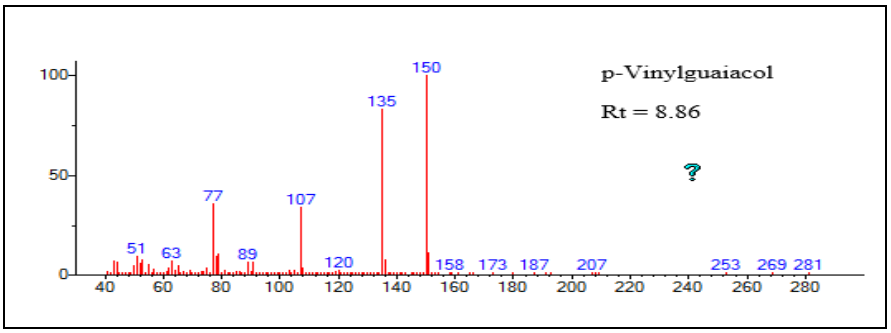
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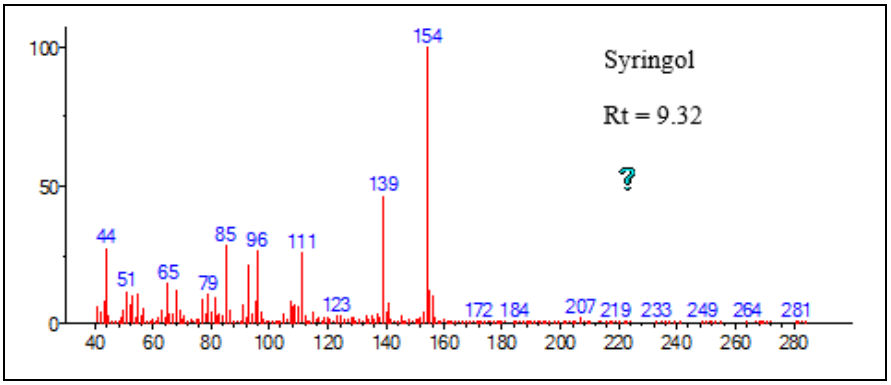
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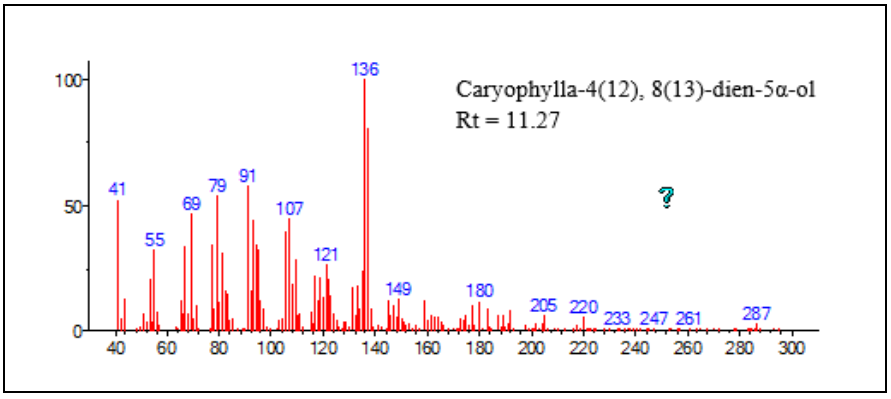
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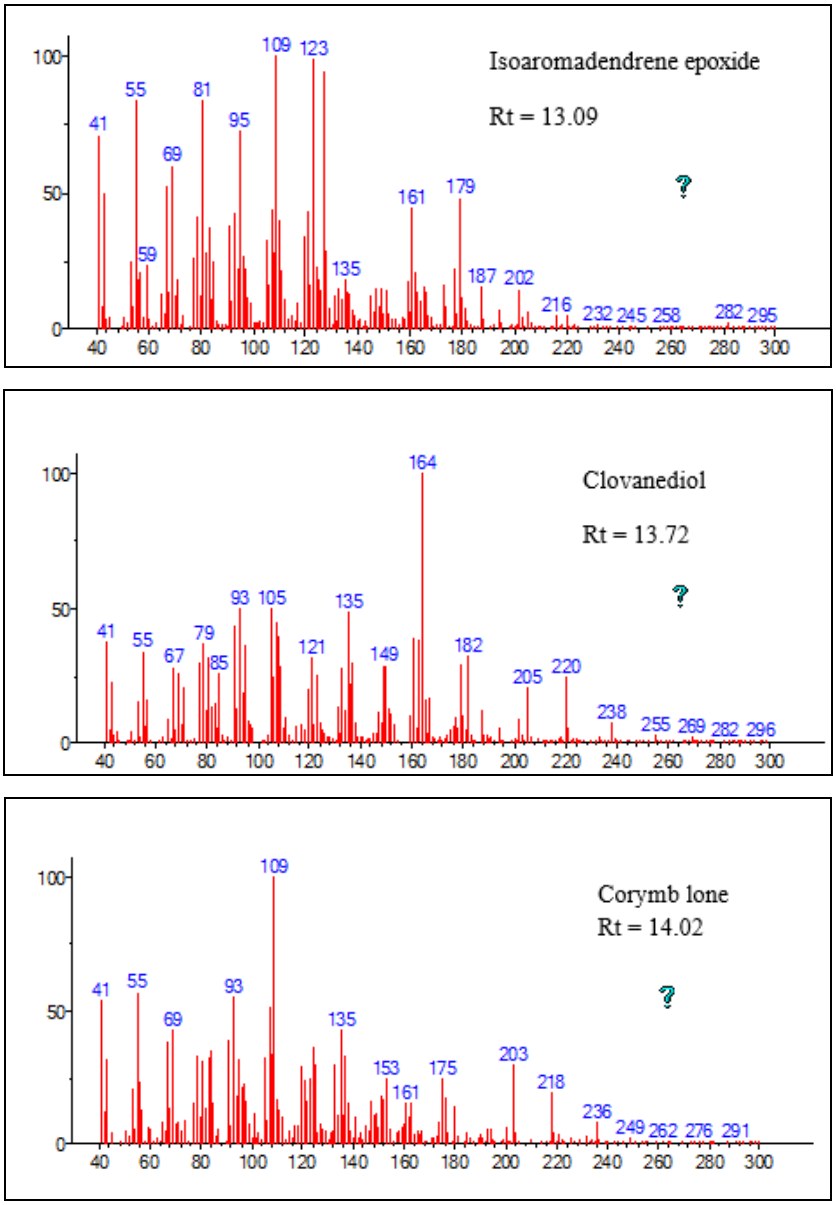

Fig 2: Mass spectra of some of the identified compounds the biological activities of some of the detected compounds are summarized in the table below.

Table 3: Biological activities of major compounds

S. No	Name of the compound	Compound nature	Biological activity	References
1	β-Pinene	Monoterpene	antimicrobial	[13]
2	Limonene	Monoterpene	antimicrobial	[14]
3	Cymene	Alkyl benzene/monoterpene	antioxidant, anti-inflammatory, antinociceptive, anxiolytic, anticancer and antimicrobial	[15]
4	α-Copaene	Tricyclic sesquiterpene	antioxidant	[16]
5	Cyperene	Tricyclic sesquiterpene	antimicrobial	[17]
6	Rotundene	Sesquiterpene	no activity reported	
7	Phenol	Phenolic	antiseptic, antioxidant, antimicrobial and disinfectant	[18]
8	2-Methoxy phenol	Phenolic	antiseptic, antioxidant, expectorant and local anesthetic	[18]
9	3-methylphenol	Alkyl benzene	flavouring agent	[19]
10	Cyperene	Tricyclic sesquiterpene	antimicrobial	[17]
11	Coumaran	Aromatic	biofumigant, antihelminthic, anti-inflammatory, antidiarrhoeal	[20, 21]
12	*p*-Vinylguaiacol	Phenolic	flavoring agent, antioxidant, antimicrobial, anti-inflammatory analagesic,	[22, 23]
13	Caryophyllene oxide	Oxygenated sesquiterpene	antitumor, anesthetic, antibacterial, anti-inflammatory, analagesic, antioxidant	[21, 24]
14	Alloaromadendrene oxide	Oxygenated sesquiterpene	no activity reported	
15	Cyperotundone	Oxygenated sesquiterpene	anti-inflammatory, antimicrobial, antiallergic, antioxidant	[25]
16	5-Eicosene	Alkene	no activity reported	
17	Clovanediol	Oxygenated sesquiterpene	no activity reported	
18	Corymbolone	Oxygenated sesquiterpene	no activity reported	

Three compounds were isolated, using chromatographic techniques, from the smoke condensate of *Cyperus esculentus* rhizome. Structures of the isolated compounds were determined using spectroscopy and chromatographic techniques such as UV, FTIR, NMR, and GCMS.

Compound 1 (*p*-Vinylguaiacol or 2-Methoxy-4-vinylphenol):

Compound 1 was isolated as a white gelatin material from the smoke trapped in methanol by using column chromatography. The UV-VIS spectrum showed maximum absorption at 260 which indicates the presence of π-π* transition of C=C double bond of the benzene ring. The FTIR spectrum data of the isolated compound 1 showed a strong absorption band at 3422.48 cm^{-1} due to O-H stretching. The strong and sharp absorption band at 2921.70 cm^{-1} is an indication for C-H stretching. The strong and sharp band at 1613.28 cm^{-1} stands for a conjugate system.

To fully elucidate the structure, one dimensional NMR was used and the data was compared with literature data [26] (Table 4). It was proposed that the isolated compound is 2-methoxy-4-vinylphenol (Figure 3).

Table 4: NMR spectral data of the isolated compound together with literature data of 2-methoxy-4-vinylphenol (*p*-Vinylguaiacol**)** [26].

C No	NMR Data of compound 1		Literature data for 2-methoxy-4-vinylphenol		Remark
	^{1}H NMR (ppm)	^{13}C NMR (ppm)	^{1}H NMR (ppm)	^{13}C NMR (ppm)	
C_1	----------	145.63	----------	145.78	Quaternary
C_2	----------	146.58	----------	146.77	Quaternary
C_3	6.93, m	108.17	6.90, m	108.21	-CH-
C_4	----------	130.28	----------	130.40	Quaternary
C_5	6.93, m	120.22	6.90, m	120.20	-CH-
C_6	6.93, m	114.49	6.90, m	114.55	-CH-
C_7	6.64, dd, J=16, 8 Hz	136.77	6.65, dd, J=17.6, 10.8 Hz	136.78	H_a
C_8	5.14, dd, J=10.8, 0.8 Hz		5.14, dd, J=10.8, 0.8 Hz		H_b
C_8	5.61, dd, J=17.6, 0.8 Hz	111.60	5.60, dd, J= 17.6, 0.8 Hz	111.57	H_c
C_{10}	3.91, s	56.03	3.90, s	56.01	O-CH_3
	5.67, s		---------		hydroxyl proton

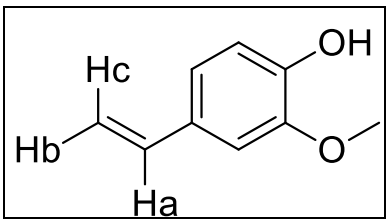

Fig 3: Chemical structure of compound 1

Compound 2 (Vanillin or 4-hydroxy-3-methoxybenzaldehyde)

Compound **2** was obtained as white solid with distinct aroma character from the methanol condensate. The UV-Vis spectrum showed absorption maxima at 228 nm, 276 nm and 308 nm, which indicates the presence of π-π* transition of C=C double bond and n-π* transition of C=O bond.

The FTIR spectrum data of compound 2 showed a weak and broad absorption band at 3406.44.48 cm^{-1} due to O-H stretching. The strong and sharp absorption band at 2923.04 cm^{-1} was an indication of C-H stretching of an aldehyde. Moreover, the strong and sharp band at 1677.97 cm^{-1}indicates the presence of α, β-unsaturated carbonyl system in the compound. Finally the identity of the compound was determined using one dimensional NMR and by comparing with literature data, to be vanillin (Table 5, Figure 4).

Table 5: NMR spectral data of compound 2 together with literature data of 4-hydroxy-3-methoxy benzaldehyde (Vanillin) [27].

C No	NMR Data of Compound 2		Literature data for Vanillin		Remark
	^{1}H NMR (ppm)	^{13}C NMR (ppm)	^{1}H NMR (ppm)	^{13}C NMR (ppm)	
C_1	9.85, s	191.05	9.83, s	191.15	Aldehyde proton
C_2	---------	130.00	---------	130.00	Quaternary
C_3	7.44, s	108.87	7.417, s	108.80	-CH-
C_4	----------	141.85	----------	147	Quaternary
C_5	----------	147.3	----------	151	Quaternary
C_6	7.06, d, J=8.2	114.53	7.04, d, J=8.5	114.5	-CH-
C_7	7.46, d, J=8.2	127.72	7.43, d, J=8.5	127.8	-CH-
C_{10}	3.99, s	56.26	3.90, s	56.3	O-CH_3
	7. 28, s	----------	6.25, s	----------	hydroxyl proton

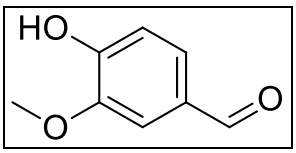

Fig 4: Chemical structure of the isolated compound **2**

Compound 3 (Cyperotundone)

The UV-Vis spectrum of isolated compound **3** from methanol extract showed a maximum absorption band at 245nm, which indicates the presence n-π* transition of a C=O bond.

The FTIR spectrum showed strong and sharp absorption band at 2919.04 cm^{-1} which was due to C-H stretching. Two bands of almost equal intensity at 1709.73 cm^{-1} and 1658.2 cm^{-1} show the presence of α-β-unsaturated ketone. The strong and sharp band at 1381.42 cm^{-1} indicates the presence of methylene group adjacent to carbonyl group in the compound.

Since the ^{1}H-NMR of the compound was not well resolved; structure of the compound was confirmed by using GC-MS to be cyperotundone (Figure 5).

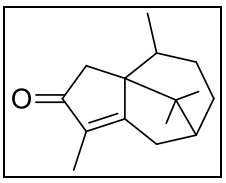

Fig 5: Chemical structure of compound 3

Conclusion

The GC-MS analysis result of the smoke condensate of *Cyperus esculentus* rhizome revealed the presence of hydrocarbons, ketones, alcohols, aldhydes, terpenes, phenols and others. Phenolic and terpene compounds account for the largest percent of the smoke constituents trapped by methanol. As reported in different literatures, many of these compounds exhibit antioxidant, anti-inflammatory, anticancer, antimicrobial, antiseptic etc. activities. The biological activities of the detected major compounds are summarized in table 3. The biological activities related to the identified compounds and the actual situation on the ground (healthy skin and absence of fungal infection among the Borana tribe) are well coincided. This supports the hypothesis that the absence of fungal infections (skin disease) could most likely be due to the smoke from *Cyperus esculentus* rhizome.

Even though several compounds were detected by GC/MS analysis of the hexane and methanol extracts, three compounds (2-methoxy-4-vinylphenole; 4-hydroxy-3-methoxy benzaldehyde; and Cyperotundone) were isolated and characterized.

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References

1. Nautiyal CS, Chauhan PS, Nene YL. Medicinal smoke reduces airborne bacteria. Journal of Ethnopharmacology. 2007; 114 (3):446-451.
2. Grolitsky L. Uses and abuses of plant derived smoke: Its Ethnobotany as Hallucinogen, Perfume, Incense, and Medicine. Journal of medicinally active plants. 2012; 1(1):44-44.
3. Adama KK, Afolayan MO, Obeerafo AA, Sunday T. Isolation and Physicochemical characterization of tiger nut (*Cyperus esculentus*) starch as a potential industrial biomaterial. International Journal of materials science and applications. 2014; 3(2):37-41.
4. Venkatachalam M, Sathe SK. Chemical composition of selected edible nut seeds. Journal of agricultural and food chemistry. 2006; 54:4705-4714.
5. Adejuyitan JA. Tiger nut processing: Its food use and health benefits. American Journal of food Technology, 2011; 6(3):197-201.
6. Rubert J, Monforte A, Hurkova K, Marrtinez GP, Blesa J, Navaroo JL *et al*. Untargeted metabolomics of fresh and heat treatment Tiger nut (*Cyprus esculents* L.) milks reveals further insight in to food qualiity and nutrition. Journal of chromatography A. 2017; 1514:80-87.
7. Patil RT, Prasad VP. A Chene morphology and its taxonomic significance in Cyperaceae of Goa, India:1. Genus Fimbrstycis. India Journal of plant science. 2016; 5(1):87-89.
8. Hussein HJ, Hadi MY, Hammed IH. Study of chemical composition of *Foeniculum vulgare* using Fourier transform infrared spectrometer and gas chromatography-mass spectrometry. Journal of pharmacognosy and phytotherapy. 2016; 8(3):60-89.
9. Karathanos VT, Mourtzinos I, Yannakopoulou K, Andrikopoulos NK. Study of the solubility, anti-oxidant activity and structure of inclusion complex of Vanillin with β-cyclodectrin. Food chemistry. 2007; 101:652-658.
10. Wong W, Zhou Y, Huang X, Zhang Q. Preparative isolation and purification of cyperotundone and α-cyperone from *cyperus rotundus* Linn. With high speed counter-current chromatography. International Journal of Medicinal and Aromatic plants. 2013; 3(2):163-168.
11. Adams RP. Identification of essential oil components by gas chromatography/mass spectrometry. Electronic version of the original 4th ed. printed by Allured publishing, 2007.
12. NIST. ttps://webbook.nist.gov/cgi/
13. Ana Cristina RS, Paula ML, Mariana MBS, Danielle CMC, Celuta SA, Daniela SA. Biological Activities of α-Pinene and β-Pinene Enantiomers. Molecules. 2012; 17:6305-6316.
14. Ana R, Marina S, Leo Van G, Jelena V, Dejan B, Mihailo SR. Antimicrobial action of limonene. Lek. Sirov. 2003; 103(23):83-88
15. Anna M, Carla RA, Ramona B, Ana SS, Seyed FN, Arold JTS, *et al*. Update on Monoterpenes as Antimicrobial Agents: A Particular Focus on p-Cymene. Materials. 2017; 10:947-962.
16. Hasan T, Kubra C, Basak T. Effects of copaene, a tricyclic sesquiterpene, on human lymphocytes cells in vitro. Cytotechnology. 2014; 66:597-603.
17. Nabil AEA, Ayman YE, Ayman AF. Chemical composition and biological activity of the essential oil of cyperus articulates. International journal of academic research. 2014; 6(5):264-269
18. Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, *et al*. Drug Bank 5.0: a major update to the Drug Bank database for 2018. Nucleic Acids Res. 2017. doi: 10.1093/nar/gkx1037.
19. WHO. Evaluations of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). 2018.
20. Yallappa R, Anjanappa R, Nandagopal B. Acetylcholinesterase inhibition by biofumigant (Coumaran) from leaves of *Lantana camara* in stored grain and household insect pests. BioMed Research International. 2014; 1:1-6.
21. Pressy PP, Ashalatha SN. Chemical fingerprint of essential oil components from fresh leaves of *Glycosmis pentaphylla* (Retz.) Correa. The Pharma Innovation Journal. 2015; 3(12):50-56.
22. Vijisaral ED, Arumugam S. GC-MS analysis of the ethanol extract of *Cyperus rotundus* leaves. International Journal of Current Biotechnology. 2014; 2(1):19-23.
23. Joint FAO/WHO Expert Committee on Food Additives. www.inchem.org/.
24. National Center for Biotechnology Information. PubChem Compound Database; CID=332, https://pubchem.ncbi.nlm.nih.gov/compound/332 (accessed Dec 29, 2018).
25. Kumar A, Chahal KK, Kataria D. A review on phytochemistry and pharmacological activities of *Cyperus scariosus*. Journal of Pharmacognosy and Phytochemistry. 2017; 6(1):510-517.

26. Marty HS, Misra K, Mahata T, Nag A. CSJ acting as a versatile highly efficient greener resource for organic transformation. Royal Society of chemistry advances. 2016; 6:24446
27. Handayami S, Arianingrum R, Haryadi W. Vanillin structure modification of isolated vanilla fruit to from vanillinacetone. Proceeding of 14th Asian chemical congress. 2011, 252-257.