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Essential oil and smoke components of *Indigofera Arrecta*

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ABSTRACT: *Indigofera arrecta,* commonly known as Buk-Buk (Amharic) in Ethiopia, is an important traditional medicinal plant among Ethiopians which is used for the treatment of different ailments. In this study, major components of the essential oil and the smoke obtained from burned *I. arrecta* were investigated. The smoke from burnt plant material was trapped in methanol and hexane solvents. Using GC-MS, about seventeen compounds were identified from the essential oil, which accounted for 95.10%. Octylacetate (28.54%) was the major component of the essential oil. On the other hand, the solid (sublimed) material obtained during hydrodistillation showed six compounds with a total percent area of 94.88%. The sublimed material was dominated by 1,2,4,5-tetrachloro-3,6-dimethoxybenzene, which accounted for 88.36%. The smoke of *I. arrecta* showed nine compounds with a combined area of 100%, of which 2, 4-di-tert-butylphenol (31.3%) and 1,2,4,5-tetrachloro-3,6-dimethoxybenzene (25.10%) compounds were the major components. The crude smoke extracts were also examined for their antioxidant activity by using 2,2-dipheneyl-1-picrylhydrizyl (DPPH). The smoke derived from *I. arrecta* showed 93.52 ± 0.09 DPPH inhibition activities at the concentration of 100 µg/mL which is closer in activity to the standard ascorbic acid. This shows a strong relationship between use of the plant smoke as medicinal incense and its antioxidant activity.

Keywords/ phrases: - anti-oxidant activity, essential oil, *Indigofera Arrecta,* medicinal smoke

INTRODUCTION

Indigofera arrecta (I. arrecta) is a flowering plant belonging to the genus *Indigofera* of the family Fabaceae (Dzoyem, McGaw *et al.* 2014). *I. arrecta* was originated from East, Central, West and Southern part of Africa and has been introduced in Laos, Vietnam, the Northern end of Philippines that is Luzon and Swaziland (Verbelen and Dossche 2015). *I. arrecta* has an indigo dye pigment which obtained from the leaflets and branches of the plant parts through fermentation (Gabriel and Onigbanjo 2010). In addition, it is used for medicine, ornamentals purposes, for erosion control, crop shading, feeding livestock, and food. *I. arrecta* is a large shrub plant that grows to a height of 3 m, erect and woody. The leaves are spirally arranged, 2 to 9 mm long, and have about 7 to 21 leaflets, usually smooth above and hairy beneath (Orwa 2009). A flower is about 5 mm long and has a pinkish, reddish, or brown color. The fruit of *I. arrecta* is a linear pod about 12 to 25 mm long and 2 mm wide, brown when ripe, and has four to eight seeds (Foden and Potter 2005).

I. arrecta is utilized as a traditional cure for treatment of ring worm, skin mycosis (Ngezahayo, Havyarimana *et al.* 2015), abdominal pain (Stangeland, Alele *et al.* 2011), anti-stomachache (Giday, Asfaw *et al.* 2010), snake bites, diphtheria, vertigo, dysentery (Tabuti, Lye *et al.* 2003) and also for the treatment and prevention of diabetes (Gyamfi, Yonamine *et al.* 1999).The leaves and roots of the plant were reported as effective to treat gum infections, gonorrhea, epilepsy and jaundice. In Ghana, leaves from young shoots is administered orally to patients with diabetes mellitus (Cos, Hermans *et al.* 2002), hepatitis, leprosy, malaria, scabies (Vlietinck, Van Hoof *et al.* 1995) and mental illness, enlargement of the liver

and spleen. It is mixed with honey, to promote the action of the bowel in children (Chhabra, Mahunnah *et al.* 1990). The phytochemical analysis of *I. arrecta* indicated the isolation of secondary metabolites such as tannins, flavonoids, alkaloids, saponnins, phenolic groups, glycosides, steroids and triterpenes (Parekh, Karathia *et al.* 2006).

Because of enormous prospective of aromatic plants as sources of radical scavenging activity and there is no prior scientific report on the chemical composition and bioactivity of the smoke from the stem of *I. arrecta,* the current investigation was conducted to determine the main constituents and the antioxidant activities of the essential oil and smoke of *I. arrecta*.

MATERIALS AND METHODS

Plant material

I. arrecta stem sample was purchased from a local market of Addis Ababa, Ethiopia. The identity of the plant material was confirmed at the Department of biology, Addis Ababa University.

Figure 1: Photo of *I. arrecta*

Chemicals and reagents

The chemicals used to conduct this research were standard ascorbic acid; dichloromethane (Fisher Scientific, UK), methanol (>99.7 %, Sigma-Aldrich, USA), n-hexane (99 %, Labachemic Pvt. Ltd, India), DPPH (ALPHA CHEMIKA, India) and anhydrous sodium sulfate were used.

Materials

In this study, mortar and pestle were used for grinding and homogenizing the sample. A digital balance, measuring cylinder, pipettes and micropipettes were used for measuring the sample and solvents. A round bottom flask (JLASSCO, Borosilicate) was used for distillation, and Clevenger apparatus was used for collecting essential oil and solid material. During the trapping and extraction of smoke products, an electrical stove, wire gauze, spatula, suction flasks (250 mL), rubber tube, inverted glass funnel, and various sizes of Erlenmeyer flask were used. The rotary evaporator (Heildolph instruments, Gmbh & Co. KG, Germany) was used for concentrating the sample.

Instrumentation

The essential oil and smoke extract were analyzed by Gas Chromatography Mass Spectrometry (GC-MS). GC-MS analysis was conducted on an Agilent Technology 7820A GC system coupled with an Agilent Technology 5977E MSD, USA. The essential oil and smoke components were identified via a library search using NIST-2014.

The DPPH assay was carried out with a UV–Vis–NIR spectrophotometer. The UV-Visible absorbance of the crude methanol extract and that of the prepared solutions were done using two side transparent quartz cuvette. The data obtained from crude mixture of plant material was analyzed by using Origin software (version 2019).

Sample preparation

Extraction of essential oil from *Indigofera arrecta*

Essential oil of *I. arrecta* was obtained by hydro distillation. The dry powder sample of *I. arrecta* (500 g) was placed into a distillation flask containing distilled water (3 L). The flask was attached to a Clevenger apparatus, which was attached to a condenser. Hydro distillation continued for 6 hours after initial boiling. During the process, a white solid (sublimed material) was distributed all over the distillation set up. The solid material (1.548 g, 0.310%) and oil were collected separately; the essential oil and the solid material were stored in a refrigerator until they were analyzed by GC-MS.

Smoke collection

The *I. arrecta* stem was chipped off into small pieces. The plant material (300 g) was burned using an electrical stove (**Figure 1**). The smoke produced from the burning plant material was collected using inverted funnel fitted with a heat resistance rubber tube as described in Melaku *et.al* (Sisay, Yaya *et al*. 2022). The advantage of the suction flask that contains hexane was to trap smoke components that escape from the methanol containing flask. The mixture (methanol and hexane) extracts were concentrated over rotary evaporator to yield (0.737 g, 0.246%) then the crude extract was stored in refrigerator until it was analyzed by GC-MS. The percent yield of hexane and methanol extracts derived from smoke was calculated using **Equation 1**.

$$Percent\ yield = \frac{Weight\ of\ crude\ extract}{Weight\ of\ plant\ material} \times 100 \quad \textbf{(1)}$$

The retention index of the different components in the essential oil and the smoke derived from *I. arrecta* was calculated as described in Melaku et. al. (Sisay, Yaya et al. 2022) using a mixture of n-alkane with the same experimental condition as that of the sample analysed. The retention index was calculated using **Equation 2**.

$$RI = 100n + 100\left(\frac{R_{t(unknown)} - R_t}{R_{t(n+1)} - R_{t(n)}}\right) \quad \textbf{(2)}$$

Where RI is retention index of the analyte, n is number of carbon atom eluting before the analyte, $R_{t\ (unknown)}$ is the retention time of the analyte, $R_{t\ (n+1)}$ is retention time of the reference elute after the analyte and $R_{t\ (n)}$ is retention time of the reference elute before the analyte

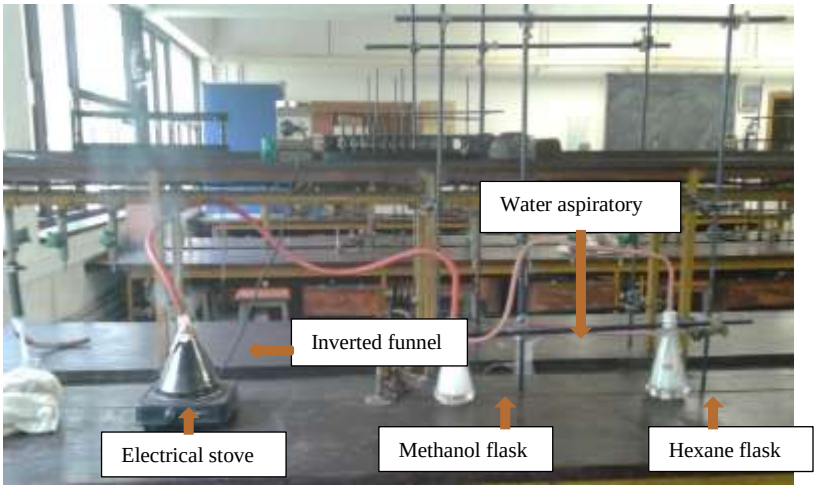


Figure 1: Smoke collection apparatus set up (Sisay, Yaya et al. 2022)

Sample preparation for GC-MS analysis

Preparation of essential oil solution and the smoke extract

In this study, 1000 µg/mL stock solutions were prepared for both the essential oil and smoke extract. From the stock solution, 20 µg/mL solution was prepared via serial dilution method and analysed by GC-MS.

Antioxidant activity determination

Antioxidant activity was measured using DPPH (2,2-diphenyl-1-picryl-hydrazylhydrate). The plant extract was weighed and dissolved in

methanol to get all the necessary working concentrations according to the procedure described in (Sisay, Yaya et al. 2022). The changes in color (from deep-violet to light-yellow) were measured at 517 nm on a UV-Visible light spectrophotometer (Yamaguchi, Takamura *et al.* 1998).

Preparation of working solution

The plant sample of different concentrations (6.5, 12.5, 25, 50, 100 and 200 µg/mL) were prepared in methanol from stock solution (2000 µg/mL)**.** 0.004% DPPH reagent was prepared by dissolving 0.01 g of DPPH in 250 mL of methanol. DPPH and sampelsolutions of different concentrations were mixed and the mixture was kept in dark for 30 minutes before the analysis (Sisay, Yaya et al. 2022). Absorbances of the solutions were then recorded at 517 nm using a UV-Vis spectrophotometer. The radical scavenging activity of the extract was measured using absorbances by ascorbic acid solutions as reference. All the measurements were performed in triplicates. The scavenging effect of DPPH free radical was calculated by using **Equation (3)**.

$$\%DPPH = \frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100 \quad \textbf{(3)}$$

Where $\mathbf{A}_{\text{blank}}$ is the absorbance of DPPH solution in methanol and $\mathbf{A}_{\text{sample}}$ is the absorbance of the test sample plus DPPH solution.

GC-MS analysis of the essential oils and the smoke extract

The amount of each component of the essential oil, solid material and smoke trapped were determined based on peak area obtained from the chromatogram. Selection of peaks was based on relative quality information obtained from NIST-14 library. Peak purity and identity check was done both automatically using the software and also manually by selecting different parts of a peak and comparing with data stored in the NIST-14 library. Number of individual components in chromatogram of different samples was compared by considering peak area, retention time and retention index.

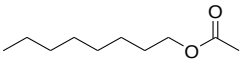
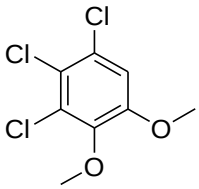
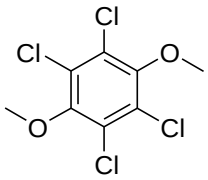
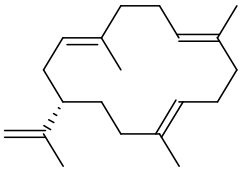
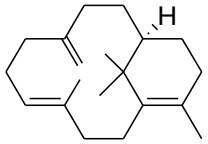
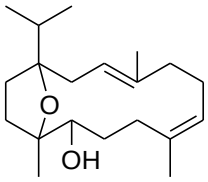
RESULTS AND DISCUSSION

Essential oil, solid material, and the smoke derived from Indigofera arrecta

[During the hydro distillation of *I. arrecta* stem, sublimed white solid, was condensed all over the Clevenger, and essential oil was collected over the water. The formation of solids was an unusual phenomenon that interested us to study it separately. The GC-MS analysis data of the solidified material **(Table 1)** revealed that it is composed of six compounds with a total percent area of 94.88. The solid part was dominated by 1,2,4,5-tetrachloro-3,6-dimethoxybenzene which accounted for 88.36% and reported to be toxic (Bidleman, Andersson *et al.* 2023).

The intense peak at 17.24 min represents the presences 1,2,4,5-tetrachloro-3, 6-dimethoxy benzene the most abundant compound in the mixture. Table 2 shows the chemical compositions of essential oil constituents of *I. arrecta.* The GC-MS analysis result of essential oil of *I. areecta* showed seventeen compounds which accounted for 95.10% of the total constituents. The oil was dominated by octylacetate (28.54%) and hexacosane (18.80%). The dominant compound, octylacetate, has been reported as an antibacterial agent (Afshar, Bakhshandeh *et al.* 2017). Other identified compounds were chlorinated methoxy benzene (6.73%), monoterpenoid (1.11%), aliphatic alcohol (2.03%) and ketone (1.66%). As shown in **Figure 3,** large intense peaks at 10.03 min were due to octylacetate

Table 1. Chemical composition of sublimed mixture.

PK	Compound	Structure	Formula	RT	A%	RI	Q
1	Octylacetate		$C_{10}H_{20}O_2$	10.03	1.37	1276.20	91
2	1,2,3-Trichloro-4,5-dimethoxybenzene		$C_8H_7C_{l3}O_2$	16.46	1.12	1800.25	76
3	1,2,4,5-tetrachloro-3,6-dimethoxybenzene		$C_8H_6C_{l4}O_2$	17.24	88.36	1863.96	62
4	(R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-yl)cyclotetradeca-1,5,9-triene		$C_{20}H_{32}$	19.43	0.77	2029.59	99
5	(S,E)-8,12,15,15-Tetramethyl-4-methylenebicyclo[9.3.1]pentadeca-7,11-diene		$C_{20}H_{32}$	20.45	1.53	2105.50	99
6	Isopropyl-1,5,9-trimethyl-15-oxabicyclo[10.2.1]pentadeca-5,9-dien-2-ol		$C_{20}H_{34}O_2$	24.39	1.73	2398.44	96

Pk=peak number, RT=retention time, RI=retention index, A=area, Q=NIST matching quality

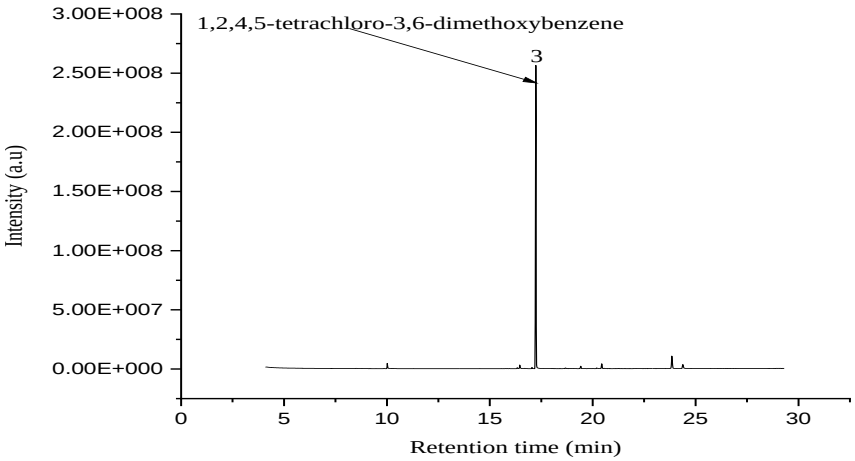
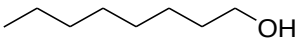
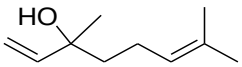
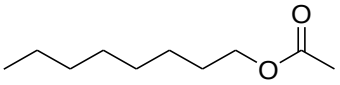
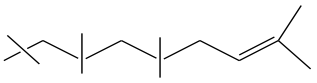
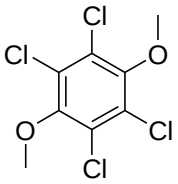
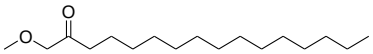
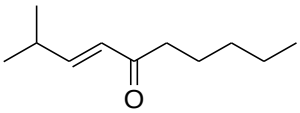
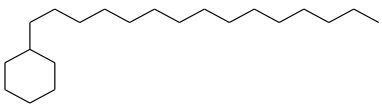
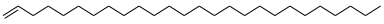


Figure 2: GC-MS Chromatogram of the sublimed compounds of *I. arrecta*

Table 2. Chemical composition of essential oil constituents of *Indigofera arrecta.*

Pk	Compound	Structure	Formula	RT	A%	RI	Q
1	1-Octanol		$C_8H_{18}O$	8.52	2.03	1172.63	91
2	3,7-Dimethylocta-1,6-dien-3-ol		$C_{10}H_{18}O$	8.79	1.11	1190.44	86
3	Octylacetate		$C_{10}H_{20}O_2$	10.03	28.54	1276.20	91
4	Heptadecane		$C_{17}H_{36}$	15.27	0.54	1691.04	90
5	2,4,4,6,6,8,8-Heptamethyl-2-nonene		$C_{16}H_{32}$	15.56	0.82	1717.17	47
6	Octadecane		$C_{18}H_{38}$	16.36	0.92	1790.91	98
7	1,2,4,5-Tetrachloro-3,6-dimethoxy Benzene		$C_8H6Cl_4O_2$	17.24	6.73	1863.64	99
8	Eicosane		$C_{20}H_{42}$	17.55	0.63	1889.19	97
9	Methyl hexadecanoate		$C_{17}H_{34}O_2$	18.94	2.34	1993.75	98
10	2-Methyldec-3-en-5-one		$C_{11}H_{20}O$	19.58	1.66	2040.74	50
11	Heneicosane		$C_{21}H_{44}$	20.41	2.30	2103.12	97
12	Nonadecane		$C_{19}H_{40}$	22.11	3.44	2229.15	95
13	Pentadecylcyclohexane		$C_{21}H_{42}$	23.97	7.11	2367.44	97
14	Tetracosane		$C_{24}H_{50}$	25.96	13.56	2515.47	95
15	1-Hexacosene		$C_{26}H_{52}$	26.86	2.53	2582.68	92
16	Hexacosane		$C_{26}H_{54}$	28.04	18.80	2670.41	98
17	Pentatriacontane		$C_{35}H_{72}$	28.73	2.04	2722.83	91

Pk =peak number,RT = retention time, RI = retention index, A=area ,Q = NIST matching quality

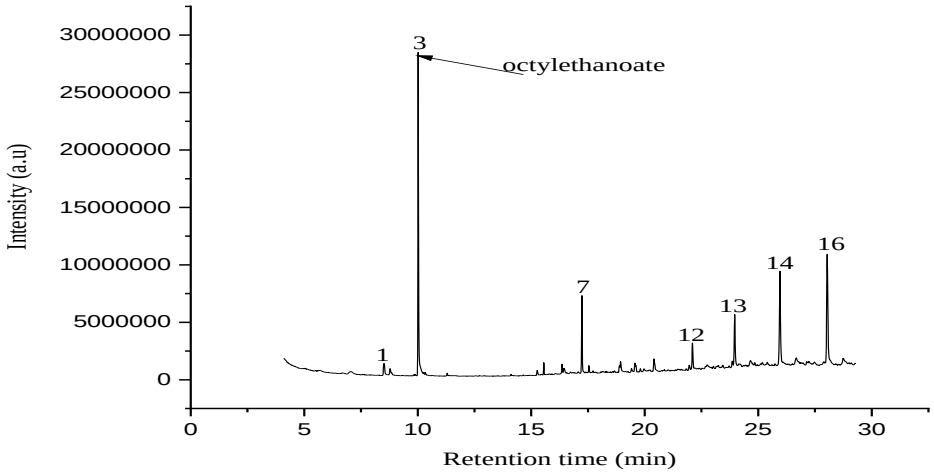


Figure 3: Chromatogram of essential oil constituents of *I. arrecta*

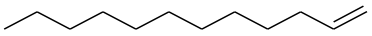
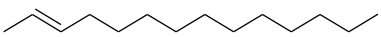
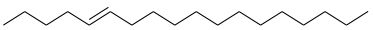
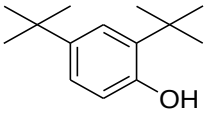
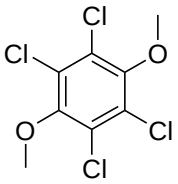
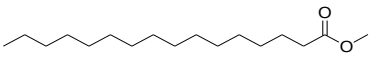
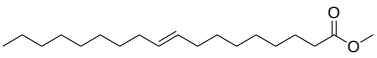
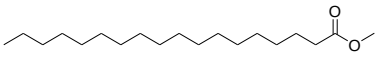
The *I. arrecta* smoke extract revealed nine compounds with a combined area of 100%. From **Table 3,** a phenolic compound and a methoxy benzene, namely 2,4-di-tertbutylphenol (31.3%) and 1,2,4,5-tetrachloro-3,6-dimethoxy benzene(25.10%) were determined as major components of the crude mixture. Other identified compounds were aliphatic esters (21.37%) and unsaturated hydrocarbons (22.23%). The 2,4-di-tertbutylphenol has been reported as anti-inflammatory, anti-bacterial, anti-viral, auto toxicity, anti-fungus, cytotoxicity and antioxidant compound (Zhao, Wang *et al.* 2020).

Figure 4 shows the GC-MS chromatogram of compounds identified in *I. arrecta* smoke extract**.** The intense peak at 15.09 min was due to 2,4-di-tert-butylphenol. The peak at 17.24 min represent 1,2,4,5-tetrachloro-3,6-dimethoxy benzene. The smaller peaks at 11.65 min, 14.16 min, and 21.96 min stand for 2-tetradecene, 5-octadecene and (9Z)-octadecenoate respectively.

The DPPH radical scavenging assay of the smoke derived from Indigofera arrecta

The radical scavenging activity of the crude extract of *I. arrecta* was assessed by DPPH. At a concentration of 50 µg/mL and 100 µg/mL, it showed inhibitions of 90.76% and 93.52% respectively. This results are comparable to that of the ascorbic acid standard that exhibited 96.26% and 96.29% of DPPH inhibition at 50 µg/mL and 100 µg/mL respectively. The recorded inhibitions were most likely due to the presence of phenolic compounds in the smoke mixture. It shows that the smoke traditionally used to fumigate the house and body may serve as a component of skin care products. The radical scavenging activities of smoke trapped by methanol is shown in **Table 4.**

Table 3: The smoke constituents of *Indigofera arrecta* .

Pk	Compound	Structure	Formula	RT	A%	RI	Q
1	1-Dodecene		$C_{12}H_{24}$	8.86	7.38	1195.05	97
2	2-Tetradecene		$C_{14}H_{28}$	11.65	8.44	1394.08	97
3	5-Octadecene		$C_{18}H_{36}$	14.16	5.40	1594.41	99
4	2,4-Ditertbutylphenol		$C_{14}H_{22}O$	15.09	31.30	1675.20	97
5	1-Nonadecene		$C_{19}H_{38}$	16.42	1.01	1795.87	95
6	1,2,4,5-Tetrachloro-3,6-dimethoxy benzene		$C_8H_6Cl_4O_2$	17.24	25.10	1863.39	99
7	Methyl hexadecanoate		$C_{17}H_{34}O_2$	18.94	5.35	1993.61	98
8	(9Z)-Octadecenoate		$C_{19}H_{36}O_2$	21.96	12.04	2218.29	99
9	Methyl octadecanoate		$C_{19}H_{38}O_2$	22.22	3.98	2237.40	99

Pk = peak number, RT = retention time, RI = retention index, A=area, Q= NIST matching quality

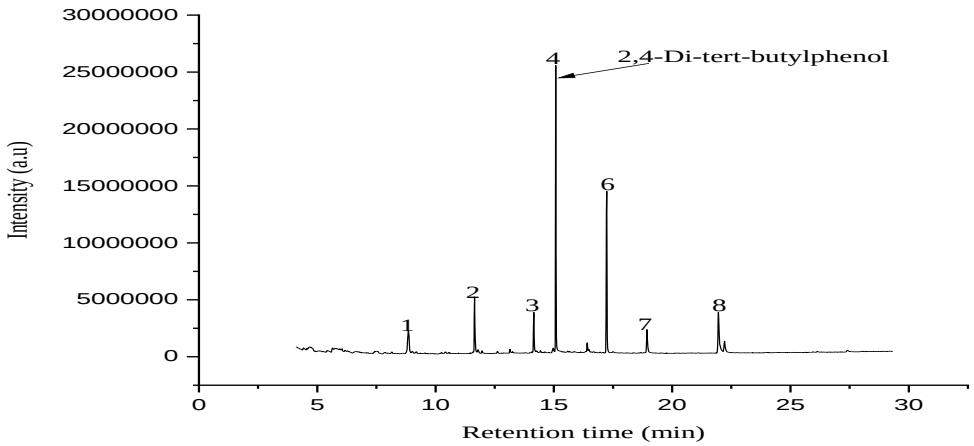


Figure 4: Chromatogram of the smoke constituents of I. arrecta

Table 4: Radical scavenging activities of the smoke trapped in methanol.

Concentration µg/mL	%DPPH Inhibition	
	I. arrecta	Ascorbic acid standard
6.25	33.69±0.55	91.30±0.31
12.5	47.48±0.34	96.06±0.12
25	70.26±1.50	96.09±0.16
50	90.76±0.62	96.26±0.06
100	93.52±0.09	96.29±0.06
200	94.13±0.16	-

The result are reported as mean ±SD of three replicates

CONCLUSION

In this study, essential oil and smoke extract obtained from *I. arrecta* were analyzed by GC-MS and the antioxidant capacity of methanol trapped smoke was also determined using 2,2-Diphenyl-1-picryldrazyl (DPPH). The detection of large amount of chlorinated compound is unusual as the plant is not a marine plant. However, it is believed that somehow the plant incorporate chlorine from soil into the biosynthetic route of the compound. The strong antioxidant activity of the smoke trapped in methanol coincides with the presence of phenolic compounds. The traditional use of the plant material, as a skin care, by mixing with butter oil makes a good sense as phenolic compounds scavenge radicals initiated by UV radiation.

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