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Effect of Oil Pollution on Chemical Composition, Anti-Inflammatory Properties of Stem Extracts of *Hyptis suaveolens* (L) POIT

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ABSTRACT

Oil pollution poses significant environmental challenges, potentially altering the chemical composition and medicinal properties of plants. This study investigated the impact of oil pollution on the phytochemical profile and anti-inflammatory activity of methanolic stem bark extracts of *Hyptis suaveolens* (L) POIT, a medicinal plant reported to possess antioxidant, anti-inflammatory, anti-cancerous, anti-diabetic, wound-healing among other uses. Plant samples were collected from polluted (Kaduna Refining and Petrochemical Company) and unpolluted (Murtala Muhammed Square) sites. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified 37 compounds in polluted stem extracts, compared to 31 in unpolluted extracts, with higher molecular weight compounds and saturated fatty acids in the former. Qualitative phytochemical screening revealed identical phytoconstituents in both extracts, though quantitative differences were statistically non-significant ($p > 0.05$). Anti-inflammatory activity, assessed via carrageenan-induced paw edema in rats, showed the unpolluted extract (300 mg/kg) exhibited a higher inhibition (49.6%) compared to the polluted extract (49.2%) at the fifth hour post-induction. These findings suggest that oil pollution increases the diversity of chemical compounds in *Hyptis suaveolens* stem extracts but slightly reduces anti-inflammatory efficacy, highlighting the need for caution in using polluted plants for medicinal purposes.

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KEYWORDS: *Hyptis suaveolens*, oil pollution, phytochemicals, GC-MS, anti-inflammatory activity, stem bark

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INTRODUCTION

Environmental pollution, particularly from oil, is a global concern due to its detrimental effects on ecosystems, human health, and plant physiology (Ezeabara *et al*., 2014; Punwong *et al*., 2017). Oil pollution, resulting from petroleum exploration, refining, and transportation, releases toxic hydrocarbons and heavy metals into the environment, potentially altering plant metabolic pathways and bioactive compounds (Adesina *et al*., 2014; Sharma *et al*., 2024). *Hyptis suaveolens* (L) POIT, a member of the Lamiaceae family, is a widely distributed medicinal plant in Nigeria, valued for its antioxidant, antimicrobial, and anti-inflammatory properties due to its rich phytochemical content, including tannins, flavonoids, and alkaloids (Mishra *et al*., 2021; Umedum *et al*., 2014). The stem bark of *Hyptis suaveolens* is used in traditional medicine for treating inflammation, infections, and wounds (Gavani and Paarakh, 2008; Iqbal *et al*., 2021). However, the plant often grows in areas affected by oil pollution, raising concerns about the safety and efficacy of its medicinal applications. Previous studies suggest that environmental stressors, such as oil pollution, may induce the biosynthesis of novel phytochemicals as an adaptive response (Diyaolu *et al*., 2022). Yet, the impact on specific plant parts, such as the stem bark, and their therapeutic properties remains underexplored. This study aimed to evaluate the effect of oil pollution on the chemical composition and anti-inflammatory activity of methanolic stem bark extracts of *Hyptis suaveolens*. Using Gas Chromatography-Mass Spectrometry (GC-MS) and carrageenan-induced paw edema models, the polluted and unpolluted extracts were compared to determine whether oil pollution alters the plant's phytochemical profile and medicinal efficacy. The findings contribute to understanding the safety of using *Hyptis suaveolens* from polluted environments in traditional medicine.

MATERIALS AND METHODS

Plant Collection and Processing: *Hyptis suaveolens* stem bark samples were collected from two sites in Kaduna, Nigeria: a polluted site near the Kaduna Refining and Petrochemical Company and an unpolluted site at Murtala Muhammed Square. The samples were authenticated, shade-dried, and ground into a fine powder.

Extract preparation: Methanolic extracts were prepared using the Soxhlet extraction method with methanol as the solvent: 50 g of the pulverized stem bark sample was extracted in 500 ml of methanol for 6 cycles at the boiling point of methanol (64.7℃). The extract obtained was evaporated to dryness and stored at 4℃ in an airtight container (Deshamukh, 2025).

Phytochemical Screening: Qualitative phytochemical analysis was conducted to detect the presence of tannins, flavonoids, alkaloids, saponins, glycosides, phenols, and other constituents using standard methods (Harborne, 1998). Quantitative analysis was performed to determine the concentrations of key phytochemicals, and statistical significance was assessed using a t-test ($p < 0.05$).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis: The chemical composition of the methanolic stem bark extracts was analyzed using an Agilent 7890A GC system coupled with a 5975C inert MSD mass spectrometer. A JandW capillary column (30 m × 0.250 mm) was used, with helium as the carrier gas at a flow rate of 1 mL/min. The oven temperature was programmed from 50℃ (held for 1 min) to 300℃ at 20℃/min (held for 8 min). Samples (0.2 µL) were injected in splitless mode at 250℃. Ionization energy was set at 70 eV, with a scan range of 50-500 amu. Compounds were identified using the NBS75K library database, and retention indices were calculated relative to n-alkanes (Ramzi *et al*., 2013).

Anti-Inflammatory Activity: Anti-inflammatory activity was evaluated using the carrageenan-induced paw edema model in Wistar rats (n = 6 per group). Rats were divided into groups receiving normal saline (control), carrageenan (negative control), indomethacin (10 mg/kg, positive control), or methanolic extracts (100, 300, or 400 mg/kg) of polluted or unpolluted stem bark. Acute edema was induced by injecting 0.1 mL of 1% (w/v) carrageenan solution into the sub-plantar tissue of the right hind paw. Paw thickness was measured hourly for 5 hours using a Vernier callipers (Sarkhel *et al*., 2016).

Percentage inhibition was calculated as: $\frac{T-To}{T} * 100$

T = thickness of paw in control (carrageenan) group;

T_o = thickness of paw edema in the test compound treated group

Statistical Analysis: Summary data were calculated as mean ± standard deviation or percentages, and expressed as bar charts in figures. Differences between polluted and unpolluted extracts were analyzed using *t*-tests for phytochemical quantities and ANOVA for anti-inflammatory activity, with significance set at $p < 0.05$.

RESULTS

Phytochemical Composition: Qualitative phytochemical analysis revealed the presence of tannins, flavonoids, alkaloids, saponins, glycosides, phenols, quinones, reducing sugars, cardiac glycosides, essential oils, carbohydrates, anthraquinones, amino acids, coumarins, and xanthoproteins in both polluted and unpolluted stem bark extracts. Aromatic acids, leucoanthocyanins, and anthocyanins were absent (Supplementary_data_1.docx). Quantitative analysis showed slightly higher levels of saponins, phenols, reducing sugars, carbohydrates, glycosides, and terpenoids in polluted extracts, while steroids were higher in polluted stem bark. Tannins, alkaloids, and flavonoids were similar in both extracts. No statistically significant differences were observed ($p > 0.05$) (Figure 1).

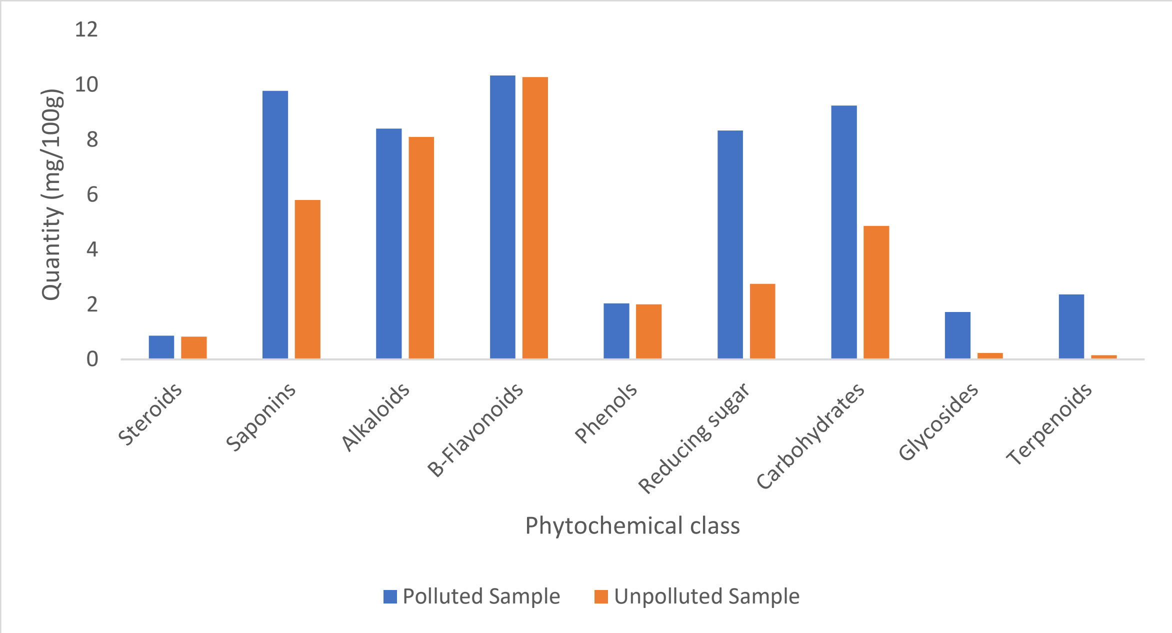

Figure 1: Quantitative distribution of phytochemicals in polluted and unpolluted stem bark extract of *Hyptis suaveolens* (tannins and cyanogenic glycosides not depicted; values above 500 mg/100g each).

GC-MS Analysis: GC-MS analysis identified 37 compounds in the polluted stem bark extract (Supplementary_data_2.docx) and 31 in the unpolluted extract (Supplementary_data_3.docx). In the polluted extract, tetrapentacontane, 1,54-dibromo- (MW 914) was the heaviest compound, and 2,4-pentadien-1-ol, 3-ethyl-, (2Z) (MW 112) was the lightest. Dominant compounds included cosanes, dodecanes, and esters, with oleic acid and oxirane also present. The unpolluted extract contained cyclodecasiloxane, eicosamethyl- (MW 740) as the heaviest compound, and dodecane, 2-methyl-, and decane, 1-(ethenyloxy)- (MW 184) as the lightest. Esters, dodecanes, cosanes, and siloxanes dominated, with methyl stearate and oleic acid identified. Polluted extracts showed a higher number of compounds and more saturated fatty acids.

Anti-Inflammatory Activity: The unpolluted extract at 300 mg/kg exhibited the highest anti-inflammatory activity, with 49.6% inhibition of paw edema at the fifth hour post-induction, followed by the polluted extract at 300 mg/kg (49.2%) (Figures 2 and 3). Indomethacin (10 mg/kg) showed 45.8% inhibition. Both extracts demonstrated time-dependent inhibition, with significant activity from the second to fifth hours ($p < 0.05$). The unpolluted extract outperformed the polluted extract at all doses, though differences were minimal at 300 mg/kg.

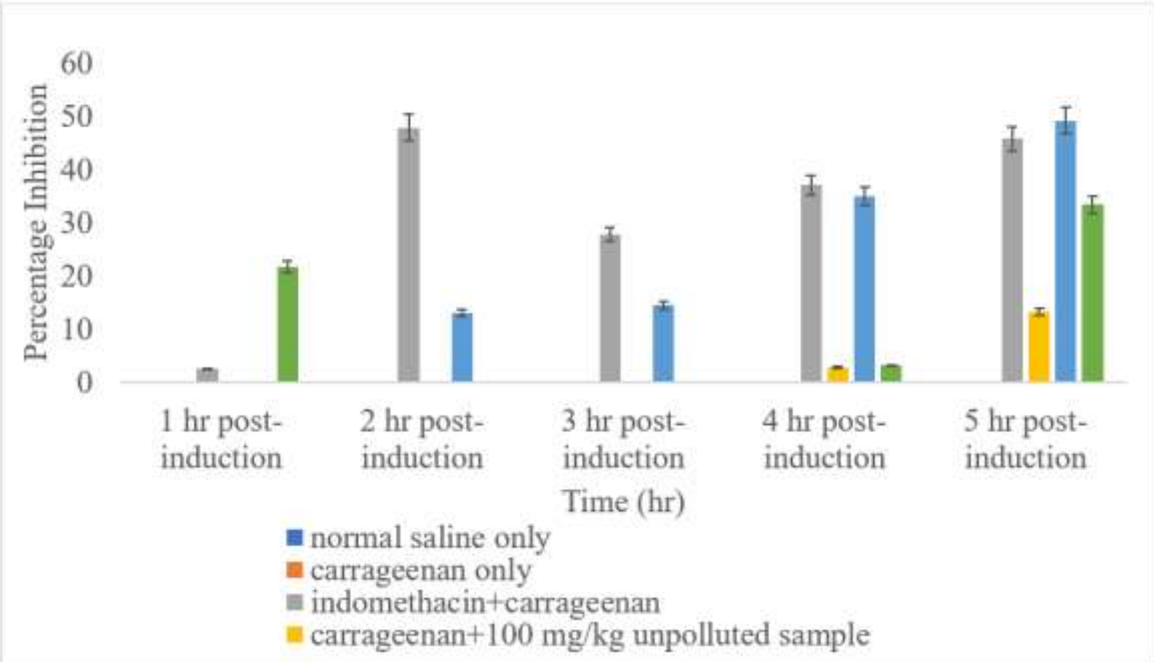

Figure 2: Percentage inhibition activity of the unpolluted extract against carrageenan inducted paw edema in rats

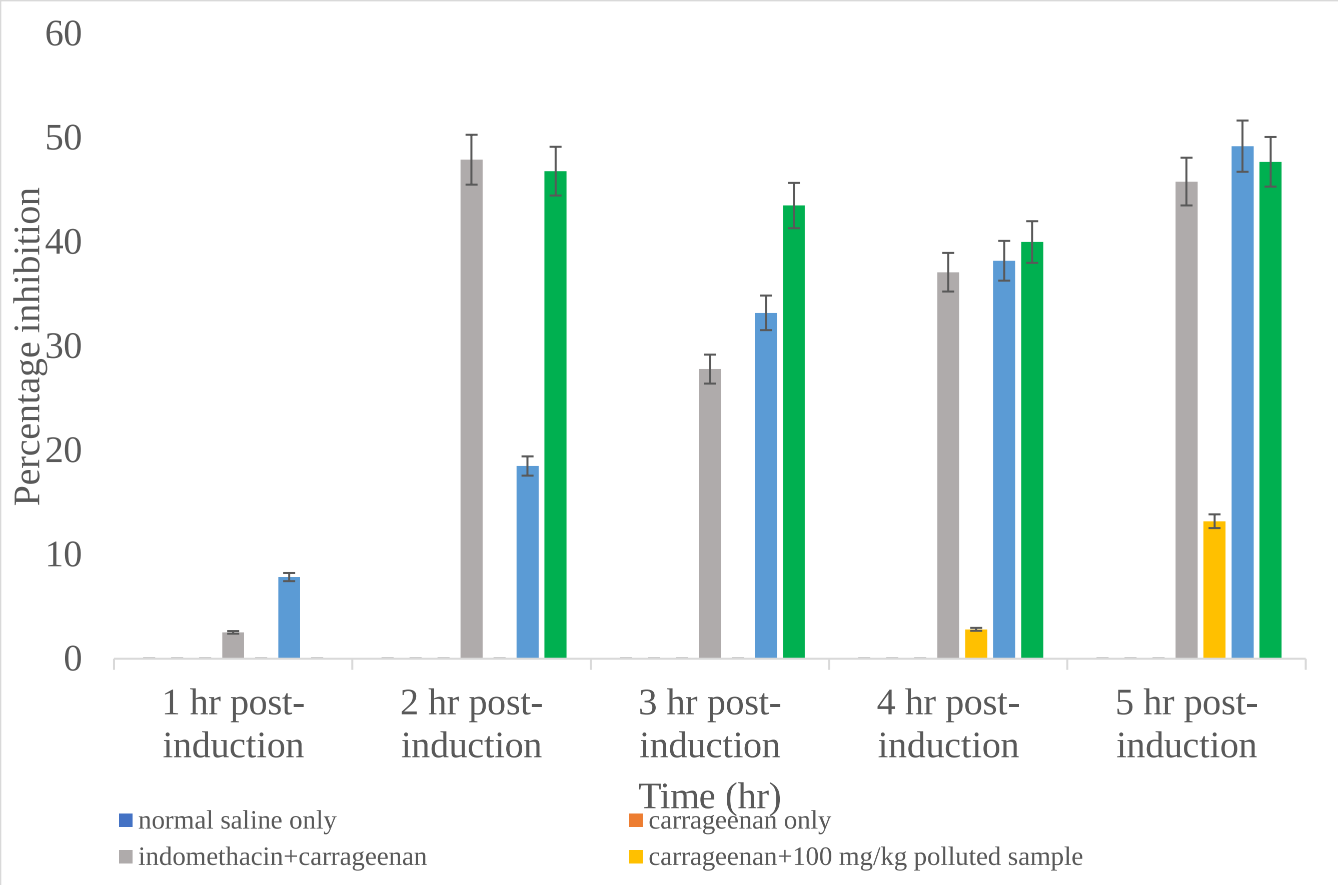

Figure 3: Percentage inhibition activity of the polluted extract against carrageenan inducted paw edema in rats

DISCUSSION

Oil pollution significantly altered the chemical composition of *Hyptis suaveolens* stem bark extracts, as evidenced by the increased number of compounds (37 vs. 31) and higher molecular weight compounds in polluted extracts. This aligns with Diyaolu *et al.* (2022), who reported that oil-polluted environments enhance the biosynthesis of phytochemicals as an adaptive response to stress. The presence of more saturated fatty acids in polluted extracts may result from hydrocarbon interactions with plant metabolic pathways, potentially strengthening phenolic rings (Odukoya *et al*., 2019). However, the qualitative phytochemical profile remained unchanged, suggesting that core bioactive constituents are resilient to pollution, though quantitative variations indicate subtle metabolic shifts (Diyaolu *et al*., 2022).

The anti-inflammatory activity of *Hyptis suaveolens* stem bark extracts was slightly reduced in polluted samples, with the unpolluted extract (49.6% inhibition) outperforming the polluted extract (49.2%) at 300 mg/kg. This difference may be attributed to difference in flavonoid and phenol contents in unpolluted extracts, which are known for their anti-inflammatory properties (Al-Khayri *et al*., 2022; Liu *et al*., 2023; Radwan *et al*., 2018). The time-dependent inhibition observed in both extracts aligns with the biphasic nature of carrageenan-induced edema, involving histamine, serotonin, and eicosanoid mediators (Karim *et al*., 2019; Widyarini *et al*., 2023). The comparable efficacy to indomethacin (45.8%) supports the potential of *Hyptis suaveolens* as a natural anti-inflammatory agent, as reported by Machado *et al*. (2021).

The increased chemical diversity in polluted extracts may enhance certain bioactivities but could also introduce toxic compounds, such as tetrapentacontane derivatives, warranting caution in medicinal use. The absence of significant differences in phytochemical quantities suggests that *Hyptis suaveolens* adapts to oil pollution without compromising its core therapeutic constituents. However, the slight reduction in anti-inflammatory efficacy in polluted extracts underscores the need for further studies on the safety of polluted plants in herbal medicine.

CONCLUSION

Oil pollution increases the chemical diversity of methanolic stem bark extracts of *Hyptis suaveolens*, with 37 compounds identified in polluted extracts compared to 31 in unpolluted ones. While qualitative phytochemical profiles remain unchanged, quantitative differences and a slight reduction in anti-inflammatory activity (49.6% vs. 49.2% inhibition) suggest that pollution subtly impacts the plant's medicinal properties. These findings highlight the need to protect *Hyptis suaveolens* from oil pollution and recommend further molecular studies to elucidate the mechanisms underlying these changes and assess the safety of polluted plants for therapeutic use.

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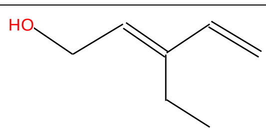
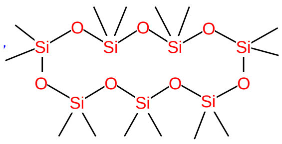
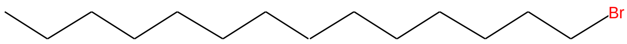
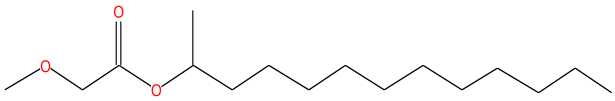
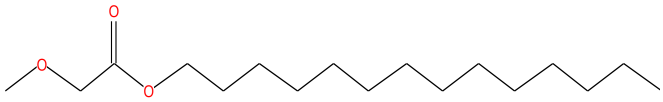
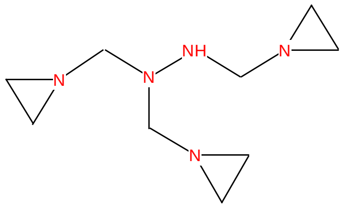
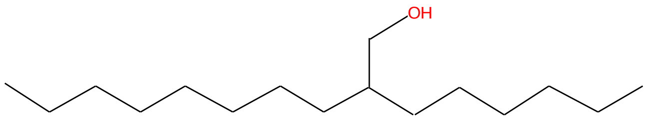
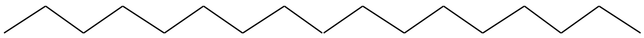
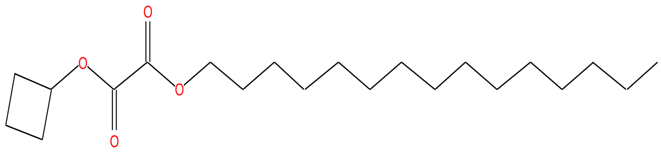
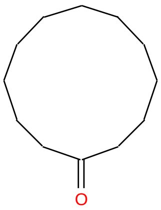
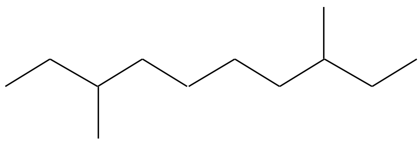
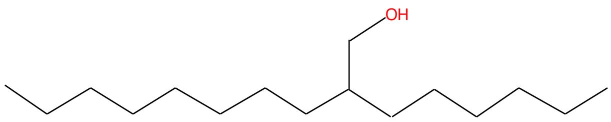
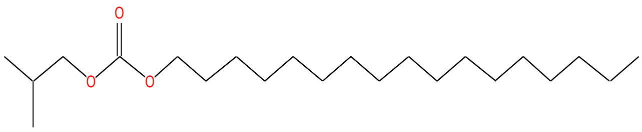
Supplementary_data_1: Qualitative Phytochemical analyses of polluted and unpolluted methanolic extract of *Hyptis suaveolens*

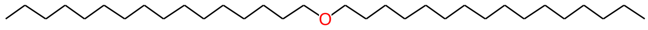
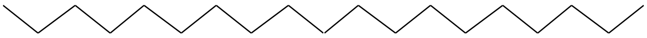
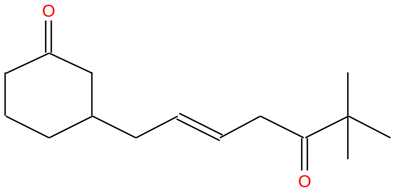
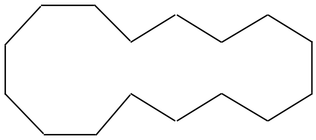
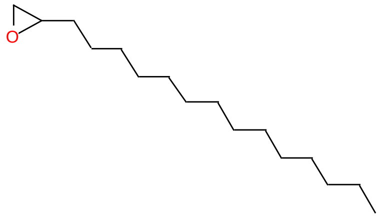
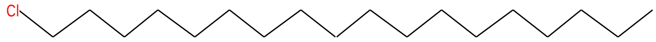
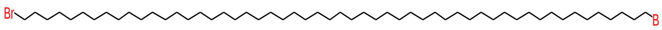
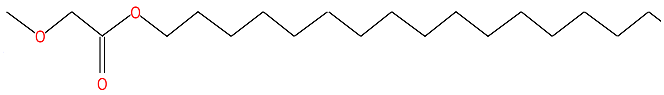
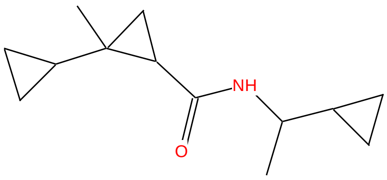
S/N	Phytochemicals	PS	UPS
1	Tannins	+	+
2	Phlobatannins	+	+
3	Steroids	+	+
4	Saponins	+	+
5	Alkaloids	+	+
6	Flavonoids	+	+
7	Glycosides	+	+
8	Cardiac glycosides	+	+
9	Quinones	+	+
10	Amino acids	+	+
11	Phenolic groups	+	+
12	Essential oil	+	+
13	Carbohydrates	+	+
14	Anthraquinones	+	+
15	Xanthroproteins	+	+
16	Aromatic acids	-	-
17	Anthocyanins	-	
18	Coumarins	+	+
19	Reducing sugars	+	+
20	Leucoanthocyanins	-	-

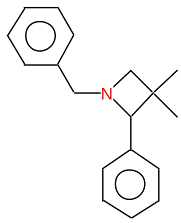
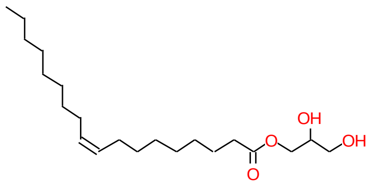
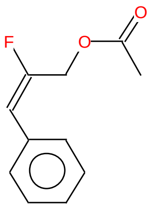
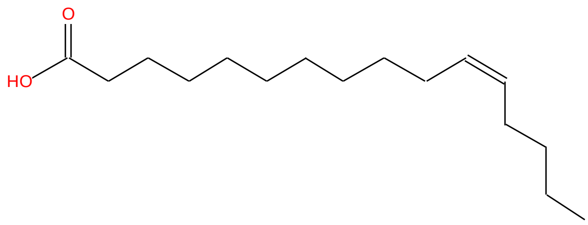
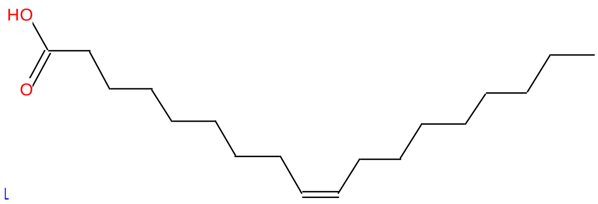
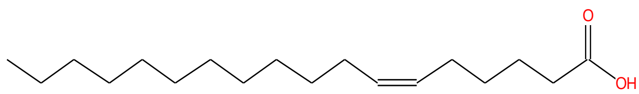
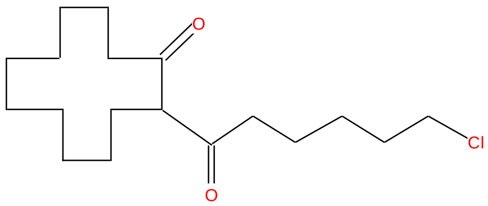
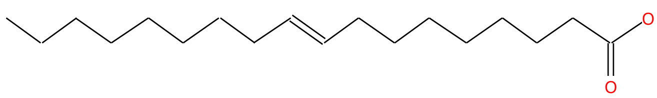
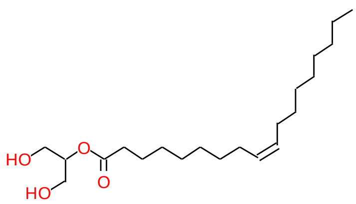
Key: PS = Polluted Stem Bark Sample, UPS = Unpolluted Stem Bark Sample; Positive (+): Indicates presence of a phytochemical; Negative (-): Indicates absence of a phytochemical

Supplementary_data_1: Chemical compounds present in methanolic extract of polluted stem bark of *Hyptis suaveolens*.

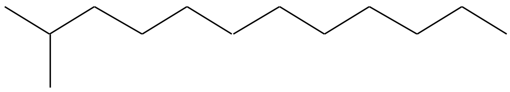
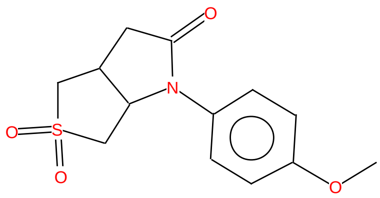
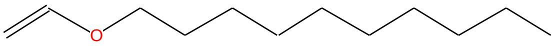
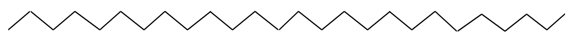
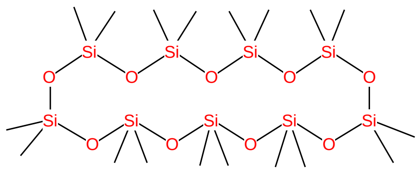
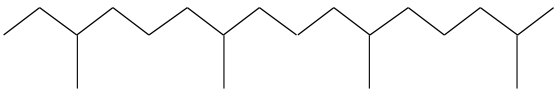
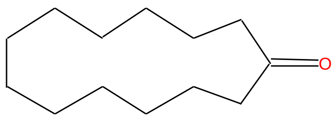
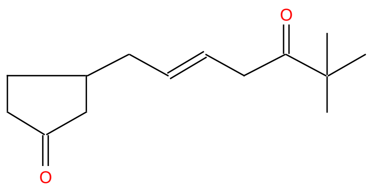
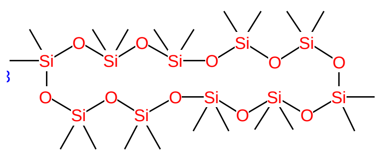
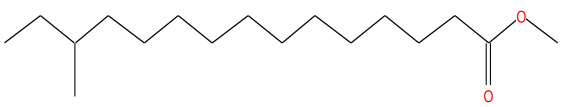
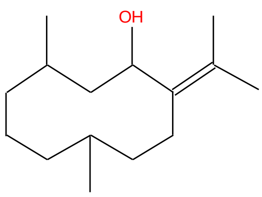
No.	Compounds	Molecular Formula	Molecular Wight	Retention Time (Min)	Area (%)	Structure
1	2,4-Pentadien-1-ol, 3-ethyl-, (2Z)	$C_7H_{12}O$	112	9.833	0.29	

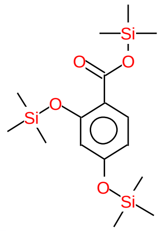
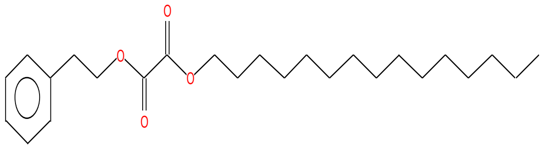
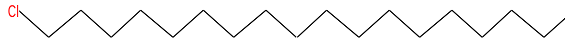
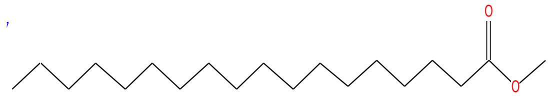
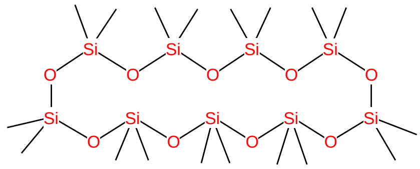
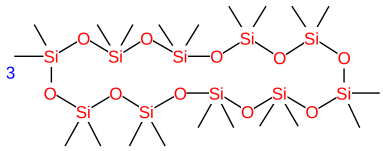
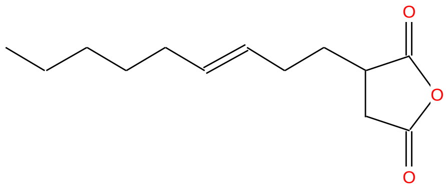
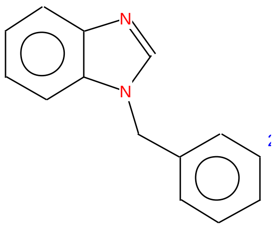
2	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	518	10.251	0.26	
3	Tetradecane, 1-bromo-	$C_{14}H_{29}Br$	276	10.892	0.44	
4	Methoxyacetic acid, 2-tridecyl ester	$C_{16}H_{32}O_3$	272	11.601	0.50	
5	Methoxyacetic acid, tetradecyl ester	$C_{17}H_{34}O_3$	286	11.721	0.19	
6	Hexadecane	$C_{16}H_{34}$	226	12.099	1.88	
7	Tris(aziridinomethyl)hydrazine	$C_9H_{19}N_5$	197	12.603	0.88	
8	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$	242	12.912	1.12	
9	Heptadecane	$C_{17}H_{36}$	240	13.249	3.99	
10	Oxalic acid, cyclobutyl pentadecyl ester	$C_{21}H_{38}O_4$	354	13.758	1.91	
11	Cyclododecanone	$C_{12}H_{22}O$	182	14.022	2.02	
12	Decane, 3,8-dimethyl-	$C_{12}H_{26}$	170	14.359	4.35	
13	1-Decanol, 2-hexyl-	$C_{16}H_{34}O$	242	14.771	2.62	
14	Carbonic acid, heptadecyl isobutyl ester	$C_{22}H_{44}O_3$	356	15.080	1.84	

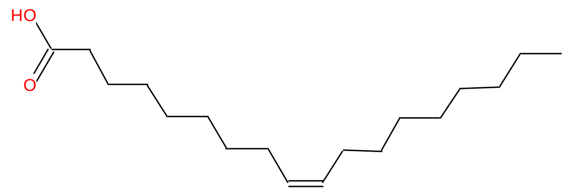
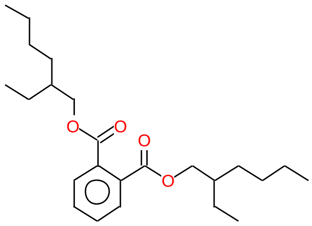
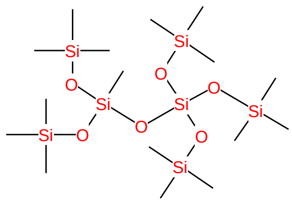
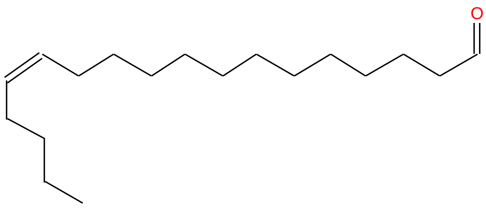
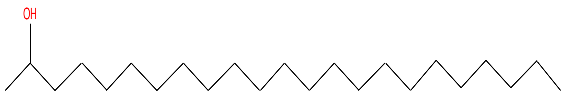
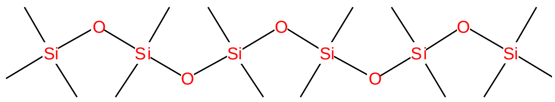
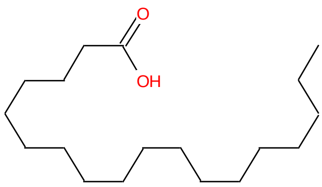
15	Hexadecane, 1,1'-oxybis-	$C_{32}H_{66}O$	466	15.223	0.98	
16	Nonadecane	$C_{19}H_{40}$	268	15.401	3.39	
17	3-(6,6-Dimethyl-5-oxohept-2-enyl)-cyclohexane	$C_{15}H_{24}O_2$	236	15.710	3.14	
18	Cyclohexadecane	$C_{16}H_{32}$	226	16.179	2.51	
19	Eicosane	$C_{20}H_{42}$	282	16.396	3.60	
20	Oxirane, tetradecyl-	$C_{16}H_{32}O$	240	16.797	1.83	
21	Octadecane, 1-chloro-	$C_{18}H_{37}Cl$	288	17.066	2.65	
22	Heneicosane	$C_{21}H_{44}$	296	17.363	4.61	
23	Tetrapentacontane, 1,54-dibromo-	$C_{54}H_{108}Br_2$	914	17.644	4.54	
24	Docosane	$C_{22}H_{46}$	310	18.273	2.64	
25	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	196	18.588	1.61	
26	Methoxyacetic acid, heptadecyl ester	$C_{20}H_{40}O_3$	328	18.845	1.48	
27	Tricosane	$C_{23}H_{48}$	324	19.154	2.58	
28	Cyclopropane carboxamide, 2-cyclop ropyl-2-methyl-N-(1-cyclopropylethyl)-	$C_{13}H_{21}NO$	207	19.778	2.65	

29	Azetidine, 1-benzyl-3,3-dimethyl-2-phenyl-	$C_{18}H_{21}N$	251	20.459	9.60	
30	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356	21.123	3.05	
31	2-Methylacetoxy-2-fluoro-1-phenylp rop-1-ene	$C_{11}H_{11}FO_2$	194	21.403	1.31	
32	Hexadecenoic acid, Z-11-	$C_{16}H_{30}O_2$	254	21.586	2.19	
33	Oleic Acid	$C_{18}H_{34}O_2$	282	22.153	3.42	
34	6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282	22.347	2.77	
35	Cyclododecanone, 2-(6-chloro-1-ox0hexyl)-	$C_{18}H_{31}ClO_2$	314	23.120	3.18	
36	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282	23.383	4.31	
37	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{21}H_{40}O_4$	356	34.901	0.06	

Supplementary_data_2: Chemical compounds present in methanolic extract of unpolluted stem bark of *Hyptis suaveolens*.

No.	Compounds	Molecular Formula	Molecular Weight	Retention Time (Min)	Area (%)	Structure
1	Dodecane, 2-methyl-	$C_{13}H_{28}$	184	10.892	0.33	
2	1-(4-Methoxy-phenyl)-5,5-dioxo-hexahydro-5.lambda. (6)-thieno[3,4-b] pyrrol-2-one	C13H15N O4S	281	12.219	4.55	
3	Decane, 1-(ethenyloxy)-	$C_{12}H_{24}O$	184	12.597	1.31	
4	Hexacosane	$C_{26}H_{54}$	366	13.249	6.35	
5	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	13.930	6.88	
6	Hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	282	14.359	4.64	
7	Cyclotridecanone	$C_{13}H_{24}O$	196	14.748	2.74	
8	Cyclopentanone, 3-(6,6-dimethyl-5-oxo-2-heptenyl)-, (E)-	$C_{14}H_{22}O_2$	222	15.246	3.84	
9	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	740	15.446	5.23	
10	Pentadecanoic acid, 13-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	15.715	3.82	
11	5,9-Dimethyl-2-(1-methylethylidene)-1-cyclodecanol	$C_{15}H_{28}O$	224	16.104	2.60	

12	Eicosane	$C_{20}H_{42}$	282	16.402	3.14	
13	Benzoic acid, 2,4-bis[(trimethylsilyl)oxy]-, trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	370	16.837	2.66	
14	Oxalic acid, pentadecyl propyl ester	$C_{25}H_{40}O_4$	404	17.060	2.27	
15	Octadecane, 1-chloro	$C_{18}H_{37}Cl$	288	17.369	5.75	
16	Methyl stearate	$C_{19}H_{38}O_2$	298	17.644	2.92	
17	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	18.101	1.40	
18	Docosane	$C_{22}H_{46}$	310	18.273	2.10	
19	Tricosane	$C_{23}H_{48}$	324	19.154	1.44	
20	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	740	19.263	1.54	
21	n-Nonenylsuccinic anhydride	$C_{13}H_{20}O_3$	224	19.583	0.53	
22	Tetracosane	$C_{24}H_{50}$	338	20.001	1.08	
23	N-Benzyl-1H-benzimidazole	$C_{14}H_{12}N_2$	208	20.459	12.62	

24	Oleic Acid	$C_{18}H_{34}O_2$	282	20.802	2.05	
25	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	21.077	2.75	
26	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	$C_{16}H_{48}O_6Si_7$	532	21.403	1.77	
27	13-Octadecenal, (Z)-	$C_{18}H_{34}O$	266	21.586	1.23	
28	Tricosan-2-ol	$C_{23}H_{48}O$	340	23.068	4.63	
29	Hexasiloxane, tetradecamethyl-	$C_{14}H_{42}O_5Si_6$	458	23.291	2.95	
30	Octadecanoic acid	$C_{18}H_{36}O_2$	284	24.304	0.63	
31	Ethyl 2-acetamido-3,3,3-trifluoro-2-(4-fluoroanilino) propionate	$C_{13}H_{14}F_4N_2O_3$	322	25.546	0.45