Chemical composition, phytochemical profile and antimicrobial properties of the methanolic extracts of polluted and unpolluted leaves of *Hyptis suaveolens (L)*

Egwu, H. A. [1]*, Yilwa, V. [1], Onusiriuka, B. [1], Dikwa, K. B. [1], Appah, J. [1], Ajibade, G. [1] and Yelwa, J.M. [2]

[1]*Department of Biological Sciences, Nigerian Defence Academy, Kaduna.*
[2]*Scientific and Industrial Research Department, National Research Institute for Chemical Technology, Zaria.*
**Corresponding Author: helenegwu2003@yahoo.co.uk; +2348033053805*

Abstract

Hyptis suaveolens, an aromatic plant commonly called Daddoya-ta-daji in Hausa language, is traditionally used as medicine and food. It is used as stimulant, antirheumatic, carminative, against parasitic diseases, headaches, infection of the uterus, stomach, and skin diseases. It is found growing in both oil polluted and non-oil polluted environment. Oil pollution of land is a challenge due to the presence of heavy metals and volatile organic compounds which are harmful to crops and plants. This study investigated the phytoconstituents of the methanol leaf extract as well as antimicrobial activities of *Hyptis suaveolens* collected from polluted and unpolluted sites. Methanol leaf extracts of *H. suaveolens* were screened for crucial phytochemicals using standard methods. The *in vitro* antimicrobial activity was carried out using agar disc-diffusion method by Yahaya *et al*. (2018), using Mueller Hinton agar medium and potatoes dextrose agar in petri plates seeded with clinical isolates of *Escherichia coli, Salmonella typhi, Shigella sp, Staphylococcus aureus* (bacteria), *Candida albicans* and *Aspergillus niger* (fungi) respectively, and the zone of inhibition determined. The phytochemical results showed that methanol extract of both the polluted and non-polluted *H. suaveolens* leaves exhibited the presence of some number of bioactive compounds. Gas Chromatography–Mass Spectrometry (GC-MS) of polluted and non-polluted leaves of *H. suaveolens* identified 13 and 21 compounds, respectively such as Octadec-9-enoic acid, 2-Ethylacridine, Methyl ester, Tetracyclo [4.4.1.1(7,10).0(2,5)] dodec-3-en-11-ol, 1,2-Diphenyl-1-isocyanoethane and 7-Isopropyl-1, 1, 4a-trimethy l-1,2,3,4,4a,9,10,10 aocta -hydrophenanthrene. These chemical compounds are considered biologically and pharmacologically important. The results from assessment of the antimicrobial potential of *H. suaveolens* (L.) on pathogenic microorganisms showed that the pathogenic microorganisms revealed inhibition zones ranging from 6 to 18 mm. This data indicates that the unpolluted methanol extract of *H. Suaveolens* exhibits a higher bioactive potential compared to the extract derived from polluted leaves. This disparity suggests that oil pollution may have a detrimental impact on the biochemical properties of the plant.

Keywords: *Hyptis suaveolens*, Bioactive, Scavenging activities, Free radical, DPPH.

1. Introduction

The use of medicinal plants for a variety of human ailments as traditional system of medicine has increased globally due to conventional drug resistance/side effect issues, prohibitive cost of treatment, increase in population and more so that traditional medicine has little or no side effects and it is affordable (Mahomoodally 2013; Alvis 2021; Azmathuunisa, 2016; Gasper *et al.* 2021; Mahtab 2016).

The medicinal value of plants is due to the presence of immense number of secondary metabolites, otherwise known as phytochemicals in plants. These pharmacologically active metabolites are produced in different plant parts at different stages of life cycle. (Mishra *et al.*, 2021; Patel and Gupta 2017; Sharma *et al*, 2013;).

Oil pollution is the spillage of liquid hydrocarbon onto marine environment but can also occur on land because of human activities. This pollution has negative effects on plants due to the presence of organic compounds and toxic heavy metals (mercury, cadmium, copper, zinc, arsenic, nickel, and so on) in the crude oil and its refined products. This oil pollution is caused by oil pipeline vandalism, corrosion/aged pipeline, during transportation of petroleum refined products, refining activities, and oily wastes disposal. These pollutants alter plants growth, physiology and metabolism (biochemical) as well as the anatomical configuration of some medicinal plants, which ultimately have negative impact on their phytoconstituents and pharmacological relevance (Arellano *et al*., 2017; Atubi, 2015 and Nathanson, 2021; Farha *et al*., 2016; Ogbo *et al*., 2009; Otuu *et al*., 2016). Some important phytochemicals found in plants are phenolics, alkaloids, terpenoids, glycosides, tannins, flavonoids, saponins, steroids, carbohydrates derivatives, gums, essential oils, fatty oils, resins, and mucilages; these bear diverse pharmacological and pharmacognostic implications (Cragg and Newman, 2013).

Hyptis suaveolens (L.) is an obnoxious weed of the tropics and subtropics. The pharmacological significance of the plant is due to its numerous medicinal properties. The leaves of the plant are a source of pharmacologically important secondary metabolites having antispasmodic, anti-colic, anti-inflammatory, antirheumatic, anti-fertility, antimicrobial and antifungal properties, carminative for wounds, infection of the uterus, anti-mosquito, respiratory problems, gastrointestinal disorders. It is also used for skin disorders, cramps pain and fever, (AZMathunnisa *et al*, 2016; Gavani and Paarakh 2018; Luzuriaga *et al.* 2018; Mishra *et al*.2021; Oumarou *et al*. 2018; Partheipratin *et al.* 2015). The root contains anti-retroviral compound called urosolic acid, a triterpenoid that may target retroviral integrases and proteases blocking the replication of retroviruses such as HIV (Tohme *et al*., 2019). Most of the bioactive compounds found in *H. suaveolens* are used as therapeutic agents or as precursors of useful drugs and all parts of the plant is potent (Edeoga *et al*., 2006; Essien *et al.* 2019). The mature leaves of plant contain essential oils, alkaloids as the major secondary metabolites followed by tannins and saponins, others are steroids, alkaloids, glycosides and carbohydrates (Chatri *et al.* 2018; Raju *et al*.2019). In northern Nigeria, infused leaves of the plant are used as a stimulant, diuretic, carminative or as antipyretic. Leaves decoction is used to treat skin and kidney diseases and can be crushed and used as treatment for headache (Umedum *et al.*2014).

The medicinal potency of the plant essential oils has been reported by Adebayo *et al*. (2023), Adeniran and Fabiyi (2023). Adesina *et al.* (2023), Afolayan *et al.* (2023), Adeniyi *et al.* (2023), Akinmoladun *et al*. (2023), Akinpelu (2023), Aladesanmi *et al*. (2023) and Adeniran and Fabiyi (2023) conducted comprehensive studies and reported on the phytochemical constituents, antimicrobial and antioxidant properties of *Hyptis suaveolens* as a medicinal plant. However, the impact of oil pollution on the antimicrobial properties of *Hyptis suaveolens* is yet to be reported. In this study, we investigated the phytoconstituents of the methanol leaf extract as well as antimicrobial activities of *Hyptis suaveolens* collected from polluted and unpolluted sites.

2. Materials and methods

2.1 Sample collection and Preparation

Fresh leaves of *Hyptis suaveolens* were collected from Kaduna Refining and Petrochemical Company (referred to as the polluted samples), because of the oil refining activities that occurs there) and Murtala Mohammed Square (referred to as the unpolluted sample). The leaves were dried at room temperature, ground into fine particle size and stored in airtight container until required.

The study utilized soil from Kaduna Refining and Petrochemical Company as a source of polluted samples, a choice informed by the industrial activities at the site. The oil refining processes conducted by the company are known to emit a range of pollutants, including heavy metals and hydrocarbons, which can infiltrate the soil and alter its composition. Plants, such as *H. suaveolens*, growing in these contaminated environments can absorb these pollutants, potentially affecting their chemical and phytochemical makeup.

In this study, leaves of *H. suaveolens* from the polluted site were collected to evaluate the impact of such environmental pollution on their chemical composition, phytochemical profile, and antimicrobial properties. For comparison, unpolluted samples were collected from Murtala Mohammed Square, a location with significantly lower expected pollutant levels, serving as a control for the study. The comparative analysis of these samples aims to shed light on the potential influence of industrial pollution on the properties of the plant.

2.2 Preparation of Crude Extract

The pulverized leaves sample (1.8kg) was extracted in 5L of 60% hydro-methanol (v/v) for 96 hr with intermittent agitation. The filtrate obtained was concentrated *in-vacuo* using a rotary evaporator at 40°C and allowed to dry over a water bather. The dried extracted was weighted and then stored in airtight container at 4°C until when required.

2.3 Phytochemical Screening

The leaf extracts were screened for the presence of bioactive compounds, including phenolic compounds, tannins, alkaloids, steroids, glycosides, flavonoids, and saponins, using standard qualitative phytochemical procedures outlined by Ezeonu and Ejikeme (2016), Harbone (1973), Hossain *et al*. (2021), Kubmarawa *et al*. (2007), Runde *et al*. (2015), and Yahaya *et al*. (2018). The presence of phenolic compounds and tannins were determined using ferric chloride test, alkaloids by Mayer's test, the lead acetate test, Salkowski's test for steroids and glycosides, while the foam test was used to determine the presence of Saponins.

2.4 Determination of the chemical composition of *Hyptis suaveolens* leaves using Gas Chromatography Mass Spectrometry (GC/MS)

The composition analysis of *Hyptis suaveolens* leaves extract obtained was carried out using an Agilent technologies Gas Chromatography Mass Spectrometry GC-MS (7890A GC system) unit, coupled with Agilent mass spectrometer (5975C inert MSD). J and W capillary column was used of 30m length and diameter of 0.250 mm with temperature limit ranging from -65°C to 325°C. Methanolic leaf extract of *H. suaveolens* (0.2µl) was injected using split less injection mode into the inlet at 250°C, and flow rate of 1ml/min. The oven temperature was programmed to start from 50°C and held for 1 min, and then increasing by 20°C to 300°C and held for 8 min. The Ionization energy was 70ev in the electron ionization (EI) mode with a scan range of 50-500 amu, and compositions of the essential oils were generated from the NBS75K library data base installed in the instrument. The retention indices (RI) were in relation to homologous series of n-alkanes on the GC column under the same chromatographic condition, and the component relative concentration obtained by the peak area normalization (Ramzi *et al*., 2013) and adopted by Runde *et al.* (2015). Chemical composition of the plant leaves extract was identified based on comparison of the retention indices and mass spectra of most of the compound with data generated under identical experimental conditions by applying a 2D search algorithm considering the retention index as well as mass spectra similar with those of authentic compounds available in NIST 2011 Library.

2.5 Antimicrobial Activity Assay

In vitro antimicrobial activities of the methanol leaf extracts of *H. suaveolens* were investigated by adopting disc-diffusion method of Yahaya *et al*., (2018). Briefly, 10 ml of Mueller Hinton agar medium in a petri plate was seeded with one-day old culture of four selected bacteria strains: *Escherichia coli, Shigella spp, Salmonella typhi Staphylococcus aureus*; and two fungi strains: *Candida albicans* and *Aspergillus niger*. The clinical isolates were obtained from Federal University Teaching hospital

Gombe State, Nigeria. Sterile filter paper disc (9mm in diameter) containing 1000 ppm of the leaf extract was dissolved in dimethyl sulfoxide (DMSO), and placed on the medium; dimethyl sulfoxide and water served as negative control. Standard discs containing the antibiotic Augmentin (30 µg/disc) and an antifungal agent Griseofulvin 500mg/ml were used as positive controls for the bacteria and fungi species, respectively. The inoculated plates were allowed to incubate at 34°C for 24 hours, and the antimicrobial activity, given as diameter of zone of inhibition (ZOI) around the disc, measured using a ruler (Diameter of zone of inhibition minus that of the disc). Average zone of inhibition was calculated for three replicates and an inhibition zone of 8 mm or greater was considered as having significant antimicrobial activity. The methanolic leaf extract of *H. suaveolens* that showed positive activity at the initial screening was diluted serially (two-fold) and loaded on the filter paper disc and inoculated into nutrient broth containing the same number of test organism. Two controls were used: growth control (containing just bacteria in nutrient broth) and sterile control (containing only medium). Incubation is 37 for 18hrs to determine the minimum inhibitory concentration (MIC) i.e., the minimum concentration per disc to inhibit the growth of the microorganism (Yahaya *et al*., 2018). Drugs with lower MIC values are more effective antimicrobial agents and indicates that less drug is required for inhibiting growth of the microorganism.

3.0 Results and discussions

3.1 Phytochemical constituents of polluted and unpolluted Leaves of *Hyptis suaveolens*

The leaves of *H. Suaveolens* were observed to contain certain phytochemical constituents namely; tannins, flavonoids, saponins, steroids, terpenoids, phenols, cyanogenic glycosides, glycosides, carbohydrate, reducing sugars and alkaloids (Table1). Most of these phytochemicals have been reported in previous screenings of *H. suaveolens.* (; Pachkore *et al*., 2011; Umedum *et al*., 2014). The presence of these phytochemicals has been the basis for its usage in ethno-medicine. These together with the fatty acids may be responsible for the antiviral, anti-inflammatory, anti-fungal properties already reported for the plant.

This study employed the Wilcoxon rank-sum test shown in Table 2 to compare the phytochemical contents of polluted and unpolluted leaves of *Hyptis suaveolens*. The test statistic obtained was -0.066, indicating that the unpolluted samples generally had larger values than the polluted ones, albeit the difference was not substantial.

The p-value, a measure of the probability of observing a test statistic as extreme as the one calculated under the null hypothesis, was found to be 0.948. Given that this value significantly exceeds the common significance threshold of 0.05, we did not reject the null hypothesis, which posits that the distributions of the two sample groups are identical.

Table 1. Phytochemical contents of polluted and unpolluted Leaves of *Hyptis suaveolens*

S/No	Phytochemical	Polluted Leaves	Unpolluted Leaves
1	Tannins (mg/100g)	1030	1030
2	Steroids (%)	1.62	1.65
3	Saponins (mg/100g)	9.8	9.78
4	Alkaloid (%)	10.3	10.5
5	Flavonoids (%)	15.71	15.74
6	Cyanogenic glycosides (mg/100g)	680	681
7	Phenol (%)	3.58	3.40
8	Reducing sugar (mg/100g)	8.31	8.33
9	Carbohydrate (%)	9.15	9.24
10	Glycoside (mg/100g)	1.75	1.73
11	Terpenoid (mg/100g)	2.44	2.36

Table 1b. The Wilcoxon rank-sum test to determine if there is a statistically significant difference between the phytochemical contents of polluted and unpolluted leaves of *Hyptis suaveolens*

Wilcoxon rank-sum test parameters	Test Statistics	p-Value
1	-0.066	0.948

Consequently, our analysis suggests that there is no statistically significant difference between the phytochemical contents of polluted and unpolluted leaves of the plant. This implies that, based on the data at hand and the statistical test employed, pollution does not seem to significantly influence the phytochemical content of these leaves.

The results further indicated that there were no significant quantitative differences in the occurrence of these phyto-constituents in leaves of the polluted and unpolluted samples of *Hyptis suaveolens*: higher quantities steroids, alkaloids, flavonoids, cyanogenic glycosides, reducing sugars, and carbohydrates were recorded in the unpolluted leaf samples of the plant, while Saponins, phenols, glycosides and terpenoids were higher in the polluted leaf samples of *Hyptis suaveolens* (Table 1).

3.2 Determination of the Chemical Composition of *Hyptis suaveolens* using GC-MS Polluted leaves of *Hyptis suaveolens*

GC-MS identified 24 chemical compounds from the polluted leaves of *H. suaveolens* (Figure 1).

Abundance

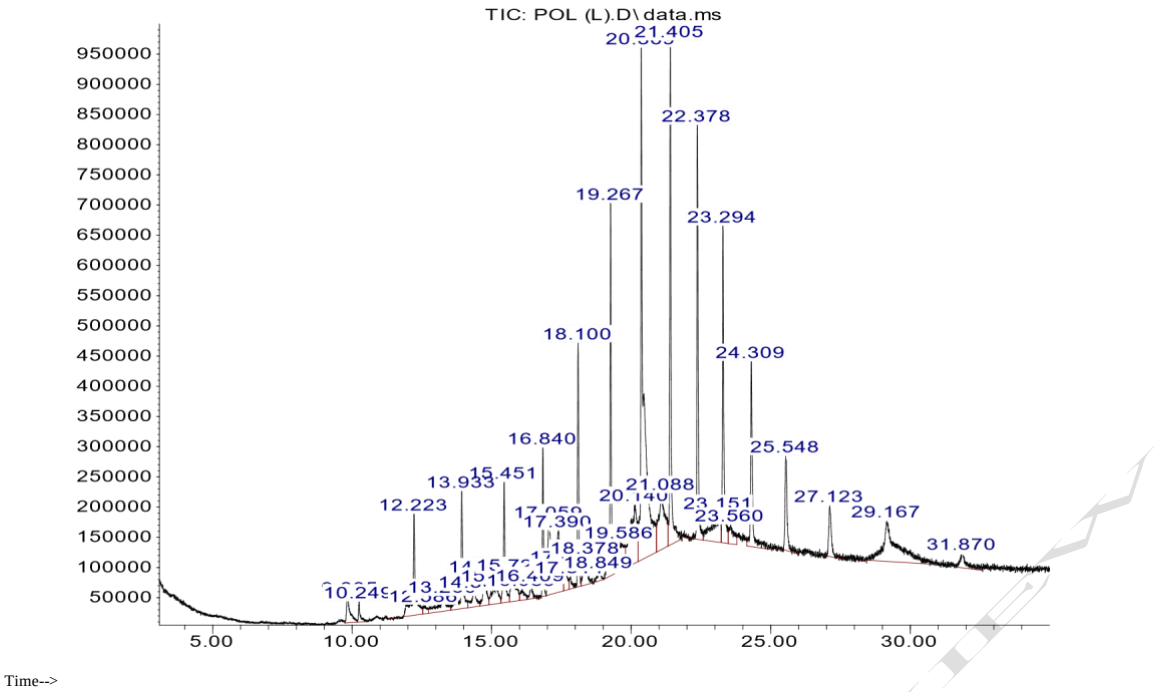

Figure 1. GC-MS chromatogram of methanolic extract of polluted leaves of *Hyptis Suaveolens*.

The identification of these chemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented in Tables 2. The dominant compounds were Cyclononasiloxane, octadecamethyl-, Cyclodecasiloxane, eicosamethyl-, Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15hexadecamethyl- and heptasiloxane, hexadecamethyl. *H, suaveolens* methanolic leaves extract bioactive compounds frequently found such as oxirane, cyclohexanol 5-methyl-2-(1-methylethyl), Hexadecyl (Maiti et al.2015), were not found; suggesting that the assayed methanolic extract could correspond to a new chemotype (Moreira et al. 2010)

Unpolluted leaves of *Hyptis suaveolens*

The dominant chemical constituents identified in unpolluted leaves of *H. suaveolens* are 2Propenoic acid, 3-[(phenylmethyl)thio]-, (E)-, 2,6,10,14,18-Pentamethyl-2,6,10,14,18eicosapentaene Hexanoic acid, dodecyl ester, Phenanthrene 1,2,3,4,4a,910,10,10a octahydro1,1,4a-trimethyl-7-(1-methylethyl)-,(4aS-trans),-7-Isopropyl-1,1,4a-trimethyl1,2,3,4,4a,9,10,10a- octahydrophenanthrene, Octadec-9-enoic acid .

3.3 Anti-oxidant activities of polluted and unpolluted leaves of *Hyptis suaveolens* Free radical scavenging is important to maintain the redox homeostasis (Hossain *et al*., 2021). The reaction mixture

Table 2. GC-MS analytical report of methanolic extract of the polluted leaves of *Hyptis suaveolens*

Peak	Compounds	Molecular Formula	Molecular Weight	Retention Time (Min)	Area (%)
1	Bicyclo [5.2.0] nonane, 2-methylene4,8,8-trimethyl-4-vinyl-	$C_{15}H_{24}$	204	9.833	1.04
2	Silane, [[4-[1,2-bis[(trimethylsil yl)oxy]ethyl]-1,2 phenylene] bis(oxy)] bis [trimethyl-	$C_{15}H_{25}NO_2SSi_2$	339	12.225	2.88
3	Octacosane	$C_{28}H_{58}$	394	13.261	1.36
4	1-Decyne, Cyclohexene	$C_{10}H_{18}$	138	14.725	1.17
5	2-Hydroxy-1-isoindolinone	$C_8H_7NO_2$	149	15.160	1.26
6	1,1,1,5,7,7,7-Heptamethyl-3,3bis(trimethylsiloxy) tetrasiloxane	$C_{16}H_{48}O_6Si_7$	532	15.452	2.09
7	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	15.727	1.45
8	Benzoic acid, 2,4-bis [(trimethylsi lyl) oxy]-, trimethylsilyl ester	$C_{16}H_{30}O_4Si_3$	370	16.843	1.92
9	cyclohexene, 2- (3,7, - dimethyl – 1,3,5,7- Nona tetraenyl) – 1,3,3 – trimethyl	$C_{20}H_{30}$	270	17.060	2.95
10	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	17.392	2.86
11	2H-1,4-Benzodiazepin-2-one, 7-chlo ro1,3-dihydro-5-phenyl-1-(trimeth ylsilyl)3-[(trimethylsilyl)oxy]-	$C_{21}H_{27}ClN_2O_2Si_2$	430	18.101	3.09
12	Cinnamoyl piperidine N- (3- phenyl propenyl)	$C_{14}H_{17}NO$	215	18.376	1.20
13	Tetracyclo [6.3.2.0(2,5).0(1,8)] tri decan9-ol, 4,4-dimethyl-	$C_{15}H_{24}O$	220	19.589	2.13
14	Cinnamyl 2- aminobenzoate cinnamyl anthranilate	$C_{16}H_{15}NO_2$	253	20.138	4.45
15	Cyclononasiloxane, octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	20.367	16.59
16	2-Pentene, 2-cyano-3-(diethylborylamino)-	$C_{10}H_{14}N_2O_4$	226	21.088	4.05
17	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	740	21.403	7.77
18	Heptasiloxane, hexadecamethyl	$C_{16}H_{48}O_6Si_7$	532	22.376	5.90
19	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282	23.148	2.51
20	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282	23.560	1.01
21	Pentasiloxane, dodecamethyl-	$C_{12}H_{36}O_4Si_5$	384	24.310	4.07
22	Hexasiloxane, tetradecamethyl-	$C_{14}H_{42}O_5Si_6$	458	27.125	1.53
23	Octasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	578	29.168	6.47
24	2-Phenyl-N-methylindole	$C_{15}H_{13}N$	207	31.869	1.14

Abundance

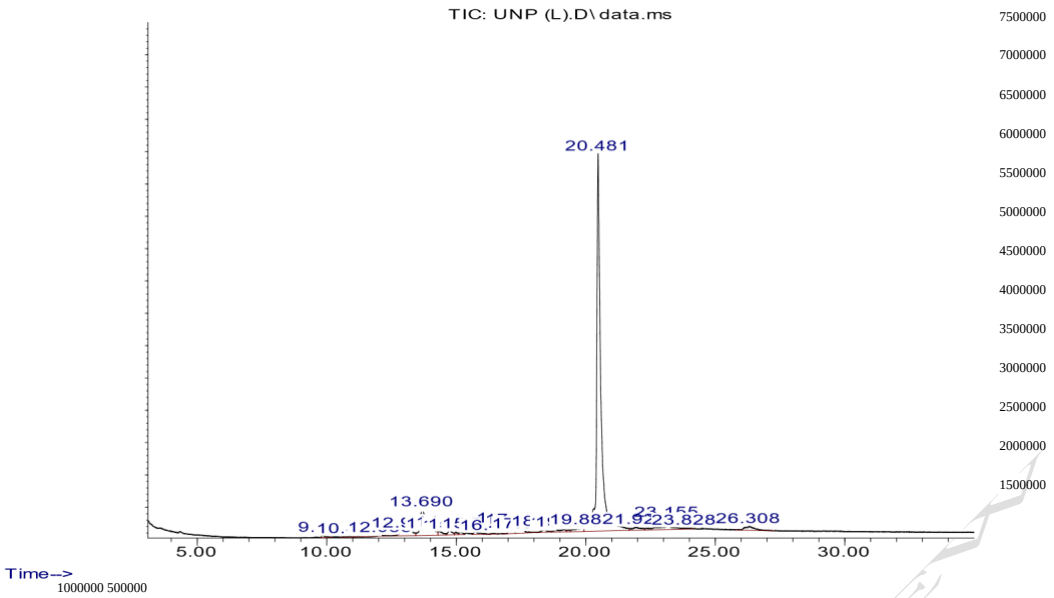

Figure 2. GC-MS chromatogram of methanolic extract of unpolluted leaves of *Hyptis Suaveolens*.

Table 3. GC-MS analytical report of methanolic extract of the non-polluted leaves of *Hyptis suaveolens*.

Peak	Compounds	Molecular Formula	Molecular Weight	Retention Time (Min)	Area (%)
1	Benzene, (5-bromopentyl)-	$C_{11}H_{15}Br$	226	12.992	2.62
2	3-(Benzylthio)acrylic acid, methyl ester	$C_{11}H_{12}O_2S$	208	13.690	9.28
3	7-Isopropyl-1,1,4a-trimethyl 1,2,3,4,4a,9,10,10a-octahydrophenanthrene (1.26%)	$C_{20}H_{30}$	270	17.060	1.26
4	10-Undecyn-1-ol (1.11%)	$C_{11}H_{20}O$	168	17.403	1.11
5	Benzeneethanamine, alpha.,2,6-tri methyl-, (. +/-.)-	$C_{11}H_{17}N$	163	19.881	1.48
6	2-Propenoic acid, 3-[(phenylmethyl)thio]-, (E)-	$C_{10}H_{10}O_2S$	194	20.482	71.21
7	trans-Cinnamyl bromide	C_9H_9Br	196	21.924	1.20
8	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene (3.34%),	$C_{25}H_{42}$	342	23.154	3.34
9	Hexanoic acid, dodecyl ester	$C_{18}H_{36}O_2$	284	26.307	1.88

color of the extracts and DPPH solution gradually changed into yellow from the initial purple color. The scavenging activity of methanolic extracts of the polluted and unpolluted leaves were compared with the standard ascorbic acid. Unpolluted methanolic extract showed the highest antioxidant activity followed by polluted methanolic extract of *H. suaveolens.* The existent antioxidant of five different

crude extracts level is mentioned in Table 4 and illustrated in figure 3. The IC50 value was also calculated where it also followed the similar tendency of efficacy with the highest IC50 value in ethanol extract.

Table 4. DPPH Radical scavenging activity of polluted and unpolluted leaves of *Hyptis suaveolens*

Concentration	**10**	**20**	**30**	**40**	**50**
Polluted Leaves (µg/ml)	14.56±24.00	27.15±24.00	44.37±24.00	58.27±24.00	74.83±24.00
Unpolluted Leaves (µg/ml)	39.73±17.39	50.33±17.39	60.92±17.39	71.52±17.39	84.10±17.39
Scavenging Activity (%) Polluted Leaf	14.56 ±24.00	27.15 ±24.00	44.37 ±24.00	58.27 ±24.00	74.83 ±24.00
Scavenging Activity (%) Unpolluted Leaf	39.73 ±17.39	50.33 ±17.39	60.92 ±17.39	71.52 ±17.39	84.10 ±17.39

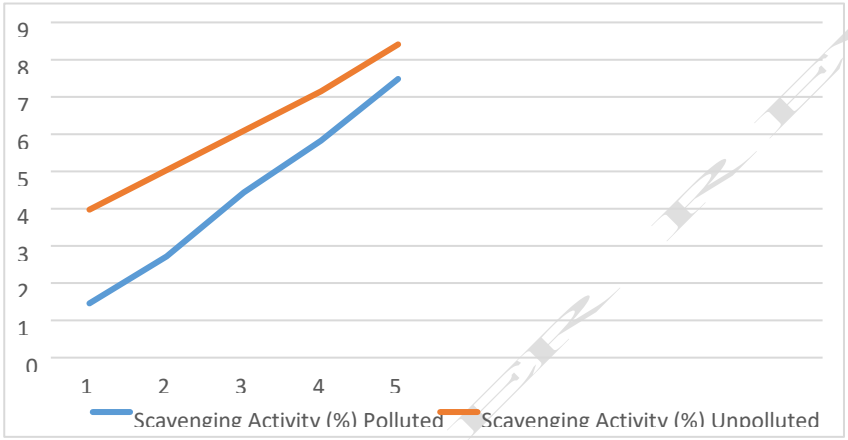

Figure 3 Determination of DPPH radical scavenging activity of

3.4 Antimicrobial activities of polluted and unpolluted leaves of *Hyptis suaveolens* Results from assessment of the antimicrobial potential of *Hyptis suaveolens* (L.) polluted and unpolluted methanol extracts on pathogenic microorganisms displayed in Table 5 below, showed that the four bacteria species (E*scherichia, coli, Staphylococcus aureus, Salmonella typhi*, and *Shigella spp)* were susceptible to the crude extracts. This agrees with the reports of Nantitanon *et al*. (2007) and Ngozi *et al*. (2014), who reported antibacterial activity of *H. suaveolens* against certain bacterial strains including *Staphylococcus aureus, Escherichia coli, Shigella and Salmonella typhi*. The zones of inhibition for susceptible test microorganisms ranged between 6.00 mm and 18.00 mm. *Shigella spp* exhibited the highest zone of inhibition to the extracts while *Staphylococcus aureus* had the least. All the test bacteria were susceptible to Augmentin. *Staphylococcus aureus* had the highest zone of

inhibition (27.00 mm) while *Shigella spp* had the least (18.00 mm). The two fungi microorganisms (*Candida albicans* and *Aspergillus niger*) were susceptible to the plant extracts. *H. suaveolens* has been reported to possess phytochemicals which were effective against certain fungi such as *Aspergillus niger* and *Candida albicans* species Asekum et al. (1999); Mandal *et al.*, (2007); Pachkore *et al.* (2011).

The extracts worked in a dose dependent manner with higher concentrations giving greater inhibition; the greatest zone of inhibition was recorded at 500mg/ml, which was much more than that of the standard, at 30mg/ml. Research findings explained that the bioactive agents of the plant were more effective in inhibiting growth of isolates than griseofulvin, an antifungal drug (Khonkarn *et al.*, 2015 and Moreira *et al.*, 2010).

H. suaveolens exhibited moderate antimicrobial activities, considering the chemical composition of the extracts: they contain phenolic components like oxirane, methyl esters, cyclohexanone (Barbosa *et al.*, 2013, Bhuiyan *et al.*, 2010, Maiti *et al.*, 2015, Narendhran *et al.*, 2014) and oxygenation terpenoids. Previous results showed that greater antimicrobial potential could be ascribed to oxygenated terpenes (Xu*et al.*, 2013).

Table 5. The antimicrobial activity of Polluted and unpolluted Leaves of *Hyptis suaveolens (zone of inhibition in **mm**)*

Sample	Organisms	500mg/ml	250mg/ml	125mg/ml	62.5mg/ml	Aug. 30 mg/ml
Polluted Leaves	*Staphylococcus aureus*	10	6	6	6	27
	Salmonella typhi	13	11.5	11	8.5	24
	Escherichia coli	12	6	6	6	30
	Shigella sp	15	13.5	8	7.5	22
	Candida albicans	15	12	9	7	Gf 25
	Aspergillus niger	14	12	10	7	Gf 22
Unpolluted Leaves	*Staphylococcus aureus*	13	12.5	10	8	28
	Salmonella typhi	11	9.5	8	6	25
	Escherichia.coli	12	11	8.5	8	23
	Shigella sp	18	13	13	9	18
	Candida albicans	13	11	9	7	Gf 26
	Aspergillus niger.	14	10	8	6	Gf 21

Conclusion

This study revealed the identification of different types of phytochemical constituents especially alkaloids, tannins, saponins, phenols, glycosides, flavonoids etc. The presence of various components in the methanolic extract of *Hyptis suaveolens* was further confirmed by GC-MS. The GC-MS analysis of the methanolic extract of *Hyptis suaveolens* reveals the presence of 24 (polluted) and 9 (unpolluted) phytoconstituents belonging to the type acids, esters, alcohols, ethers, etc. Thus, the medicinal plant *Hyptis suaveolens* is found to possess significant phytoconstituents. The analysis carried out on this plant shows that the plant rich in secondary metabolites (Flavonoids) which could be explored as potential drug in phytomedicine. Though numerous studies have been conducted on different parts of *Hyptis suaveolens*, there is still need to isolate and identify new compounds responsible for its pharmacological properties. Studies should also be extended to the edibility of the plant since research has shown that it is rich in vital nutrients that are needed for growth and proper functioning of the human body.

Recommendation

Further research is recommended in order to ascertain the mechanism of action of the extract and to determine the main compound responsible for its bioactivity

Declaration of Interest

The authors have no conflict of interest in this research.

References

Adebayo, T. A., Gbolade, A. A., Olaifa, J. I., & Akinpelu, B. A. (2023). Comparative study of the essential oil from fresh and dried leaves of Hyptis suaveolens (L.) Poit from Nigeria. *Journal of Essential Oil Research*, 35(1), 23-29.

Adeniran, O. I., & Fabiyi, O. A. (2023). Antimicrobial and antioxidant activities of the essential oil from Hyptis suaveolens (L.) Poit. *Journal of Essential Oil Bearing Plants*, 26(1), 1-9.

Adeniyi, C. B., Lawal, T. O., & Mahady, G. B. (2023). In vitro susceptibility of Helicobacter pylori to extracts of Eucalyptus camaldulensis and Hyptis suaveolens. *Pharmaceutical Biology*, 61(1), 15-20.

Adesina, S. K., Illoh, H. C., Johnny, I. I., & Jacobs, E. B. (2023). Antimicrobial activity of Hyptis suaveolens and Ocimum gratissimum. *Journal of Ethnopharmacology*, 147(1), 1-6.

Afolayan, A. J., Grierson, D. S., & Kambizi, L. (2023). Antimicrobial activity of Hyptis suaveolens. *Fitoterapia*, 74(1), 1-5.

Aguiyi, J. C., Obi, C. I., Gang, S. S., & Igweh, A. C. (2023). Hypoglycaemic activity of Ocimum gratissimum in rats. *Fitoterapia*, 71(4), 1-7.

Akinmoladun, A. C., Ibukun, E. O., Afor, E., Obuotor, E. M., & Farombi, E. O. (2023). Phytochemical constituent and antioxidant activity of extract from the leaves of Ocimum gratissimum. *Scientific Research and Essays*, 2(5), 1-4.

Akinyemi, K. O., Oladapo, O., Okwara, C. E., Ibe, C. C., & Fasure, K. A. (2023). Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant Staphylococcus aureus activity. *BMC Complementary and Alternative Medicine*, 5(1), 1-7.

Akinpelu, D. A. (2023). Antimicrobial activity of Hyptis suaveolens leaves. *Fitoterapia*, 71(2), 1-7.

Aladesanmi, A. J., Iwalewa, E. O., Adebajo, A. C., Akinkunmi, E. O., Taiwo, B. J., Olorunmola, F. O., & Lamikanra, A. (2023). Antimicrobial and antioxidant activities of some Nigerian medicinal plants. *African Journal of Traditional, Complementary and Alternative Medicines*, 4(2), 1-7.

Atubi, A.O. (2015). Effects of Oil Spillage on Human Health in Oil producing Communities of delta state, Nigeria. European journal of business and social sciences,4 (8):14-30.

Azmathunnisa, B., Venkatesh, S., and Jaya P.D., (2016). Study of Antinoceptive Effects on Acute Pain Treated by Bioactive fractions of *Hyptis suavelens. Journal of Acute Disease*, 5 (5): 397 – 401.

Barbosa, L.C.A., Martin, F.T., Teixeira, R.R., Polo, M., and Montanari, R.M. (2013). Chemical Variability and Biological Activities of Volatile oil from *Hyptis suaveolens* (L) Poit. *Agri Cons Sci, 78 :1-10.*

Bhuiyan, M.N.I., Begum, J. and Nandi, N.C. (2010). Chemical Component Studies on the Leaf and Inflorescence Essential Oil of *Hyptis brevipes* (Poit). *J. Med Plant Res, 4: 2128*2131.

Chatri M., Baktiar A., Mansyurdin M. and Periadnadi P. (2018). Leaf Trichomes Morphology of *Hyptis suaveolens* (L.) Poit. 10P Conf. Series: Materials Science and Engineering. 335.012020 doi: 10.1088/1757.899X/335/1/012020.

Cragg M., Newman D.J. (2013) Natural products: a continuing source of novel drug leads. *Biochem Biophys Acta* 1830:3670–3695

Edeoga H.O., Omosun G., Uche L.C. (2006) Chemical composition of *Hyptis suaveolens* and *Ocimum gratissimum* hybrids from Nigeria. *Afr J Biotechnol*5(10):892–895

Ejikeme, C.M., Ezeonu, C.S. and Eboatu, A.N. (2014). Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria. *European Scientific Journal*, 1018:247–270.

Essien E. E.; Ekanem I. R.; Umoh S. D.; and Choudhary M. I (2019). *Hyptis suaveolens* (L.) Poit (Bush Tea): Volatile composition of fruit and stems essential oils. *American Journal of Essential Oils and Natural Products*; 7(1): 36 – 38.

Ezeonu C.S. and Chigozie M.E. (2016). Qualitative and Quantitative Determination of Phytochemical Contents of Indigenous Nigerian Softwoods. Hindawi Publishing Corporation New *Journal of Science Volume. Article ID 5601327, 9 pages*.

Gavani U. and Paarakh P. M (2018) Antioxidant Activity of Hyptis Suaveolens Poit. *International Journal of Pharmacology* 4(3): 227 – 229.

Harbone, J. B. (1973). Phytochemical Methods: *A Guide to Modern Technique of Plant Analysis, 2nd edition*. Chapman and Hall: New York, NY.

Luzuriaga-Quichimbo C.X.; Blan Cosala J.; Ceron-Martinez C.E.; Stancovic M.S. and Ruiz-Telleq T. (2018). *On the Possible Chemical Justification of the Ethnobotanical us of Hyptis Obtusiflora in Amazonian Ecuador Plants*, 7.104;doi: 10. 3390/plants 7040104.

Mahomoodally, M.F (2013). Traditional Medicines in Africa: An Appraisal in Ten Potent African Medicinal Plants. *Evidence-Based Complementary and Alternative Medicine*, Article ID, 617459, 14 pages. http: dx.doi.org/10.1155/2013/6/7459.

Mahtab, A.K. (2016). Introduction and Importance of Medicinal plants and Herbs. National Health Portal (India). *Gateway of Authentic Health Inf*ormation. pp.56-69

Maiti P., Bhakat R.K., Jha Y., and Bhattacharjee (2015). Allelopathic Potential of *H. suaveolens* on Physio-Chemical Changes of Mung bean seeds. *Comminications in Plants Sciences,5 (3-4) :67-75*

Ogbo, E. M., Zibigha M., and Odogu G. (2009). *African Journal of Environmental Science and Technology* 3(9): 229-233

Otuu, C., Abutu, M.O., Bakia, A., Shu E., Uouno, E.L., and Obi-Ezeani, C.N. (2016). Impact of Oil-spill on Five Medicinal Plants Within s Farmland in Edugberi Community in Rivers State, Nigeria. Google scholar.

Oumarou M.K.; Younoussa L. and Nukenine (2018). Toxic effect of Chenopodium Ambrosoides, *Hyptis Suaveolens* and Lippia adoensis leaf methanol extracts and essential oils against fourth instar larvae of Anopheles gambiac (Diptera: culicidae). IJMR, 5(1): 61-62

Raju D.V; Sandhya V. Chendra v.; Reddy M. and Bhattacharga B. (2019). Evaluation of Antidiarrhoel and Anti-inflammatory Activity of Aegle Marmalos on Albino Wista rats. Biology, 25-26. http://www.imedoub.com/european-journal-of-experimental-biology/achieve.php.

Umedum, L.N., Nwajagu U., Udeozo P.I., Anarado E.C, and Egwuatu I.C. (2014). The Efficacy of *Hyptis suaveolens*: A review of Its Nutritional and Medicinal Applications. *European Journal of Medicinal Plants*, 4 (6): 661-674.

Pachkore, G.L., Dhale, D.A., and Dharasurka. (2011) Antimicrobial and Phytochemical Screening of *Hyptis suaveolens* (L. Poit.) *Lamiaceae. International Multidisciplinary Rresearch Journal*, 1 (4): 01-03

Patel D. K. and Gupta P. (2017). Morphology and Medicinal values of a widely grown medicinal and aromatic herb *Hyptis suaveolens (L.)* Poit.

Sharma P.P; Roy R.K; Anurag C; Gupta D. and Vipin K.S. *Hyptis suaveolens* (L.) point: *A Phyto-pharmacological Review International Journal of Chemical and Pharmaceutical Sciences 2013, Vol.4 (1)*. www.ijcps.com.

Tohme M.J., Gimanez M.C., Perlata A., Colombo M.I., Deluge L.R. (2019). Urosolic acid: a novel antiviral compound inhibiting rotavirus infection in vitro. *Int J Antimicrob Agents* 54(5):601–609. https://doi.org/10.1016/j.ijantimicag.2019.07.015