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Potential Application of Essential Oils in the Treatment of Neurodegenerative Diseases: a Case Study of Essential oil from *Hyptis Suaveolens* (L.) Poit

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Abstract

The main method for treating nerve-signaling disorders like Alzheimer's disease has been chemical suppression of acetylcholinesterase. Limited treatment options have driven the research into extracts from indigenous plants. In this study, the chemical constituents, anti-cholinesterase, and radical scavenging potentials of the essential oil and the polar constituents (decoction water) of a Nigerian variety of the medicinal plant, *Hyptis suaveolens* (L.) Poit, were investigated. Essential oil from the leaves of the medicinal plant was obtained through hydro-distillation, and the chemical composition was determined using gas chromatography-mass spectrometry (GC-MS). Radical scavenging activity potential and acetylcholinesterase inhibition activity were carried out using the colorimetric method. The GC-MS analysis revealed 24 chemical compounds, including β-Caryophyllene (15.31%), β-Phellandrene (9.73%), trans-α-Bergamotene (6.94%) and Fenchone (5.79%) as major components. The phytochemicals present in the polar constituent (the decoction water) of the leaves of *H. suaveolens* were alkaloids, glycosides, flavonoids, tannins, steroids, terpenoids, and saponins. Bioactivity assessment revealed significant antioxidant activities in essential oil extracts with values ranging from (DPPH = 38.80 ± 0.04 to 69.33 ± 0.12 %, ABTS = 2.84 ± 0.01 to 11.26 ± 0.26 mg TEAC /g, FRAP = 10.89 ± 0.01 to 15.79 ± 0.33 mg AAE /g and NOx = 26.06 ± 0.03 to 71.87 ± 0.21 %). Higher acetyl-cholinesterase inhibition activity was recorded for the essential oil compared to the polar constituents with values ranging from 10.42 ± 0.12 to 46.50 ± 0.19 %. Findings from this study highlight the antioxidant and anticholinesterase potential of extracts from *Hyptis suaveolens* (L.) Poit.

Keywords: Essential oil, Bioactivity, β-Caryophyllene, Decoction Water.

1. Introduction

Up to 2% of persons in developed civilizations with a high standard of living, particularly in Western nations, are affected by neurodegenerative disorders like Alzheimer's disease (AD), which cause a severe loss in cognitive functions and the degradation of synapses and death of neurons [1, 2]. The activity of acetylcholinesterase (AChE), the primary enzyme that hydrolyzes acetylcholine in human tissues, has been extensively studied since the discovery of decreased amounts of acetylcholine in the tissues of Alzheimer's disease (AD) patients [3]. The literature on several investigations on AD patients suggests that the tissues of those with the disease experience significant alterations in AChE activity [4].

Oxidative stress brought on by free radicals like reactive oxygen species has also been discovered to accelerate the onset of Alzheimer's disease (AD) and other age-related disorders [5]. As a defense mechanism against the damaging effects of free radicals in the tissues of vertebrate beings, the use of synthetic antioxidants (reducing agents) like thiols, ascorbic acid, or polyphenols [6] has been demonstrated [7]. Additionally, a substantial body of research has established that consuming antioxidants produced from plants helps prevent degenerative illnesses brought on by oxidative stress, such as cancer, Parkinson's disease, Alzheimer's disease, or atherosclerosis [8].

Accordingly, the use of medicinal plants with high antioxidant components levels has now been suggested as a different strategy for the improvement of free radical-mediated diseases [9]; consequently, mixtures of compounds with anticholinesterase and antioxidant activity, as used in traditional herbal medicine, have been considered beneficial in treating the entire disorder as opposed to single isolated symptoms of the disease [10].

Hyptis suaveolens (L.) Poit, also known as Pignut black, is endemic to tropical Asian and African nations as well as Nigeria, where it is classified as a weed. Some of these nations eat the leaves as vegetables [11]. The herb has qualities that are anti-inflammatory, antinociceptive, antiplasmodial, antirheumatic, anticonvulsant, antiulcerogenic, carminative, and lactagogue [12]. Additionally, the plant is used to cure nausea and gall bladder infections [13]. Its ability to lessen calcium oxalate crystallization is equivalent to that of branded medications used to dissolve kidney stones [14].

In this work, the essential oil and water-soluble extracts (decoction) from the leaves of *Hyptis suaveolens*, a plant that grows uncontrolled in southwest Nigeria, were investigated for their antioxidant and anticholinesterase properties.

2. Materials and Methods

2.1. Plant Collection and Identification

The plant "*Hyptis suaveolens"* was collected from a site around the premises of the Federal University of Technology Akure and identified.

2.2. Sample preparation

The fresh leaves of *H. suaveolens* were separated from the rest of the plant and air-dried for two weeks. The dried plant parts were chopped into small pieces and subjected to hydro-distillation. The essential oil separation was done using Clevenger-type apparatus. In a round bottom flask of 1000 mL capacity, 100 g of the fresh leaves were taken, and 200 mL of water was added. The separation of the oil was carried out in 10 batches at 40 °C. Prior to analysis, the oil was collected into a glass sample bottle and refrigerated.

The water extract was partially evaporated under reduced pressure using a rotary evaporator as a semi-solid sample, which was collected in a plastic sample that was also stored in a refrigerator prior to further analyses.

2.3. Chemical Composition of the Essential Oil

2.3.1. GC-MS Analysis

Essential oil of *Hyptis suaveolens* was analyzed using Agilent 7890B Gas chromatograph coupled with Mass spectrometer, fitted with a DB-1 (fused silica) capillary column coated with 5% Phenyl Methyl Siloxane (30*h* 0.32 0.25 µ*h*) (Agilent Technologies, U.S.A). Sample volume was 1µL injected in splitless mode at temperature of 220°, pressure of 2.84 and flow rate of 23.38/s. Oven temperature was initially programmed at 100 ° (5) then ramped at 7 ° / 225° (20). Helium was used as the carrier gas at constant flow rate of 1.0/s.

The identity of the constituents of the essential oil was established by comparing their mass spectra with those reported in literature [15] and by computer matching with NIST & WILEY libraries.

2.3.2. Phytochemical Screening

The polar constituents (decoction water) were screened for secondary metabolites using standard procedures as described by Sofowora [16], Momeni, *et al.* [17], Aguoru, *et al.* [18].

2.3.2.1. Test for Tannins

2 mL of the water extract was stirred with chloride solution, 2 mL of distilled water and few drops of ferric solution. The formation of a green precipitate was an indication for the presence of tannins

2.3.2.2. Test for Saponins

5 mL of the water extract was shaken vigorously with 5 mL of distilled water in a test tube and warmed. The formation of stable foam was taken as an indicative of the presence of saponins.

2.3.2.3. Test for Phlobatannins

2 mL of the water extract was added to 2 mL of 1% concentrated Hydrochloric acid and the mixture was boiled. Deposition of a red precipitate was taken as an evidence for the presence of phlobatannins.

2.3.2.4. Test for Flavonoids

1 mL of the water extract was added to 1 mL of 10% lead acetate solution. The formation of a yellow precipitate was taken as a positive test for flavonoids.

2.3.2.5. Test for Terpenoids

2 mL of the water extract was dissolved in 2 mL of chloroform and evaporated to dryness after which 2 mL of concentrated sulphuric acid was added and heated for about 2 min. A greyish colour indicated the presence of terpenoids.

2.3.2.6. Tests for Steroids

2 mL of the water extract was dissolved in 2 mL of chloroform and 2 mL concentrated sulphuric acid. A red colour produced in the lower chloroform layer indicated the presence of steroids.

2.3.2.7. Test for Alkaloids

3 mL of the water extract was stirred with 3 of 1% Hydrochloric acid on a steam bath. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

2.3.2.8. Tests for Glycosides

Salkowski's test: 2 mL of the water extracts was dissolved in 2 mL of chloroform. Then, 2 mL of sulphuric acid was added carefully and shaken gently. A reddish brown colour indicates the presence of a steriodal ring (that is, aglycone portion of glycoside).

2.3.2.9. Antioxidant Activity of the Essential Oil and the Decoction Water (water extract)

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity

The free radical scavenging activity of the essential oil and decoction water against DPPH radical was carried out by a method previously described in literature [19]. 1 of 0.4 methanolic solution of the DPPH was mixed with 1 of different concentration of the essential oil of *Hyptis suaveolens* diluted in methanol. The mixture was shaken vigorously and kept at room temperature for 30 in the dark. Absorbance of the reaction mixture was measured at 515 spectrophotometrically. The same procedure was carried out on the water extract diluted with distilled water and not methanol.

The percentage of free radical-scavenging capacity was calculated by the following equation (eqn 1).

$$\text{Radical scavenging (\%)} = \frac{A\text{blank} - A\text{sample}}{A\text{blank}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{ eqn 1}$$

Where A_{sample} is the absorbance of DPPH mixed with essential oil and A_{blank} is the absorbance of DPPH without the essential oil. All measurements were performed in duplicate and reported as the average value. To determine the IC_{50} a calibration curve was plotted with % radical scavenging capacity versus the different concentration.

2,2-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assay

The ABTS radical cation decolorization assay, a technique described by Re, *et al.* [20], was also used to assess the essential oil's capacity to scavenge free radicals. The ABTS cation radical was created via the reaction of 2.45 mM potassium persulfate with 7 mM ABTS in water, which was then held at room temperature in the dark for 16 hours before use. The absorbance was measured at 734 after adding 0.2 mL of diluted essential oil in methanol to 2.0 mL of ABTS solution for 15 minutes. The same process was used to the water extract that had been thinned out with distilled water rather than methanol. Results were expressed as mg of trolox equivalent antioxidant capacity per gram of dry weight using trolox as the benchmark.

2.3.2.10. Ferric Reducing Antioxidant power (FRAP)

The reducing property of the extract was determined through a procedure described by Pulido, *et al.* [21]. 0.25 mL of essential oil was combined with 0.25 mL of pH 6.6, 0.2 M sodium phosphate buffer, and 0.25 mL of 1% potassium ferricyanide [K3Fe(CN)6] solution. The mixture was incubated at 50 °C for 20 min; thereafter, 0.25 mL of 10% trichloroacetic acid was also added, and it was centrifuged at 2000 rpm for 10 min. Then 1.0 mL of the supernatant was mixed with 1.0 mL of distilled water and 0.5 mL of 0.1% ferric chloride, and the absorbance was measured at 700 nm using a UV-visible spectrophotometer. Ascorbic acid was used as a reference standard. The reducing power was then calculated and expressed as mg of ascorbic acid per gram of dry weight. The same procedure was carried out on the water extract diluted with distilled water, not methanol.

2.3.2.11. Nitric Oxide (NOx) Radical Scavenging Assay

The procedure was performed based on the method reported by Sreejayan and Rao (1997) Sumanont, *et al.* [22]. Sodium nitroprusside (10 mM)) in phosphate-buffered saline, was mixed with 1ml different concentrations of essential oil extract dissolved in methanol and incubated at room temperature for 150 min. Griess reagent (0.5 mL), containing 1% sulfanilamide, 2% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride, was added to the mixture after incubation time. The absorbance of the reaction mixture was read at 546 nm using UV Spectrophotometer. Gallic acid was used as the positive control. The same procedure was carried out on the water

extract diluted with distilled water not methanol. The percentage inhibition was calculated using the following formula (eqn 2).

$$\text{Inhibition \%} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100 \quad \text{.....eqn 2}$$

Where $A_{control}$ = absorbance of control sample and A_{sample} = absorbance in the of the sample.

2.3.2.12. Acetylcholinesterase Inhibition Activity (AChE)

Inhibition of AChE was assessed by Ellman colorimetric method [23], with some modifications. The AChE activity was determined in a reaction mixture containing 200 μL of a solution of AChE (0.5 U/mL), 100 μL of 3.0 mM solution of 5,5-dithio-bis(2-nitrobenzoic) acid, 200 μL of essential oil extract and 500 μL of phosphate buffer (0.1 M, pH 8). After incubation for 20 min at 25°C, 100 μL of 1.83 M acetylthiocholine iodide was added as the substrate. The absorbance of the reaction mixture was read at 412 nm using UV Spectrophotometer. The same procedure was carried out on the water extract. The AChE inhibitory activity was expressed as percentage inhibition.

$$\text{AChE inhibition (\%)} = \frac{A\text{blank} - A\text{sample}}{A\text{blank}} \times 100 \quad \text{.....eqn 4}$$

Where A_{sample} is the absorbance of enzyme mixed with essential oil and A_{blank} is the absorbance of enzyme solution without the sample.

2.3.2.13. Estimation of IC_{50} values

The IC_{50} values (i.e., concentration of test compounds that inhibits the free radicals and enzyme by 50%) were determined by spectrophotometric measurement of the effect of increasing concentrations of test compounds (essential oil extract and water extract). Determinations were carried out in duplicates. To calculate the IC_{50} values, each sample was assayed at 5 concentrations (0.25, 0.5, 0.75, 1.0, and 1.25 mg/mL). The IC_{50} values were obtained by linear regression.

3. Results

3.1. Phytochemical Screening of Essential Oil and the Decoction Water

The essential oil of the leaves of the *Hyptis suaveolens* obtained by hydro-distillation showed colourless volatile oil with strong aromatic odour. The percentage yield was 0.30% (v/w on dry weight basis). GC-MS profiling of chemical composition of the oil revealed a total of 24 compounds with β–Caryophyllene (15.31%), β– Phellandrene (9.73%), trans-α–Bergamotene (6.94%), Caryophyllene oxide (4.14%), Fenchone (5.79%) and α-Cadinol (5.72%) constituting major components (Table 1).

The qualitative phytochemical screening of the water extract of *H. suaveolens* indicated the presence of tannins, flavonoids, saponins, terpenoids and glycosides while alkaloids, steroids and phlobatannins were not detected (Table 8).

Table-1. Chemical Composition of Essential Oil of *H. suaveoelens*

S/N	Compounds	Retention index	Area (%)	Hydrocarbon subclass
1	α- Pinene	937	1.17	Monoterpene
2	β- Phellandrene	1004	9.73	Monoterpene
3	β- Pinene	979	3.35	Monoterpene
4	p-Cymene	1025	0.37	Monoterpene
5	α- Terpinene	1017	0.27	Monoterpene
6	D-Limonene	1030	1.38	Monoterpene
7	γ- Terpinene	1060	0.61	Mononterpene
8	β- Terpineol	1188	0.63	Oxygenated monoterpene
9	Terpine-4-ol	1177	3.26	Oxygenated monoterpene
10	β- Elemene	1390	0.94	Sesquiterpene
11	β-Caryophyllene	1420	15.31	Sesquiterpene
12	Humulene	1450	2.62	Sesquiterpene
13	Linanool	1099	0.21	Oxygenated monoterpene
14	γ- Elemene	1436	3.37	Sesquiterpene
15	d- Cardinene	1523	2.22	Sesquiterpene
16	Fenchone	1086	5.79	Oxygenated monoterpene
17	Caryophyllene oxide	1580	4.14	Oxygenated sesquiterpene
18	Spathulenol		1.43	Oxygenated sesquiterpene
19	α- Cardinol	1654	5.72	Oxygenated sesquiterpene
20	*Trans*- α-bergamotene	1435	6.94	Sesquiterpene

21	*Cis*-Pinane	983	0.52	Monoterpenene
22	Nerolidyl acetate	1632	0.31	Ester
23	β- Thujene	927	0.31	Monoterpenene
24	D-Farnescene	1490	0.40	Sesquiterpene

3.2. Bioactive Properties

Examining the ability of essential oils and polar extracts to scavenge DPPH radicals revealed that the amount of DPPH that could be scavenged increased with increasing essential oil or polar extract concentrations. However, essential oils greatly outperformed polar extracts in terms of the number of DPPH radicals they were able to scavenge per concentration. Assessment of potency factor showed that greater potency of essential oils over polar extracts for DPPH radical scavenging ability was greater at concentrations below 1.00mg/mL (Table 2, Fig. 2).

Both the essential oils and the polar extracts used to test the ABTS radical scavenging activity displayed concentration-dependent activity. At the lowest doses (0.25 and 0.5 mg/L), water extracts outperformed essential oils, but at 0.75 mg/L, essential oils outperformed water extracts the most. The efficacy of essential oils relative to water extracts reduced as concentrations rose (Table 3).

Table-2. DPPH radical scavenging ability (%) of the essential oil and water extract of *H. suaveolens* at different concentrations

Samples	Essential oil	Water extract	Relative potency
0.25 mg/mL	38.80 ± 0.04^{a}	22.04 ± 0.02^{a}	1.76
0.5 mg/mL	50.39 ± 0.01^{b}	23.98 ± 0.05^{b}	2.10
0.75 mg/mL	55.43 ± 0.00^{c}	31.45 ± 0.05^{c}	1.76
1.00 mg/mL	58.29 ± 0.03^{d}	34.49 ± 0.01^{d}	1.69
1.25 mg/mL	69.33 ± 0.12^{e}	47.85 ± 0.04^{e}	1.44

Data are represented as (mean value ± SD) n = 2; Concentrations along the same column with the same superscript are not significantly different.

Table-3. ABTS radical scavenging activity of the essential oil and water extract of *H. suaveolens* at different concentrations

Samples	Essential oil	Water extract	Relative potency
0.25 mg/mL	2.84 ± 0.01^{a}	2.97 ± 0.06^{a}	1.04
0.5 mg/mL	3.26 ± 0.09^{b}	3.94 ± 0.16^{b}	1.20
0.75 mg/mL	6.20 ± 0.00^{c}	4.36 ± 0.21^{b}	1.42
1.00 mg/mL	6.44 ± 0.03^{d}	5.74 ± 0.08^{c}	1.12
1.25 mg/mL	11.26 ± 0.26^{e}	10.70 ± 0.03^{d}	1.05

Data are represented as (mean value ± SD) n = 2; Concentrations along the same column with the same superscript are not significantly different.

Likewise, the ferric-reducing antioxidant properties of both essential oils and water extracts showed concentration-dependent properties. However, water extracts showed a greater potency compared to essential oils at all concentrations except at the highest concentration of 1.25 mg/mL. More importantly, it was observed that the potency of water extract compared to essential oil was greatest at the lowest treatment concentration (Table 4).

NO_X percentage inhibition measured at different concentrations of essential oil and water extracts compared to other bioactive properties also showed concentration-dependent activities. Polar extract showed greater potency of inhibition over essential oils at lowest concentration (0.25mg/mL), while essential oils showed greater NOx inhibitory potency at higher experimental exposure concentrations compared to the polar extracts. Greatest potency of essential oils compared to the polar extracts was observed at the highest exposure concentration (1.25mg/mL) (Table 5, Fig 3).

Table-4. Ferric reducing antioxidant properties of the essential oil and water extract of *H. suaveolens* at different concentrations FRAP

Samples	Essential oil	Water extract	Relative potency
0.25 mg/mL	10.89 ± 0.01^{a}	14.63 ± 0.09^{a}	1.34
0.5 mg/mL	13.00 ± 0.00^{b}	14.78 ± 0.02^{b}	1.14
0.75 mg/mL	13.88 ± 0.11^{c}	14.82 ± 0.03^{b}	1.06
1.00 mg/mL	14.00 ± 0.03^{c}	14.83 ± 0.00^{b}	1.05
1.25 mg/mL	15.57 ± 0.33^{d}	14.85 ± 0.03^{b}	0.95

Data are represented as (mean value ±SD) n=2; Concentrations along the same column with the same superscript are not significantly different

Table-5. NO_X Percentage inhibition of the essential oil and water extract of *H. suaveolens* at different concentrations of Essential oil and Decoction water (Water extract)

Samples	Essential oil	Water extract	Relative potency
0.25 mg/mL	26.46 ± 0.03[a]	28.27 ± 0.02[a]	1.07
0.5 mg/mL	47.07 ± 0.12[b]	37.74± 0.09[b]	1.25
0.75 mg/mL	53.40 ± 0.01[c]	48.60 ± 0.33[c]	1.09
1.00 mg/mL	56.58 ± 0.33[d]	53.20 ± 0.11[d]	1.06
1.25 mg/mL	71.87 ± 0.21[e]	53.84 ± 0.06[e]	1.33

Data are represented as (mean value ± SD) n = 2; Concentrations along the same column with the same superscript are not significantly different

Assessment of AChE inhibition activity of essential oils and water extracts both showed concentration-dependent trends. Essential oils showed an AChE inhibition capacity that exceeded the potency of water extracts by more than two-fold for most of the exposure concentrations (Table 6, Fig. 1).

Comparative IC_{50} values of the Antioxidant and Acetylcholinesterase activities of the essential oil and water extract of *H. suaveolens* (Table 7) showed that compared to water extracts, essential oils had at least a 2-fold capacity to inhibit 50% oxidative stress due to DPPH. The IC_{50} values for NOx showed that essential oils and water extracts had there was no significant difference in the amount of extract needed to achieve 50% inhibition. As regards AChE, the smaller IC_{50} values for essential oils compared to water extracts reveals that 50% inhibition of AChE was achievable with 3-fold smaller quantity than water extracts (Table 7).

Table-6. AChE inhibition activity of the essential oil and water extract of *H. suaveolens* at different concentrations

Samples	Essential oil	Water extract	Relative potency
0.25 mg/mL	10.42 ± 0.12[a]	4.22 ± 0.22[a]	2.46
0.5 mg/mL	16.46 ± 0.31[b]	10.35 ± 0.15[b]	1.59
0.75 mg/mL	25.86 ± 0.02[c]	12.53 ± 0.08[c]	2.06
1.00 mg/mL	35.19 ± 0.28[d]	17.35 ± 0.32[d]	2.02
1.25 mg/mL	46.50 ± 0.19[e]	20.15 ± 0.03[e]	2.30

Data are represented as (mean value ± SD) n=2; Concentrations along the same column with the same superscript are not significantly different.

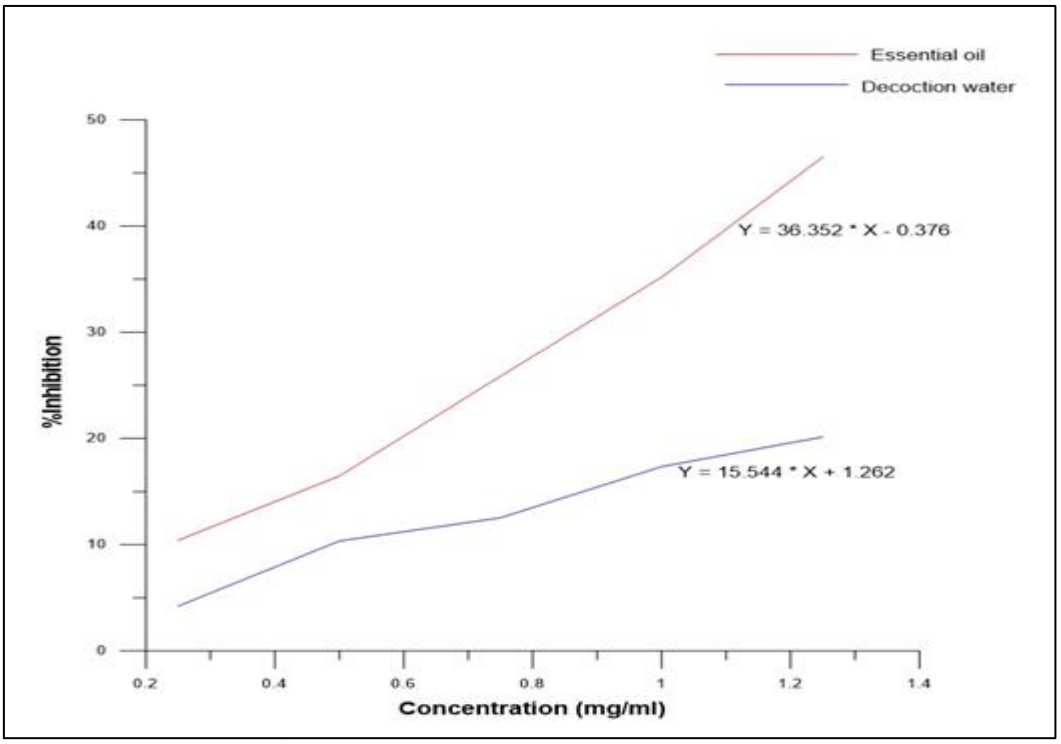

Figure-1. Plot of % AChE Inhibition vs. Essential Oil Concentration and Decoction Water

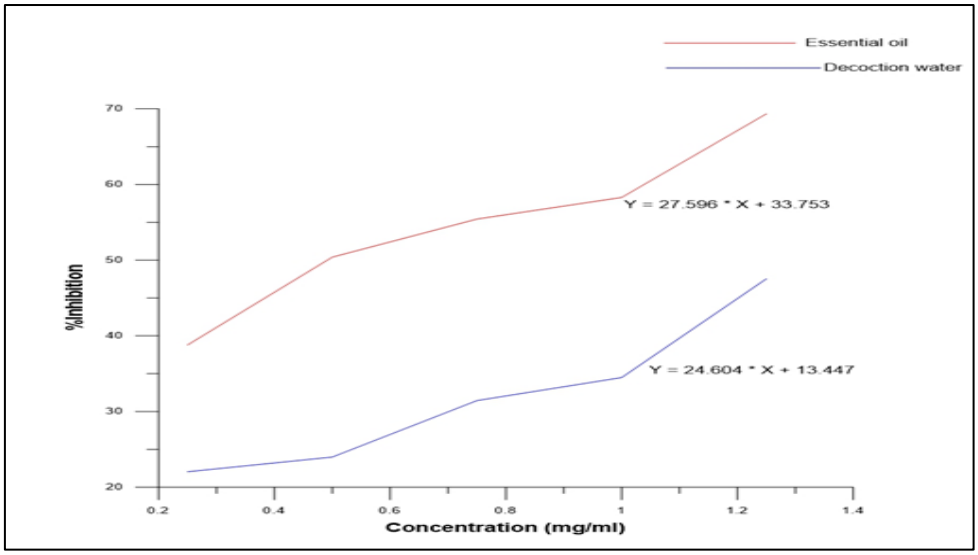

Figure-2. Plot of % DPPH Inhibition against Essential oil and decoction Water

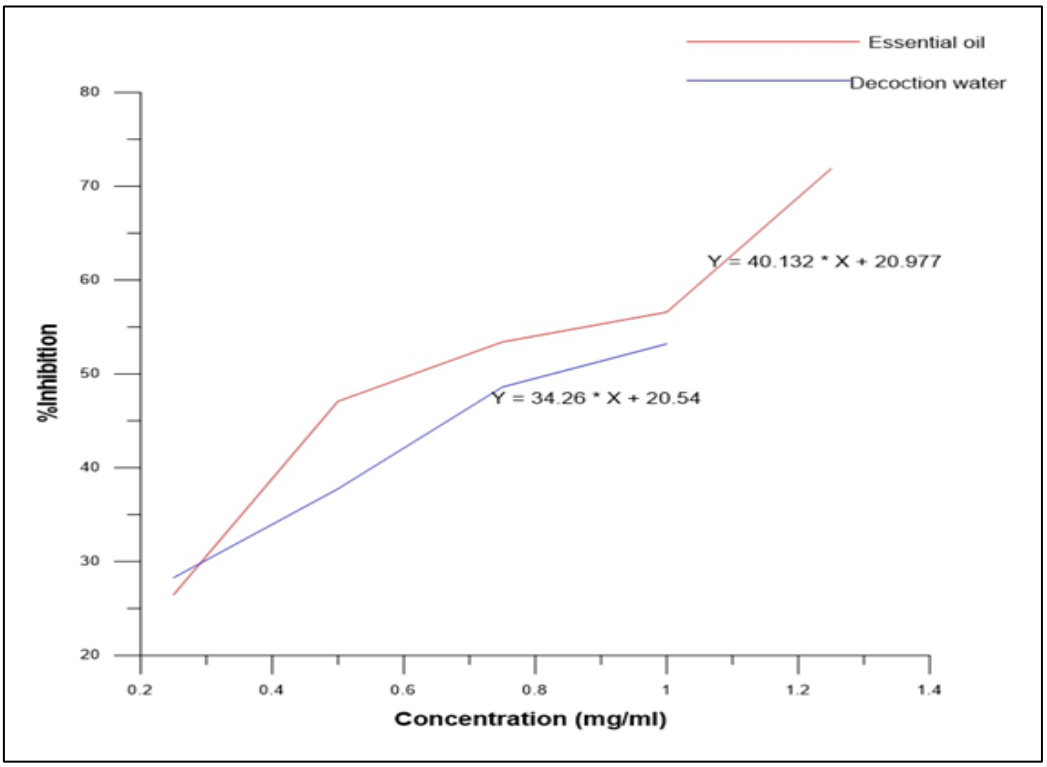

Figure-3. Plot of % NOx Inhibition against Essential oil and decoction Water

Table-7. IC_{50} values (mg/mL) of the Antioxidant and Acetylcholinesterase activities of the essential oil and water extract of *H. suaveolens*

	DPPH	NOx	AChE
Essential oil	0.59 ± 0.03^{a}	0.72 ± 0.21^{a}	$1.36 \pm .13^{a}$
Water extract	1.49 ± 0.16^{b}	0.85 ± 0.08^{a}	$3.14 \pm .22^{b}$

Data are represented as (mean value ± SD) n = 2; Concentrations along the same column with the same superscript are not significantly different.

Table-8. Qualitative phytochemical screening of the Decoction water of *H. suaveolens*

Phytochemical	Decoction water
Saponins	+
Tannins	+
Alkaloid	-
Steroids	-
Flavonoids	+
Terpenoids	+
Glycosides	+
phlobatannins	-

4. Discussion

The GC-MS analysis revealed that the essential oil from *H. suaveolens* was rich in β-Caryophyllene (15.31%), β-Phellandrene (9.73%), trans-α-Bergamotene (6.94%) and Fenchone (5.79%) as major components. The same primary constituent, Caryophyllene, of *H. suaveolens* essential oils was found in Malaysia [24-27]. In another report [28], it was determined that caryophyllene and trans-bergamotene were the two main constituents of *H. suaveolens* essential oil from other climes, and Hema, *et al.* [29] discovered that sabinene and 1,8-cineol were the two main volatile chemicals in the leaves of *H. suaveolens*. Eugenol and germacrene-D have been reported also Van Hac, *et al.* [30], as the rimary ingredients of some other samples. Although the occurrence of chemotype differences probably constitute the major factors for the reported variations in essential oil compositions, other factors like agricultural practices and analytical techniques could significantly affect the compositions of essential oils. Our results generally support studies that monoterpenes and sesquiterpenes make up the majority of the compounds in essential oils [31, 32]. The phytochemical content of the water extract indicated the presence of tannins, flavonoids, saponins, terpenoids and glycosides (Table 8). The phytochemical composition was comparable to what had previously discovered [33, 34].

Some plants are used as medicines because they contain flavonoids and saponins [35-37]. Thus, this may support the traditional healers in south-west Nigeria who utilize *H. suaveolens* to treat illnesses. It was thought that the presence of flavonoids in the aqueous extract was what gave it its antioxidant properties.

The radical scavenging activity of essential oil and water extract of *H. suaveolens* were studied by their ability to decolorize the DPPH, ABTS and NOx free radicals. The radical scavenging ability of the essential oil and water extract increases with increasing concentration. The DPPH and NOx radical scavenging abilities were expressed as IC_{50}. The antioxidant capacity of the essential oil in the DPPH and NOx radical assays were more than the water extract with IC_{50} values 0.59 ± 0.03 and 0.72 ± 0.21 mg/mL respectively. Regarding this, it has previously been stated that *H. suaveolens* essential oil exhibits effective DPPH radical scavenging activity [38]. The essential oil also showed high ABTS radical scavenging capacity at the highest concentration of 1.25 mg/mL (11.26 ± 0.26).

Different ways that essential oils showed the ability to scavenge free radicals included limiting chain reactions, termination of peroxides, stopping further hydrogen absorption, quenching the creation of singlet oxygen, and binding to catalysts made of transition metal ions [39, 40]. The ferric reducing power of the essential oil increases with increasing concentration. Maximum reducing potential (15.57mg AAE/g) was found in the essential oil, whereas, at highest concentration of the water extract minimum reducing potential (14.85 mg AAE/g) was noticed. The results showed comparable antioxidant potentials for *H. suaveolens* from other locales [41]. The results of the acetyl-cholinesterase assay indicate that the essential oil and water extract showed significant inhibitory activity. However, the essential oil showed higher inhibitory effect than the water extracts with IC_{50} values 3.14 ± 0.22 compared to the value of 1.36 ± 0.13 mg/mL recorded for the water extracts.

5. Conclusion

The results obtained in this study showed that the essential oil of the studied Nigerian variety of *Hyptis Suaveolens* (L.) Poit was composed of β-Caryophyllene, β-Phellandrene and *trans*-α-bergamotene as the major chemical principles. The essential oil showed high proclivity for scavenging different free radicals in different systems. Hence, the essential oil of *H. suaveolens* may be useful as prophylactic against oxidative stress and neurodegenerative diseases. More importantly, this study highlights the potential for polar extracts to be more bioactive at lower exposure concentrations compared to essential oils. Furthermore, the greater relative potency of essential oils as regards AChE inhibition compared to antioxidant activity, suggests that essential oils may be of greater value in the management of neurodegenerative diseases compared to decoction water.

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