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Chemical Characterization and Antioxidant Activity of Essential Oil from the Aerial parts of *Hyptis suaveolens* (L.) Poit.

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ABSTRACT: *Hyptis suaveolens (L.) Poit. (American mint or Vilayati Tulsi) is an aromatic horehound weed known for its medicinal and ethnobotanical use in various parts of world. In the present study, the chemical composition of hydrodistilled oil (yield ~ 0.09%, w/v), from the aerial parts were analysed through Gas Chromatography (GC) and Gas Chromatography Mass Spectroscopy (GC–MS). The main constituents were sabinene (~17.22%), β–caryophyllene (~14.35%), abietadiene (~6.48%), limonene (~6.05%), α-terpineole (~4.65%) and abetatriene (~4.14%). Further, the antioxidant activity of in the essential oil of H. suaveolens was evaluated in terms of 2,2-diphenyl-1-picryl hydrazyl (DPPH), hydroxyl radical (•HO) it with hydrogen peroxide (H_2O_2), Fe^{2+} chelating activity, and ferric reducing antioxidant power (FRAP) assay. The antioxidant activity was measured in terms of percent scavenging activity (%), and further IC_{50}values were also calculated. The present study depicts that the essential oil of the test plant exhibit great scavenging activity towards •HO, H_2O_2, FRAP, DPPH and Fe^{2+} chelating activity.It is, thus, clear that the essential oil of H. suaveolens possesses good antioxidant activity and holds apromising potential to replace synthetic antioxidants in further studies.*

Keywords: *Hyptis suaveolens, Essential oil, Gas Chromatography Mass Spectrometry (GC-MS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ferric ion reducing power (FRAP),Hydroxyl radical (•HO) and Hydrogen peroxide(H_2O_2).*

INTRODUCTION

Reactive oxygen species (ROS) are a group of free radicals, reactive molecules, and ions that are derived from oxygen. It includes singlet oxygen ($^1O_2$), superoxide ion ($O_2^{\bullet-}$), hydroxyl ion ($^{\bullet}HO$) and hydrogen peroxide (H_2O_2). ROS plays an important role in biological system, as these are the by products of normal metabolism. They act as intermediates in various enzymatic reactions [1]. ROS at low or moderate concentration are reported to be harmless and have been implicated as second messengers in intracellular signaling cascades [2] but at higher concentration, these can damage various bio-molecules (proteins, lipids, enzymes, and nucleic acids), resulting in oxidative injury, disease induction and ultimately leading to cell death [3, 4]. Living cells possess an excellent scavenging mechanism for antioxidants where they neutralize the toxic effects generated by ROS. However, with increasing age and exposure to various external stresses these endogenous mechanisms become inefficient and dietary supplementation of synthetic antioxidants is required. In recent years, safety and efficacy of these synthetic antioxidants used in the food industry is frequently questioned due to toxicological concerns associated with them.

Therefore, the quest for interest in finding naturally occurring antioxidants induced by ROS has intensified [5]. In this context, various aromatic and medicinal plants are generally preffered over the synthetic antioxidants [6]. Since these are safer, ecofriendly and has minimal toxic effect on human health [7]. The medicinal and aromatic plants, apart from being a good resource for drug development, they also serve as rich sources of essential oils, minerals, and vitamins, etc. [8]. According to [7] essential oils extracted from the aromatic plants serve as a powerful scavenger of free radicals. Now a days,

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main focus of research is on exploring antioxidant properties of essential oil from medicinal and aromatic plants [9]. Since essential oils contain a variety of volatile components called as secondary metabolites and are known for their significant role in plant defense mechanism. Recent biochemical *in-vivo* studies have characterized some of the compounds as natural antioxidants [10, 11]. Keeping these things in mind, a study was conducted on *Hyptis suaveolens* (L.) Poit. commonly known as bushmint, pignut or ganga tulsi, an aromatic herb native to tropical America. It occurs in various parts of India such as Deccan peninsula, North East India, Andaman and Nicobar Island. It grows as a weed along the rail tracks, roadsides [12] or foothills of open forests and forest clearings [13]. The plants is also known for its medicinal values and and used as a stimulant, carminative, for curing wounds, against infection of uterus and parasitic skin diseases etc [14, 15]. The essential oil has been used for various biological activities such as antifungal [16], antibacterial [17], anticancerous [13], antiulcer [18], antinociceptive [19], and anticonvulsant [20]. Besides these, it also possesses insecticidal properties [21]and tumorigenic properties [22]. Although, there are several reports on the chemical composition of the essential oil of *H. suaveolen s*however, little work has been done to explore the antioxidant activity of this essential oil. Therefore, the present study was conducted to investigate the chemical composition, antioxidant and free radical scavenging activity of the essential oil of *H. suaveolens* to justify its ethno-medicinal use and expose more avenues for its exploitation.

MATERIAL AND METHODS

Plant Materials

The aerial parts of *H. suaveolens* were collected during flowering stage from the outskirts of Chandigarh, India. The plant was identified and verified from the herbarium of Panjab University, Chandigarh where the voucher specimen was also deposited (PAN# 18048).

Extraction of Essential Oil

The essential oil was extracted from the chopped aboveground flowering shoots by hydro-distillation using Clevenger's apparatus. Nearly 2 kg of freshly collected plant material was mixed with 2 L of water. The mixture was boiled for 3 h and oil was collected from the nozzle of the condenser. The obtained essential oils was dried over anhydrous sodium sulphate and stored at 4°C until tested and analysed by Gas Chromatography Mass Spectroscopy (GC-MS) for identification of its components.

Essential Oil Analysis

The essential oil was analyzed by GC-MS, as described by [23]. GC was done using Shimadzu GC-17A gas chromatograph equipped with a Flame Ionization Detector (FID) and a DB-5 column [60 m × 0.25 mm (inside diameter), film thickness 0.25 um]. Helium (He) was used a carrier gas at a split ratio of 1:20 and the column rate was 1ml min^{-1}. The temperature of injector and ion source were fixed at 250°C and 280°C, respectively. The oven temperature was initially 50°C which was held isothermally for 2 minutes and then increased to 260°C at the rate of 4°C per minute and finally held at 260°C for three minutes. The relative amounts of different constituents were determined by computerized peak areas normalization based upon three injections of the oil, without any correction factor. The peaks were compared with data on GC-MS.

Identification of Compounds

Different constituents of the essential oil of *H. suaveolens* were identified by matching their mass spectra and RI with that of pure reference samples consulting the libraries of Wiley 275, NIST 98 and the compilation by Adams [23].

Radical Scavenging and Antioxidant Activity

DPPH (2, 2-diphenyl-1-picryl hydrazyl) radical scavenging activity

The DPPH scavenging activity was measured as per the method given by [24] with slight modification. Various concentrations of sample solution (50–400μg ml^{-1}) were mixed with 1 ml of 0.1 mM alcoholic DPPH solution. After this, the samples were incubated in dark at room temperature. The reaction mixture was vortexed thoroughly, left in the dark at 25°C for 30 min and measured at 517 nm. Decrease in absorbance of the samples with increasing concentration of the oil indicates the DPPH scavenging activity. The ability of the essential oil to scavenge DPPH radical was calculated as % inhibition by the following equation:

DPPH scavenging or (%) inhibition

$$= [(A_{control} - A_{sample})/A_{control}] \times 100$$

Hydroxyl radical (˙HO) scavenging activity

For assaying the hydroxyl radical scavenging activity, method given by [25] was followed. To 0.5 ml of sample solutions (50–400μg ml^{-1}), 2.5 ml of the reaction mixture containing 3 mM 2-deoxyribose, 0.1 mM $FeCl_3$, 1 mM H_2O_2, 0.1 mM EDTA, 0.1 mM ascorbic acid, and 0.02 M phosphate (pH 7.4) buffer was added to make a final volume of 3 ml followed by incubation for 1h at 37°C. Then, 1 ml of tert–butyl alcohol (TBA) and 1 ml of trichloroacetic acid (TCA) were added to the test tubes and these were heated at 100°C for 20 min. After cooling the mixture, absorbance was read at 532 nm against a blank containing buffer and 2-deoxyribose.The percent scavenging activity was calculated and expressed as inhibitory concentration IC_{50}. The percent scavenging was calculated by using the formula shown below:

Hydroxyl radical (•OH) scavenging (%)

$$= [(A_{control} - A_{sample})/A_{control}] \times 100$$

Hydrogen peroxide scavenging (H_2O_2)

H_2O_2 scavenging activity was determined as per the method of [26] with slight modification. 40 mM solution of H_2O_2 was prepared in a phosphate buffer (pH 7.4). Different concentrations of sample solution (50"400 μg ml^{-1}) were added to 0.6 ml of H_2O_2 solution and the absorbance was read at 230 nm after 10 min against a blank containing phosphate buffer without H_2O_2. The percent scavenging was calculated and further, the activity was expressed as effective concentration IC_{50}. The percent scavenging of H_2O_2 was calculated using the formula:

H_2O_2 scavenging (%)

$$= [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Iron chelating (Fe^{2+}) activity

This test was done as per the method given by [27] with slight modifications. For this, 0.05ml of $FeCl_2$ (2mM) was added to1ml of samplesolution(50–400mg ml^{-1}).After 30 seconds, 0.1 ml of ferrozine (5 mM) was added further, after 10 minutes at room temperature, the absorbance of the complex was measured at 562 nm spectrophotometrically. The metal chelating activity of sample solution was calculated (as percent) and further, the activity was expressed as inhibitory concentration IC_{50}. The metal chelating activity was calculated by using the formula:

Fe^{2+}chelating activity (%)

$$= [(A_{control} - A_{sample})/A_{control}] \times 100$$

Ferric ion reducing antioxidant power (FRAP) activity

Ferric ion reducing antioxidant power (FRAP) assay was done by the method given by [28]. Sample solution (50–400μg ml^{-1}) equivalent to 0.2 ml was taken and to this, 0.6 ml of 0.2 M phosphate buffer (pH 6.6) and 0.6 ml of potassium ferricyanide solutions were added. The mixture was incubated for 30 min at 50 °C temperature. After this, 0.6 ml of trichloroacetic acid (TCA) solution was added in each test tube followed by centrifugation for 10 min at 3000 rpm. From this, 0.6 ml of supernatant was taken and to this was added 0.6 ml of distilled water and 0.125 ml of freshly prepared $FeCl_3$ solution. The concentration of FRAP was determined spectrophotometrically at 700 nm. Percent inhibition was calculated as:

$$(\%)\ \text{Inhbition} = [(A_{sample} - A_{control})/A_{sample}] \times 100$$

Statistical Analysis

The data are presented as mean ± SE and analyzed by one-way ANOVA followed by the comparison of mean values using post-hoc Tukey's test at $p \leq 0.05$. The IC_{50} values were calculated from a linear regression analysis.

RESULTS AND DISCUSSION

Chemical Composition of the Essential Oil

The essential oils extracted by hydrodistillation from the aerial parts of *H. suaveolens* were found to be pale yellow in colour (0.09%, v/w, fresh weight basis). The chemical composition of the oils can be seen in Table 1. It shows the constituents of the essential oil, their percentage composition and Kovats Index (KI). The values are listed in order of elution on the DB-5 column. The GC-MS analysis of the *Hyptis* oil depicted the presence of 42 components which were eluted between 11.28 and 48.13 min, constituting 96.11% of the oil. The major components present in the essential oil were sabinene (17.22%), β–caryophyllene (14.35%), abietatriene (6.48%), limonene (6.05%), α-terpineole (4.76%) viridiflorol (4.53%) and abietadiene (4.14%). Essential oil was

Table 2
Composition of the essential oil of *H. suaveolens* by chemical class

Chemical class	*% in essential oil*
Monoterpene hydrocarbons	*33.58*
Oxygenated monoterpenes	16.54
Total monoterpenoids	50.23
Sesquiterpene hydrocarbons	22.61
Oxygenated sesquiterpenes	12.65
Total sesquiterpenoids	35.26
Diterpenes	10.62
Other unidentified compounds	3.89
Total	96.11

mixture of monoterpenes (~50.23), sesquiterpenes (~35.26%) and diterpenes (~10.62%).These results for chemical composition of *H. suaveolens* essential oil are in agreement with other studies [22, 29, 30, 31, 32, 33, 34, 35] where sabinene, β-caryophyllene, limonene,terpinolene and abietatriene were reported as one of the major components of the oil.

According to [36], eugenol (68.2%) and germacrene D (11.0%) were the major constituents of *H. suaveolens* oil. But our results are not concordant with their findings since we could not report eugenol and germacrene D was also not one of the major components of the oil. These variations among the chemical constituents regarding their compositions could be attributed to various factors such as climate, location, and season, age of plant and soil type. Apart from these, the methods used for drying and extraction of oil from the plant material were also responsible for variation in chemical constituents of plant [37, 29, 38].

Antioxidant Activity

In this study, various antioxidant assays, were applied to evaluate the antioxidant activity of essential oil. The antioxidant activity was measured in terms of percent scavenging activity (%) and further their IC_{50} values were also calculated.

DPPH (2, 2-diphenyl-1-picryl hydrazyl) radical scavenging activity

The free radical scavenging activity of the essential oil of *H. suaveolens* was evaluated using DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay. DPPH assay is a widely used method to investigate the free radical scavenging activity of the plant samples [39]. In the present study, the antioxidant activity of the essential oil and standard BHT (Butylated hydroxytoluene) were studied to evaluate DPPH radical scavenging activity (Fig.1). The percent scavenging activity of the test plant increased with increasing concentration of the oil. The DPPH radical scavenging activity (89%) in the essential oil of the test plant at the highest concentration of 400 ìg ml^{-1} was higher than that of BHT (78.13%). Parallel to this, it has been reported earlier also, that the essential oils from the plants of family Lamiaceae possessed good scavenging activity towards DPPH [23, 40]. Further, the IC_{50} values of the essential oil

Table 1
Chemical composition of essential oil from aerial parts of *H. suaveolens*

R.I[a]	*Components*[b]	*RT (min)*[c]	*% age*[d]
953	*α*-Thujene	11.289	0.40
959	*α*-Pinene	11.547	2.29
1000	Sabinene	13.249	17.22
1001	*β*-Pinene	13.322	3.65
1016	*β*-Myrcene	13.952	0.33
1027	*α*-Phellandrene	14.404	0.20
1035	Δ^3Carene	14.727	0.83
1040	*α*-Terpinene	14.944	0.35
1044	*p*-Cymene	15.106	1.63
1050	1,8-Cineole	15.379	1.77
1054	Limonene	15.538	6.05
1081	*γ*-Terpinene	16.671	0.63
1086	*cis*-Sabinene hydrate	16.877	0.21
1111	*α*-Terpineole	17.938	4.76
1115	*trans*-Sabinene hydrate	18.092	0.15
1130	Fechyl alcohol	18.741	0.55
1160	Isoisopulegol	19.983	1.85
1163	Citronellal	20.125	1.01
1170	Isopulegol	20.423	0.79
1191	Terpinene-4-ol	21.276	3.11
1201	Linalyl propionate	21.709	0.32
1239	*β*-Citronellol	23.284	1.65
1344	Citronellyl acetate	27.715	0.48
1385	*β*-Elemene	29.430	0.11
1410	*β*-Caryophyllene	30.495	14.35
1473	Germacrene D	30.983	3.99
1488	α-Humulene	31.482	1.50
1495	Aromadendrene	31.689	0.11
1509	α-Selinene	32.150	0.08
1514	Germacrene B	32.306	0.08
1520	β-Selinene	32.496	1.00
1530	*trans* (Z) β Guaiene	32.819	1.39
1603	(+) Spathulenol	35.170	0.90
1607	(–)-Caryophyllene oxide	35.296	2.05
1620	Virdifloral	35.730	4.53
1661	*tau*-Cardinol	37.050	0.15
1669	*β*-Eudesmol	37.313	0.57
1674	*α*-Eudesmol	37.450	0.80
1709	Bergamotol	38.581	3.65
2053	Abietatriene	47.381	6.48
2083	Abietadiene	48.131	4.14

(a) Retention index relative to n- alkanes (C_{10-40}) on the DB-5 capillary column.
(b) Compounds present in order of elution from DB-5 capillary column.
(c) Retention time of chemical compounds
(d) Percentage area.

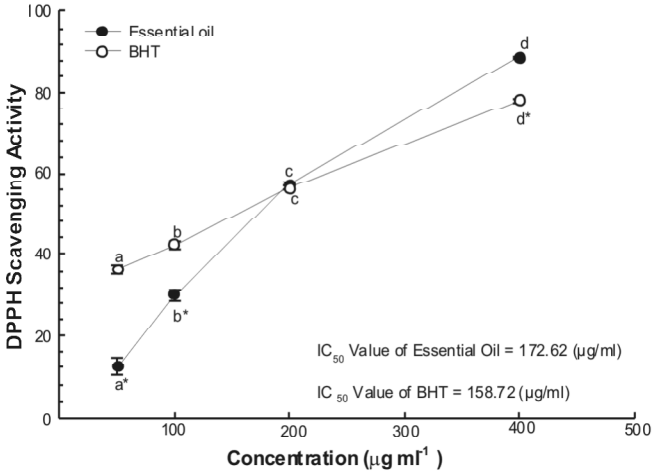

Figure 1

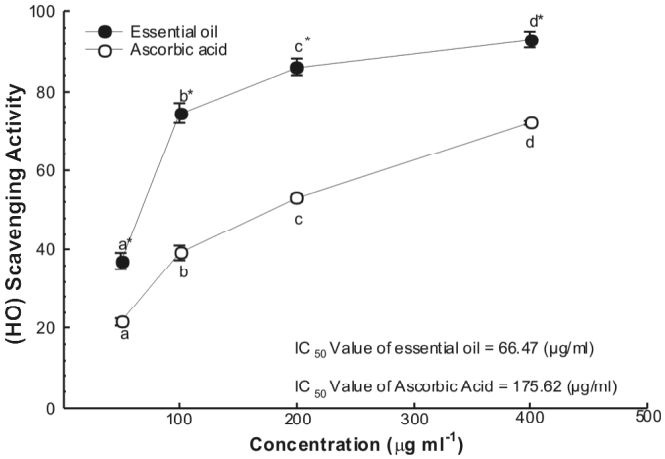

Figure 2

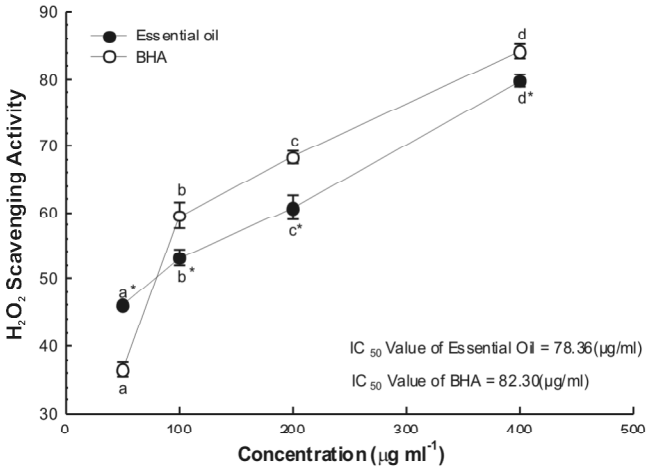

Figure 3

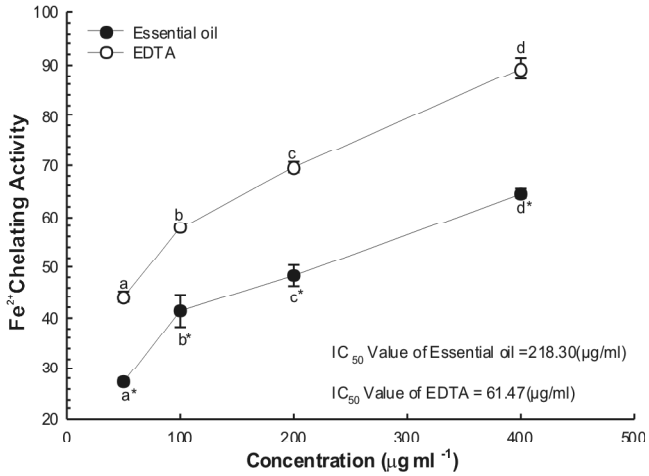

Figure 4

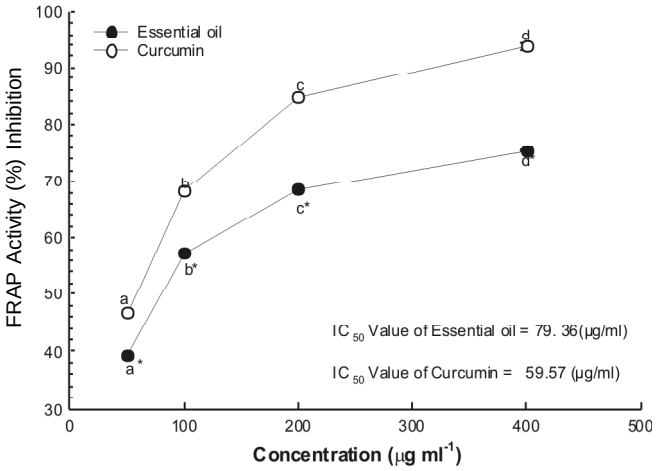

Figure 5

Figures. 1–5: Represents the antioxidant activity of the essential oil (50–400 mg ml^{-1}) of *H. suaveolens*1) 2,2-diphenyl-2-picryl-hydrazyl free radical (DPPH), 2) Hydroxyl ($^{\cdot}$HO)radical scavenging activity 3) Hydrogen peroxide (H_2O_2), 4) Fe^{2+} Chelating activity 5) Ferric ion reducing antioxidant power (FRAP) activity. Data analysed by using one-way ANOVAfollowed by the comparison of mean values using post-hoc Tukey's test at $p \leq 0.05$. Different alphabetical letters represented for each concentration.*Represents significance at $p \leq 0.05$.

and standard, BHT were calculated. Minimum IC_{50} (IC_{50} = 158.72 μg ml^{-1}) was reported in the standard andcompared to this, the IC_{50} value of the essential oil was 172.62 μg ml^{-1}.

Hydroxyl (˙HO) radical Scavenging activity

Hydroxyl radical is one of the most reactive molecules which can damage all the essential biomolecules such as amino acids, proteins and DNA [41, 42]. According to [43] the hydroxyl radical combines with nucleotides in DNA and causes strand breakage which leads to the carcinogenesis, mutagenesis and cytotoxicity. Therefore, it is important to investigate the potential of natural products to scavenge this radical. So, in this direction, we explored the hydroxyl radical scavenging activity of the essential oil of *H. suaveolens*. The present study reported a significant increase in •OH scavenging activity with increasing concentrations of the test oil.The ˙HO radicalscavenging activity of the oil was in the range of 37 to93%, whereas; in case of standard it was 21.63 to72%. The IC_{50} value of the oil (66.47 μg ml^{-1}) was calculated and found to be lesser than that of the ascorbic acid, used as a standard (175.62 μg ml^{-1}). This observation indicates that the oil possessed better scavenging ability than that of standard (Fig.2). The current findings are in accordance with the previous findings of [44, 45] where the essential oil from *Oregano*and *Portuguese thyme* speciesexhibited good scavenging potential for ˙HO radical.

Hydrogen peroxide (H_2O_2) Scavenging activity

Hydrogen peroxide (H_2O_2) a weak oxidizing agent upon entering the cell, inactivates various enzymes and leads to the formation of hydroxyl radical [46]. Present study reported that the positive control BHA (Butylated hydroxyanisole) exhibited maximum scavenging activity (84.12%) at highest concentration, which was followed by the test oil (80%). The IC_{50} value of the oil was calculated to be 78.36μg ml^{-1} as compared to BHA (82.30 μg ml^{-1}).In the present study, the minimum IC_{50} value was obtained in case of oil and was found to be better than the standard (Fig.3). The current findings are supported by the study reported by [47] who investigated the H_2O_2 scavenging activity in the essential oil of *Laurus nobilis* and concludedthat the oil possessed better scavenging activity than the standard.

Iron chelating (Fe^{2+}) activity

Iron is considered as an essential mineral for normal physiology but at higher concentrations it acts as a pro-oxidant in the form of ferrous ion and catalyzes the various oxidation reaction in biological system [48]. Therefore, it is important to remove this with the help of natural antioxidant. The current study suggested the protective role of *H. suaveolen s*oil against oxidative damage sequestered by Fe (II) ions. The chelating ability of the oil was estimated directly by studying the absorbance of Fe^{2+} *via* ferrozine complex formation.The percent scavenging activity of the oil and EDTA (Ethylenediaminetetraacetic acid- used as standard) were studiedat various concentrations ranging from 50to 400 μg ml^{-1}. The maximum chelating activity was exhibited by EDTA (44.12–89.21%) whereas the oil represented the minimum chelating activity (27.39–64.54%) (Fig.4.). The IC_{50} values for essential oil and EDTA were found to be 218.30 μg ml^{-1} and 63.47μg ml^{-1} respectively. Analogous results pertaining to iron chelating activity wasreported in the essential oil of *Rosmarinus officinalis* [49].

Ferric ion reducing antioxidant power (FRAP) activity

The antioxidant activity of oil was also determined through its reducing power expressed as the reduction of Fe^{3+} to Fe^{2+} ions at low pH which results in the formation of blue colored ferrous–tripyridyltriazine complex [50]. The reducing power of the test oil increased with increasing concentrations. The ferric ion reducing ability of the essential oil and standard (curcumin) was studied at various concentrations *i.e.* 50–400 μg ml^{-1}. Maximum reducing potential was found in the positive control (93.70%) whereas, at highest concentration of the essential oil minimum reducing potential (75.42%) was noticed (Fig. 5). Further, the IC_{50} of the oil was compared with standard and minimum IC_{50} value (59.57ìg ml^{-1}) was reported in the standard as compared to that of oil (79.36 μg ml^{-1}). Similarly, [51] studied the antioxidant potential of the methanolic extract of *H. suaveolens*on the basis of various assay including FRAP and concluded that this plant possessed good antioxidant activity. Additionally, [52] reported that the members of Lamiaceae family possess strong ferric ion reducing potential.

CONCLUSIONS

The essential oil of *H. suaveolens*possesses good radical scavenging activity due to presence of various chemical constituents. The *in-vitro* antioxidant activity shows that the oil has the ability to scavenge DPPH,

$^{\bullet}HO$ radicals and H_2O_2, along with strong ferric ion reducing potential and good chelating activity. Thus, it holds a promising use in the management of free radical mediated diseases and serves as a resource for drug development.

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