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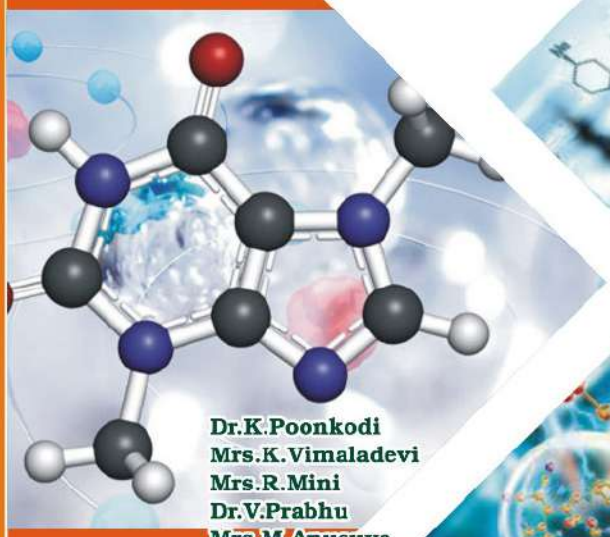

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CONTENTS

Chemical Profile and *In vitro* Radical Scavenging Activity of Essential Oil from *Hyptis Suaveolens* (L.) Poit

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Abstract -- *In a biological system, an antioxidant is defined as any substance that when present at low concentrations compared with those of an oxidizable substrate significantly delays or prevents oxidation of that substrate. The antioxidant activity of plant extracts is attributed to phenolics and flavonoids, many essential oils have been reported to possess antioxidant activity. The present study highlights the chemical composition and antioxidant potential of the essential oil of Hyptis Suaveolens. The volatile oil composition was analyzed by GC/MS analysis. A Total of 26 components were identified. The major compounds present in the oil were Eucalyptol (15.2 %), beta.-Phellandrene (13.3 %), Caryophyllene (9.9%), Alpha Phellandrene (7.0 %), Phenanthrene (6.9 %), Beta - Pinene (3.6 %), α-humulene (3.5 %). The radical scavenging activity of essential oil from Hyprtis Suaveolens was performed by DPPH and ABTS scavenging radical activity assay's showed the IC_{50} values of 112µg/mL and 156µg/mL respectively. Ascorbic acid was used as a standard with IC_{50} values of 13.8µg/mL and 14.4µg/mL. The obtained results in the present study indicates that the essential oil of Hyptis Suaveolens scavenge free radicals and could serve as a mild source of natural antioxidant.*

Key words*: Hyptis Suaveolens, GC/MS, in vitro, DPPH, ABTS, Antioxidant*

I. INTRODUCTION

Natural products have long been implemented as alternative health care treatment and in discovery of modern drugs [1]. Plants and plant-derived medicinal products have been used to help humankind to continue its healthiness from the dawn of medicine. Over the past century, the phytochemicals in plants play an important role in pharmaceutical discovery [2]. Essential oils have been used for thousands of years in various cultures for medicinal and health purposes. Essential oil uses range from aromatherapy, household cleaning products, personal beauty care and natural medicine treatments [3]. The plant *Hyptis suaveolens* (L.) Poit commonly known as *Wilayati tulsi* belongs to the family and it is one of the underexplored valuable medicinal plant used to treat various ailments in traditional system of medicine. The leaves of the plant are the source of pharmacologically important secondary metabolites having antispasmodic, anti-colic, anti-rheumatic, and anti-fertility properties [4,5]. The plant has various pharmacological activities like antifungal, antibacterial, antioxidant and mosquito repellant activity [6-10]. Therefore, this present study aimed to evaluate the chemical composition and *in-vitro* antioxidant activity of this plant.

II. MATERIALS AND METHODS

A. Plant Material

Fresh leaves of *H. suaveolens* were collected from near Pollachi, Tamil Nadu, South India. The plant sample was identified and authenticated by Dr.P.Sathishkumar, Assistant Professor, Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi and the voucher specimen was preserved in the chemistry department.

B. Isolation of essential oil from H. suaveolens

About 500g of fresh leaves were taken in a round bottom flask and subjected to hydrodistillation using Clevenger type apparatus for 4h. The essential oil was dried over

anhydrous sodium sulphate (Merck) until the last traces of water were removed and then stored in a container at 4˚C prior to GC-MS analysis.

C. GC-MS Analysis

GC-MS analysis of the phytoconstituents of *H. suaveolens* was carried out using thermo GC –trace ultra-version: 5.0 coupled with thermo MS DSQ II instrument. Compounds were separated on DB-35, MS capillary standard non – polar column (30m × 0.25 mm), film thickness 0.25µm. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 700C and held for 2 minutes and the temperature of the oven was raised to 2600C for 10min and raised 60C/MIN and final temperature was 3500C for 10 min. The sample of 100mL was dissolved in 1mL of acetone and injected with split less mode. Mass spectra were recorded over 50-500 amu range with electron impact ionization energy 70 eV, while injector and MS transfer line temperature were set at 2800C respectively.

D. Identification of phytoconstituents

The components were identified by comparison of their mass spectra with those of National Institute of Science and technology (NIST) mass spectral library version 2.0d, as well as on their comparison of their retention time either with those of authentic compounds or with their literature values.

E. Determination of in vitro antioxidant activity

DPPH radical scavenging activity

Different volumes of essential oil with ethanol were made up to 40µL with DMSO and 2.96mL DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm by UV-Vis Spectrophotometer 3ml of DPPH was taken as control. To measure emulsifying capacity, an amount of surimi powder was added to 25 mL of distilled water and 25mL of corn oil to give a final concentration of about 0, 0.5, 1, 1.5 and 2%. The mixture was blended (Waring Commercial blender, Stamford, CT, USA) for 1 min and transformed to a 50 Ml calibrated centrifuged tube and centrifuged (Hettich Universal 30 RF) at 7500g for 5 min.

Emulsifying stability was determined by the same procedure except that before the sample was centrifuged, the emulsion was

$$\% \text{ RSA} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where, RSA is the Radical Scavenging Activity; Abs control is the absorbance of DPPH radical + ethanol; Abs sample is the absorbance of DPPH radical + sample essential oil.

ABTS·+ Decolorization Assay

The working solution of ABTS·+ radical was made by reacting ABTS (9.5 mL, 7mM) with potassium persulfate (245 µL, 100 mM), and raising the volume to 10 mL with distilled water. The solution was kept in the dark at room temperature for 18 h, and then diluted with potassium phosphate buffer (0.1M, pH 7.4) to an absorbance of 0.70 (±0.02) at 734 nm. Samples were prepared in methanol with dilutions 50-1250 µg/mL. A sample (10 µL) was placed in a test tube and mixed thoroughly with 2.99 mL ABTS radical working solution. Absorbance of the resulting clear mixture was recorded at 734 nm.

The percent antioxidant activity of the sample was determined using the following formula:

$$\% \text{ Antioxidant activity} = [(Ac-As)/Ac] \times 100$$

Where Ac and As are the absorbance's of the control and sample, respectively, the control was prepared by adding 10 µL of methanol in place of the sample.

III. RESULTS AND DISCUSSION

The chemical composition of essential oil of fresh leaves of *H. suaveolens* was analyzed by GC/MS method. The GC/MS chromatogram was shown in fig. 1. A total of 26 compounds were identified (Table 1). The major compounds present in the oil were Eucalyptol (15.2 %), beta.-Phellandrene (13.3 %), Caryophyllene (9.9%), Alpha Phellandrene (7.0 %), Phenanthrene (6.9 %), Beta - Pinene (3.6 %), α-humulene (3.5 %) and minor components were Copaene (2.3%), Fenchyl acetate (2.1%), Spathulanol (1.6%), Androst-5-en-4-one (1.5%), alpha.-Farnesene (1.3%), Eicosane (1.3%), L-Fenchone (1.0%).

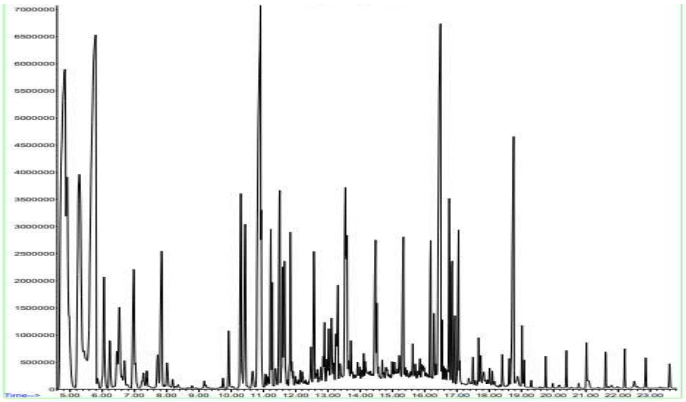

Fig.1. GC/MS chromatogram of essential oil from *H. suaveolens*

Table 1: Components in essential oil from *H. suaveolens*

S. No	*Compound Name*	*R.T*	*Percentage %*
1	beta.-Phellandrene	4.842	13.3
2	Beta - Pinene	4.909	3.6
3	Alpha Phellandrene	5.287	7.0
4	Eucalyptol	5.796	15.2
5	Beta Terpineol	6.231	0.6
6	L-Fenchone	6.531	1.0
7	alpha.-Pinene	6.687	0.3
8	Fenchyl acetate	6.975	2.1
9	Borneol	7.720	0.5
10	Alpha cubebene	9.919	0.5
11	Copaene	10.297	2.3
12	Caryophyllene	10.908	9.9
13	aromadendrene	11.275	0.6
14	Alpha Cadinol	12.475	0.4
15	alpha.-Farnesene	12.564	1.3
16	tau.-Muurolol	13.252	0.4
17	Globulol	13.308	0.8
18	Eicosane	16.185	1.3
19	Phenanthrene	16.496	6.9
20	Spathulanol	16.763	1.6
21	Androst-5-en-4-one	17.052	1.5
22	Heptadecane	17.674	0.3
23	α-humulene	18.763	3.5
24	abietatriene	19.018	0.4
25	Heptacosane	21.018	0.5
26	Nonacosane	22.207	0.3

The antioxidant efficiency of essential oil of *H. suaveolens* were performed by DPPH and ABTS scavenging radical activity assay's with IC_{50} values of 112 µg/mL and 156 µg/mL respectively. Ascorbic acid was used as a standard with IC_{50} values of 13.8 µg/mL and 14.4 µg/mL. It showed a concentration dependent antiradical activity given in the table 1 and table 2 respectively. The percentage inhibitions of the essential oil of *H. suaveolens* were shown in fig 1 and fig 2. From the results the essential oil of *H. suaveolens* showed significant antioxidant potential which may due the presence of various complex terpenes in it.

Table 2: In vitro antioxidant activity of essential oil of *H. suaveolens* -DPPH Assay

Concentration (µg/mL)	**DPPH % inhibition**	**Standard % inhibition**
25	12.2	64.5
50	23.0	87.5
75	34.6	90.1
100	45.1	95.2
150	66.0	125.3
IC50 (µg/mL)	**112 µg/mL**	**13.8µg/mL**

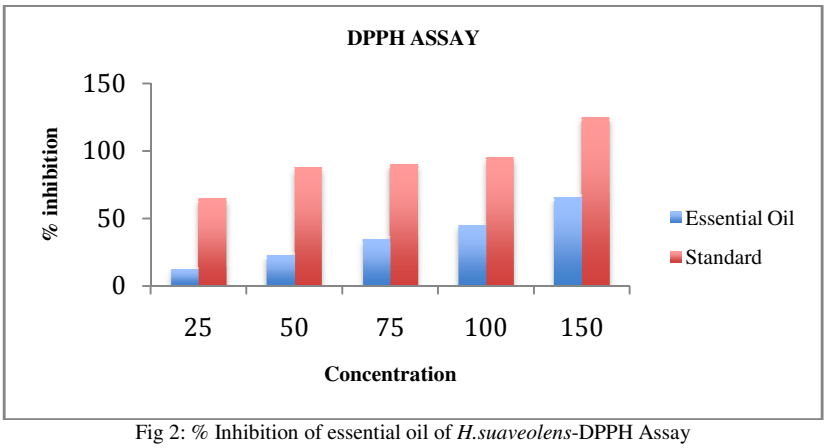

Fig 2: % Inhibition of essential oil of *H.suaveolens*-DPPH Assay

Table 3: In vitro antioxidant activity of essential oil of H. suaveolens - ABTS Assay

Concentration (µg/mL)	**ABTS·+ % inhibition**	**Standard % inhibition**
25	9.8	69.5
50	17.4	90.5
75	25.9	97.1
100	32.0	105.2
150	48.5	125.3
IC50 (µg/mL)	**156 µg/mL**	**14.4µg/mL**

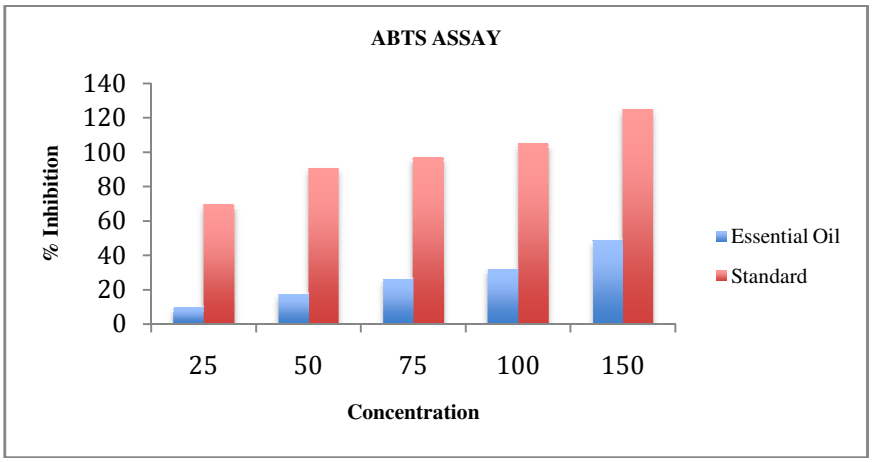

Fig 3: % Inhibition of essential oil of *H. suavelones*-ABTS Assay

IV. CONCLUSION

The chemical composition of essential oil from *H.suaveolens* was analyzed by GC/MS method. A total of 26 components was identified The *in vitro* antioxitant activity of essential oil from *H. suavelones* was tested. The essential oil showed a concentration dependent activity with IC_{50} value of 112 µg/mL for DPPH assay and 156 µg/mL for ABTS assay. From the results of the DPPH assay and ABTS assay of essential oil showed mild *in vitro* antioxidant property.

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