RESEARCH ARTICLE

Antioxidant activity and Phytochemical analysis of *Hyptis suaveolens (L.) Poit*

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ABSTRACT

Hyptis suaveolens (L) Poit. commonly known as Vilayati tulsi,belongs to the family Lamiaceae. Production of reactive oxygen species (ROS) causes various diseases and cellular anomalies in human beings. Antioxidants inhibit generation of reactive species, or scavenge them, or raise the levels of endogenous antioxidant defenses. In this study the antioxidant activity as well as phytochemical analysis of *Hyptis suaveolens L.* was undertaken. Results revealed that *Hyptis suaveolens* (L) Poit. has potent antioxidant ability of 69.46% at 100 µg/ml concentration and IC_{50} value at 40.91 µg/ml concentration and a good correlation was found to exist between concentration of extract and % inhibition with r^2=0.995. Phytochemical analysis revealed the presence of alkaloids, carbohydrates, reducing sugars, flavonoids, glycoside, tannin, phenolic compounds, protein, amino acids, triterpenoids and steroids as well as 0.88 mg/gm of photosynthetic pigments, 0.0004275 mg/g of ascorbic acid while 0.105 mg/g of foliar phenol content was found to be present.

Keywords: Tulsi, antioxidant, oxidative stress, diseases, phytochemical.

INTRODUCTION

Reactive oxygen species (ROS) is a term that encompasses all highly reactive, oxygen containing molecules including free radicals, which includes the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical and various lipid peroxides. All these are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules resulting in cellular damage. To protect the cells and organ system of the body against ROS, humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components both endogenous and exogenous in origin that function interactively and synergistically to neutralize free radicals and include nutrient derived antioxidants (Vitamin C, E , beta carotene and polyphenols), antioxidant enzymes, metal binding proteins and numerous other plant derived phytonutrients. The human body has multifaceted structure of natural enzymatic and non-enzymatic antioxidant resistance, which counteracts the detrimental effects of free radicals and other oxidants. Substantial data indicate that food rich in antioxidants may be of chief significance in disease prevention. [1]

Oxidative stress has been implicated in the pathogenesis of many human diseases like Alzheimer's , Parkinson's, neurodegenerative diseases, the pathologies caused by diabetes, rheumatoid arthritis, and cancer. It also mediates a wide range of renal impairments, from acute renal failure, obstructive nephropathy, hyperlipidemia, and glomerular damage to chronic renal failure. [2] Studies show that oxidative stress is increased along with increased lipid peroxidation and decrease in antioxidative defense in acute renal failure patients undergoing hemodialysis and urolithiasis. The positive role of Vitamin E was found to be synergistic with ascorbic acid to ameliorate oxidative stress. [3-4] In a study by Felix Grases et.al it was concluded that the antioxidant activity of herbal extracts could have an important role in avoidance of calcium oxalate monohydrate papillary calculi formation. [5-6]

Several laboratories reported that oxalate causes renal tubular injury by increasing generation of free radicals. In a study by Sivagnanum et.al showed that

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vitamin E which is considered to be a potent antioxidant, completely prevented calcium oxalate deposition, by preventing peroxidative injury and restoring renal tissue antioxidants and glutathione redox balance. [7] Antioxidant refers to a compound that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions and which can thus prevent or repair damage done to the body's cells by oxygen.[8]

Thus the use of antioxidants in pharmacology is intensively studied, particularly as treatments for neurodegenerative diseases and renal diseases etc. [9] Therefore, it is important to assess antioxidant activity of the plants used in the herbal medicine either to elucidate the mechanism of their pharmacological action or to provide information on antioxidant activity of these herbal plants. All the natural anti-oxidants though safer but show lower antioxidant activity than the synthetic anti-oxidants like BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoulene), but as these are considered to be promoters of carcinogenesis so need exists for safer, economic natural anti-oxidants with high anti-oxidant activity. The use of DPPH as a reagent for screening the antioxidant activity of small molecules and pure compounds or plant extracts has been reported. [10] The phytochemicals from plants, particularly flavonoids and other polyphenols, have been reported to inhibit the propagation of free radical reactions, to protect the human body from disease, and to retard lipid oxidative rancidity.[8] They are primary or secondary metabolites having a range of biochemical and physiological effects. Because many phytochemicals are not well characterized and their mode of action is not well established so research on these physiologically active plant components is currently an area of intense effort.

Hyptis suaveolens (L) Poit. commonly known as Vilayati tulsi belongs to the family Lamiaceae or the Mint family. It is a shrubby, scented plant with blue or purple small flowers. The plant has been considered as a weed, distributed throughout the tropics and subtropics. Common in open uncultivated areas, rocky dry substratum, on roadsides and waste grounds. Flowering and fruiting occurs during October till January.

Almost all parts of this plant are being used in traditional medicine as well as it has immense ethnomedicinal importance for treating various diseases. And the lot of research work done on this plant has also revealed various other activities in this plant.

Its leaf methanolic extract has shown various activities like antimicrobial and [11] antibacterial activity [12] it was found effective against methicillin-resistant Staphylococcus aureus [13] Similarly its toxicity and hypoglycemic activity was studied. [14] It was also screened for antifungal activity [15] while leaf and seed methanolic extracts was screened for insecticidal activity. [16] Various researchers have studied its antioxidant activity. Pradeep et.al (2011) [17] found that aqueous extract of *Hyptis suaveolens L.*showed a protective effect on the antioxidant status of the animals which was evident from the low lipid peroxidation levels, similarly the alcoholic extract [18] and petroleum ether leaf extract [19] showed antioxidant activity.

The antioxidant activity of the essential oil was determined by using two complementary methods: DPPH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) free radical decolorization assay. *Hyptis suaveolens* L. showed IC50 values of 3721 µg/ml. [8] similarly essential oil was screened by DPPH assay and was found to show an IC50 value of 3.72mg/ml whereas the TEAC value determined by ABTS assay was 65.02µM/mg. [20] The aqueous extract of *Hyptis suaveolens L.* leaf showed 98.06 % inhibition in DPPH radical. [21] The methanolic extract of leaves of *Hyptis suaveolens* exhibited potent antioxidant activity in DPPH assay with IC_{50} value of 14.04µg/ml. [22] Novel phenolic compound,from *Hyptis suaveolens L.*was found potent against free-radical-associated oxidative

damage and related degenerative diseases involving metabolic stress and genotoxicity. [23]

The polyphenol extracts of *Hyptis* leaf (0.4–1.6 µg/ml) caused a dose-dependent significant decrease in the MDA (malondialdehyde) contents in the brain. [24] The plant has been reported to be rich in plant chemicals. Phytochemical screening of methanol fraction of whole plant of *Hyptis* showed the presence of alkaloids, flavones, flavonols, terpenoids, tannins, aldehydes and ketones. The methanol soluble fraction of the plant showed the least growth inhibitory effect on fungi- Candida albicans and Aspergillus niger. [25] Similarly methanolic leaf extract showed the presence of alkaloids, tannins, along with steroids, triterpinoids, reducing sugars, phenolic compounds, flavonoids, saponins, anthraquinones and amino acids. [26] Thus the present study was undertaken to re-investigate the antioxidant activity and quantitative and qualitative phytochemical screening of alcoholic extract of *Hyptis suaveolens* (L) Poit. growing in Bhopal district.

MATERIALS AND METHOD

Plant collection, identification and preparation of extract

The plant was collected from Kolar road, Bhopal, Madhya Pradesh, during the month of January 2013 and plant was identified with the help of regional floras [27] and taxonomists and finally confirmed with the herbarium of Botanical Survey of India (BSI), Allahabad, voucher specimen No. 1234-126.01-605.

Fresh plant, after collection was shade dried at room temperature. Plant material was then grinded in a Crompton & Greaves mixer and grinder, and then the powdered plant material 100 gm was extracted with 250ml alcohol by Soxhlet apparatus for 72 hours. Then the extract was concentrated in vacuo to dryness at 30-40°c temperature, obtaining dried extract. The dried extract was stored in refrigerator until used for further analysis.

Measurement of antioxidant activity

DPPH radical scavenging assay-

The procedure for estimating the antioxidant activity involved UV-Spectrophotometric determination.[28] Three solutions i.e. standard, test and control were prepared.

Different solutions (25 - 100µg/ml) of ascorbic acid were prepared in methanol. 1.5 ml of each solution of ascorbic acid were mixed with 1.5 ml of 200µM DPPH solution and incubated for 30 min at room temperature in dark. Absorbance of each solution was taken against blank at 517 nm. Different solutions of the given sample and control were prepared to give concentrations (50 - 200µg/ml). 1.5 ml of each solution of given sample was mixed with 200 µM DPPH solution and incubated at room temperature in dark. Absorbance of each solution of given sample was taken against blank at 517 nm.

Percentage antioxidant activity of plant extract and Ascorbic acid was calculated by using

formula:

$$I\ \% = \frac{Ac - (At - Ab)}{At} \times 100$$

where

I% = percentage inhibition

Ac = absorbance of control (methanol and 200 µM DPPH solution)

At = absorbance of ascorbic acid / given sample with DPPH solution.

Ab =absorbance of ascorbic acid / given sample without DPPH solution.

Phytochemical analysis

Phytochemical testing was performed to assess the various phytoconstituents present in alcoholic extract of *Hyptis suaveolens L.* Quanlitative analysis [29] of its extract was performed to determine the presence or absence of carbohydrates, proteins and amino acids, glycosides, alkaloids, flavonoids, saponin, triterpenoids and steroids, tannin and phenolic compounds. While quantitative analysis was performed to determine the amount of chlorophyll, ascorbic acid as well as foliar phenols present in fresh plant material.

Qualitative analysis:

Tests for carbohydrates and reducing sugars:

Molish test:

2 ml of aqueous extract was treated with 2 drops of alcoholic α-naphthol solution in a test tube and then 1 ml of concentrated sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicate the presence of carbohydrates.

Barfoed's test:

1 ml of extract and Barfoed's reagent were mixed in a test tube and heated on water bath for 2 minutes. Red colour due to formation of cupric oxide indicates the presence of monosaccharide.

Fehling's test:

To 1 ml of aqueous extract, 1 ml of Fehling's A and 1 ml of Fehling's B solutions were added in a test tube and heated in the water bath for 10 minutes. Formation of red precipitate indicates the presence of reducing sugar.

Benedict's test:

Equal volume of Benedict's reagent and extract were mixed in a test tube and heated in the water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicated the presence of reducing sugar.

Tests for protein and amino acids:

Ninhydrin test:

3 ml of the test solution was heated with 3 drops of 5% Ninhydrin solution in a water bath for 10 minutes. Formation of blue colour indicates the presence of amino acids.

Tests for glycosides:

Borntrager's test:

To 3 ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and shake it welled. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammonical layer indicates presence of anthraquinone glycosides.

Legal's test:

1 ml of test solution was dissolved in pyridine. 1 ml of sodium nitropruside solution was added and made alkaline using 10% sodium hydroxide solution. Formation of pink to blood red color indicates the presence of Cardiac glycosides.

Keller-killiani test:

To 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were added in a test tube. Add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Formation of blue color in the acetic acid layer indicates the presence of Cardiac glycosides.

Tests for alkaloids:

To the extract, dilute hydrochloric acid was added, shake it well and filtered. With the filtrate, the following tests were performed.

Mayer's test:

To 2-3 ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of white or creamy precipitate indicates the presence of alkaloids.

Hager's test:

To 1-2 ml of filtrate, few drops of Hager's reagent were added in a test tube. Formation of yellow color precipitate indicates the presence of alkaloids.

Wagner's test:

To 1-2 ml of filtrate, few drops of Wagner's reagent were added in a test tube. Formation of reddish brown precipitate indicates the presence of alkaloids.

Tests for flavonoids:

Lead acetate test:

The extract was treated with few drops of lead acetate solution. Formation of yellow precipitate may indicate the presence of flavonoids.

Alkaline reagent test:

The extract was treated with few drops of sodium hydroxide separately in a test tube. Formation of intense yellow color, which becomes color less on addition of few drops of dilute acid, indicate presence of flavonoids.

Shinoda test:

To the extract, 5 ml (95%) of ethanol was added. The mixture was treated with few fragments of magnesium turning, followed by drop wise addition of concentrated hydrochloric acid. Formation of pink color indicate presence of flavonoids.

Test for saponin:

Foam test:

The extract was diluted with distilled water and shaken in graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

Tests for triterpenoids and steroids:

Salkowski's test:

The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layers turns red, sterol are present. Presence of golden yellow layer at bottom indicates the presence of triterpenes.

Libermann-burchard's test:

The extract was treated with chloroform. To this solution few drops of acetic anhydride were added, boiled and cooled. Concentrated sulphuric acid was added through the sides of the test tube. Formation of brown ring at the junction of two layers, if upper layer turned green, indicate presence of steroids and formation of deep red color indicate presence of triterpenoids.

Tests for tannin and phenolic compounds:

Ferric chloride test:

Some amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.

Lead acetate test:

Some amount of extract was dissolved in distilled water. To this solution few drops of lead acetate solution was added. Formation of white precipitate indicates presence of phenolic compounds.

Dilute iodine solution test:

To 2-3 ml of extract, few drops of dilute iodine solution were added. Formation of transient red color indicates presence of phenolic compounds.

***Quantitative analysis*:**

Photosynthetic pigments content:-

Photosynthetic pigments in O.G. leaf was analyzed by the method proposed by Machlachalan & Zalik (1963) and Duxbury & Yentsch (1956). Chlorophyll-a, Chlorophyll-b and carotenoids were determined by extracting the pigments from fresh leaf samples. Leaves were washed with distilled water and were cut into small pieces, 100 mg of these leaf pieces were put in 5 ml of 80% acetone and were crushed in a mortar pestle with little acid washed sand. The resultant extract was centrifuged at 5000 rpm for 5 minutes. Supernatant was collected and sediment was washed with 1 ml of 80% acetone and centrifuged again. The supernatant thus obtained is added to previous one and the total final volume of the supernatant was made upto 10 ml by adding 80% acetone. Optical density of the supernatant so obtained was recorded at 480, 510, 645 and 663 nm wavelengths, using Systronics Digital spectrophotometer Model-166, against blank carried out throughout the process.

The amounts of Chlorophyll-a, Chlorophyll-b and carotenoids were estimated in terms of mg/gm fresh weight of the leaf was calculated by using the following formulae (Machlachalan & Zalik, 1963; and Duxbury & Yentsch, 1956):

$$\text{Chlorophyll-a (mg/gm fresh wt.)} = \frac{12.3\,D663 - 0.86\,D645}{d \times 1000 \times w} \times V$$

$$\text{Chlorophyll-b (mg/gm fresh wt.)} = \frac{19.3\,D645 - 3.6\,D645}{d \times 1000 \times w} \times V$$

$$\text{Total Chlorophyll (mg/gm fresh wt.)} = \text{Chlorophyll-a} + \text{Chlorophyll-b}$$

$$\text{Carotenoides mg/gm fresh wt.)} = \frac{7.6\,D480 - 1.49\,D510}{d \times 1000 \times w} \times V$$

Where V, is the volume of chlorophyll solution in acetone, d, is the light path, and w, is the fresh weight of plant part in gms.

Ascorbic acid content:-

Ascorbic acid content was estimated by using method proposed by Schaffert and Kingsley (1955). Reagents required for estimation were as follows: 4 % TCA

(Trichloro acetic acid), 2 % 2-4 DNPH (2-4 Dinitro phenyl hydrazine), 85 % Sulphuric acid, 10 % Thiourea

Extraction of ascorbic acid:

2 gms of plant material was crushed with 100 ml of 4 % TCA and then the contents were centrifuged at 5000 rpm. 20 ml of supernatant was taken and was mixed with ½ teaspoon of activated charcoal. This was shaken well and then filtered. From the filtrate, 4 ml filtrate was used for further analysis. To 4 ml extract 1 drop of thiourea and 1 ml of 2-4 DNPH were added, the test tube containing sample was placed in a boiling water bath exactly for 10 minutes. After which the tubes were placed in a beaker containing crushed ice and 5 ml of 85% sulphuric acid was added to the sample slowly drop by drop and mixed by rotating the test tube placed in crushed ice. The sample was kept as such for 10 minutes and then optical density of this sample was read by using Systronics digital Spectrophotometer, Model- 166 at 515 nm against blank carried throughout the process. The µg ascorbic acid content at a given optical density was determined with the help of a standard curve prepared by taking known amounts of ascorbic acid.

Standardization:

0.1 % solution of ascorbic acid was prepared by adding 100 mg of ascorbic acid in 100 ml of 4 % TCA. This served as the stock solution. From this stock solution, working ascorbic acid solution of 0.002 % concentration was prepared by mixing 0.02 ml of stock solution with 98 ml of 4% TCA. Aliquots of this working ascorbic acid solution were prepared as follows in separate test tubes. 0.1 ml, 0.15 ml, 0.2 ml, 0.25 ml, 0.3 ml, upto 1.0 ml. Each aliquot was diluted upto 4 ml by 4 % TCA. 1 drop of thiourea and 1 ml of 2-4 DNPH was added to each sample and the test tubes were placed in boiling waterbath for 10 minutes. Optical density of the samples was read at 515nm. From these optical densities and solutions of known concentrations, standard curve was plotted. With the help of standard curve ascorbic acid contents in samples were obtained. Total amount of ascorbic acid present in the sample was calculated by using the following formula:

$$\text{Total ascorbic acid} = \frac{\mu g \text{ ascorbic acid} \times V}{W \times 1000}$$

Where, µg ascorbic acid = Concentration obtained from standard curve

V = Total volume of the sample

W= Weight of the plant sample.

Foliar phenol content:

Foliar phenols were estimated by the method proposed by Bray & Thorpe (1954). 200 mg of fresh plant material was homogenized with 10ml of 70% ethanol and centrifuged at 6000 rpm for 10 minutes. Residue was subjected to repeated washings with 70 % ethanol followed by centrifugation each time. Supernatant so obtained was concentrated by allowing evaporation overnight and by using a separating funnel the concentrate was partitioned through light petroleum to remove chlorophyll. To 1 ml extract in a test tube, 2 ml of 20% sodium carbonate solution and 1 ml 1N Folin Phenol reagent were added. The reaction mixture was immediately kept in boiling water bath exactly for 1 minute and cooled to room temperature before the optical density of this blue colored solution was measured at 650nm by using Systronics digital spectrophotometer, Model-166.

The µg phenol content at a given optical density was determined with the help of a standard curve prepared with known amount of quinine.

Total amount of phenol in the sample was obtained by using the following formula:

$$\text{Phenol content (mg/gm of fresh wt.)} = \frac{\mu g \text{ phenol} \times V}{W \times 1000}$$

Where, µg phenol = concentration obtained from the standard curve

V = total volume of the mixture

W = weight of the sample.

Results

In the present work, DPPH assay was utilized for evaluating the free radical scavenging action of *Hyptis suaveolens L.* In this assay, ascorbic acid was used as a

standard compound and its IC_{50} value was determined to be 4.69 µg/ml (Figure1). Results show that, methanolic extract of leaf of *Hyptis suaveolens L.* has potent antioxidant ability with an IC_{50} value at 40.91µg/ml concentration and minimum % inhibition of 45.30% at 25 µg/ml and highest % inhibition was found to be 69.46% at 100 µg/ml concentration (Figure 2) which is comparable with that of ascorbic acid 82.05% at100 µg/ml. It was also found that a good correlation exists between the concentration of extract and % inhibition, with r^2 = 0.995 and the regression equation shows that the % inhibition is the dependent variable while concentration is independent and the % inhibition of DPPH free radical, increased with the increase in the concentration of extract.

The results of qualitative phytochemical analysis of *Hyptis suaveolens L.*methanolic extract revealed the presence of alkaloids, carbohydrates, reducing sugars, flavonoids, glycoside, tannin, phenolic compounds, protein, amino acids, triterpenoids and steroids (Table1). While quantitative phytochemical analysis showed the presence of 0.88 mg/gm fresh weight of photosynthetic pigments, 0.0004275 mg/g fresh weight of ascorbic acid while 0.105 mg/g fresh weight of foliar phenol content.

DISCUSSION

The DPPH assay is a very simple method for screening small anti-oxidant molecules, because the reaction can be observed visually and intensity can be analyzed using common spectrophotometric assay. The stable radical DPPH has been used widely for the determination of primary anti-oxidant activity. The DPPH anti-oxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of anti-oxidants. Qualitative and quantitative phytochemical analysis showed that the plant is rich in flavonoids and phenols that are considered to be potent anti-oxidants, so this activity may be attributable to the presence of these chemicals in the extract. As the earlier studies by Felix G et.al (2006) [5] and [6] Bharathi B.K (2013) have shown that the antioxidant activity of herbal extracts have an important role in avoidance of calcium oxalate monohydrate papillary calculi formation**,** thus this extract will be further screened for its antiurolithiatic potential in our near future studies. And these in vitro results should be confirmed in vivo so as to develop potent antioxidant from this plant, as this property of the extract will be advantageous in preventing oxidative stress and in preventing the various other diseases caused by it. Further the extract could be fractioned and fractions could be analyzed for extracting the particular active principle responsible for this activity.

CONCLUSION

In the present work the inhibition of DPPH free radicals and phytochemical screening of *Hyptis suaveolens Poit.* methanolic extract was studied. The extract showed potent radical scavenging ability and the percentage inhibition was found directly proportional to the increase in concentration or percentage of the plant extract. The extract may contain phytochemicals that cause inhibition mainly phenols and flavonoids. This property of plant may be important in preventing oxidative stress related diseases. These in vitro results should be confirmed in vivo.Literature review has shown that very few studies on antioxidant potential of *Hyptis suaveolens Poit.* methanolic extract has been undertaken and to the best of our knowledge till now, no such study on antioxidant activity of *Hyptis* by DPPH assay has been done in Bhopal district. The mechanism by which it exerts its effects remains unknown, so the mechanism as well as chemicals responsible could be isolated and studied in future.

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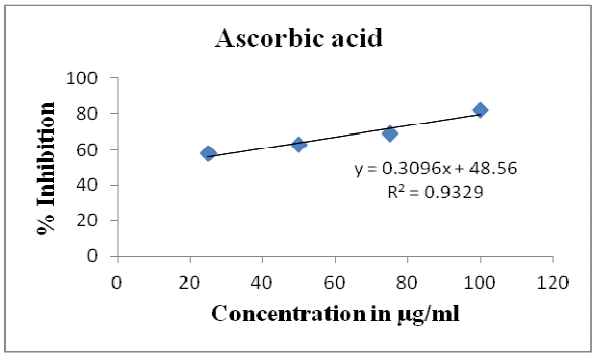

Fig. 1: Graph represent regression curve of Ascorbic acid by DPPH assay

Table1: Qualitative phytochemical screening of *Hyptis suaveolens L*

S. No.	Name of phytochemical test	Result
1.	**Carbohydrates and reducing sugars**	
	Molish test	+ve
	Barfoed's test	+ve
	Fehling's test	+ve
	Benedict's test	+ve
2.	**Protein and Amino acids**	
	Ninhydrin Test	+ve
3.	**Glycosides**	
	Borntrager's Test	+ve
	Legal's Test	+ve
	Keller-Killiani Test	+ve
4.	**Alkaloids**	
	Mayer's Test	+ve
	Hager's Test	+ve
	Wagner's Test	+ve
5.	**Flavonoids**	
	Lead Acetate Test	+ve
	Alkaline Reagent Test	+ve
	Shinoda test	+ve
6.	**Saponin**	
	Foam Test	-ve
7.	**Triterpenoids and Steroids**	
	Salkowski's Test	+ve
	Libermann-Burchard's Test	+ve
8.	**Tannin and Phenolic compounds**	
	Ferric Chloride Test	+ve
	Lead Acetate Test	+ve
	Dilute Iodine Solution test	-ve

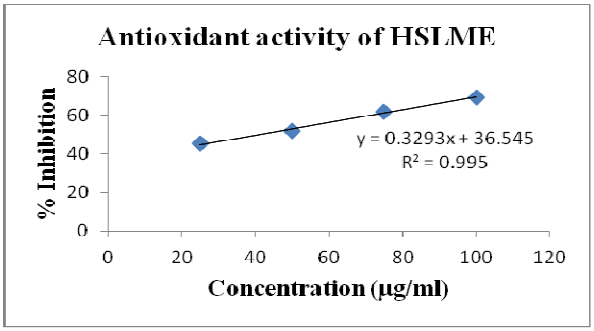

Fig. 2: Graph represent regression curve of Hyptis suaveolens leaf methanolic extract (HSLME) by DPPH assay method

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