



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(2): 1440-1444
Received: 01-01-2018
Accepted: 02-02-2018

C Sivaraj
Post Doctoral Fellow, ARMATS
Biotek Training and Research
Institute, Chennai, Tamil Nadu,
India

Nikhishaa Sree R
Student, Department of
Biotechnology, Vel Tech High
Tech Dr. Rangarajan Dr.
Sakunthala Engineering College,
Chennai, Tamil Nadu, India

PK Gayathri
Associate Professor, Department
of Biotechnology, Vel Tech High
Tech Dr. Rangarajan Dr.
Sakunthala Engineering College,
Chennai, Tamil Nadu, India

P Arumugam
Director, ARMATS Biotek
Training and Research Institute,
Chennai, Tamil Nadu, India

Antioxidant activities and GC-MS analysis of essential oil extracted from *Salvia officinalis* L.

C Sivaraj, Nikhishaa Sree R, PK Gayathri and P Arumugam

Abstract

Salvia officinalis L., the common sage, is considered to be the most representative species among the *Salvia* species. The aim of the present study was to evaluate the antioxidant activity and identify the bioactive compounds by performing GC-MS analysis of essential oil extracted from *Salvia officinalis*. The oil was extracted by employing chloroform as solvent in Soxhlet apparatus. The phytochemical screening was carried out by standard methods which revealed the presence of important phyto constituents such as alkaloids, terpenoids, steroids, and phenolic compounds. The antioxidant assays such as DPPH[•] radical, ABTS^{•+} radical cation, OH[•] radical scavenging assays, Phoshomolybdenum reduction and Fe³⁺ reducing power assays were studied for the essential oil of *Salvia officinalis*. GC-MS analysis showed the presence of volatile compounds. The IC₅₀ values DPPH[•] radical, ABTS^{•+} radical cation, OH[•] radical scavenging assays were found to be 10.47, 10.32 and 68.27 µg/mL concentration respectively. The results of this study portray the effective antioxidant activity of *S. officinalis*.

Keywords: *Salvia officinalis*, phytochemical screening, Antioxidant, GC-MS, Radical, DPPH

Introduction

Essential oils could be extracted from leaves, stems, flowers, roots, herbs, brushes, and trees via distillation. For many years, essential oil has been used for medicinal and healing purposes all over the world. The popularity of aromatherapy has lead to interest in essential oils that has increased in recent decades, which proves the beneficial effects of essential oil and other aromatic compounds. They are widely used in perfumes, cosmetics, soaps, cleaning products and other products and also for flavouring of foods and drinks^[1-4].

The genus *Salvia* (sage) is one of the largest and the most important aromatic and medicinal genera of the Lamiaceae family which is present throughout Mediterranean region, South-East Asia and Central America^[5-7]. Some members of this genus are used as food spices to flavour meats and as flavouring agents in perfumery and cosmetics. The most common uses of sage are antioxidant, anti-inflammatory, antihydrotic, spasmolytic, anticholinesterase, antiseptic, insecticide and also in the treatment of mental and nervous conditions. *Salvia* is a rich source of phytochemicals including phenolic acids, polyphenols, flavonoid glycosides, anthocyanins, sesquiterpenoids, diterpenoids, sesterterpenes and triterpenes^[8-12]. *Salvia officinalis* is one considered to be one of the essential plant herb with an unique savoury and slightly peppery flavour. Sage leaf contains various phyto constituents such as flavones, flavonoid glycosides, cornsole, cornsolic acid, fumaric acid, chlorogenic acid, caffeic acid, and estrogenic substances^[13]. Solvent extraction is commonly used an alternative method to steam distillation but it involves issues leading to the destruction of thermally liable constituents due to application of high temperatures^[14]. In order to overcome the disadvantages, Soxhlet Apparatus was utilized to extract the essential oil from the selected species which includes *Salvia officinalis* L. The antioxidant and anticancer activities have been studied to find out the potential of the active compounds for their medicinal use.



Fig 1a: Habitat of *Salvia officinalis*

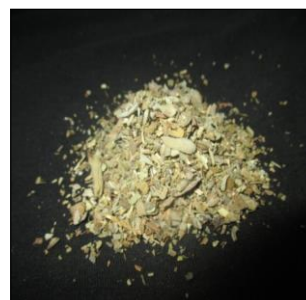


Fig 1b: Dried Powder

Correspondence

C Sivaraj
Post Doctoral Fellow, ARMATS
Biotek Training and Research
Institute, Chennai, Tamil Nadu,
India

Materials and Methods

Collection of plant material

The plant species of *Salvia officinalis* was collected from the local store in Chennai, Tamil Nadu, India. The collected raw material was stored in a container for further use.

Preparation of essential oil

The essential oil was extracted from the plant material by utilising Soxhlet apparatus and employing suitable solvent system, (here), chloroform. The freshly prepared essential oil was stored in a container for later use.

Qualitative phytochemical screening procedure

The chloroform extract of *S. officinalis* was subjected to preliminary phytochemical screening using standard methods [15]. The extract was screened for different classes of phytoconstituents such as alkaloids, steroids, terpenoids, flavonoids and phenolic compounds using specific standard reagents [16, 17].

In vitro antioxidant assays

DPPH[•] radical scavenging assay

The antioxidant activity of chloroform extract of *Salvia officinalis* was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhydrazyl (DPPH) free radical [18]. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of various concentrations (20-120 µg/mL) of essential oils. The mixture was then allowed to stand for 30 min incubation in dark. Ascorbic acid was used as the reference standard. One mL methanol and 1 mL DPPH solution was used as the control. The decrease in absorbance was measured using UV-Vis Spectrophotometer at 517 nm. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of DPPH}^{\bullet} \text{ radical inhibition} = [(\text{Control} - \text{Sample}) / \text{Control}] * 100$$

ABTS^{•+} radical cation scavenging assay

The ABTS^{•+} radical cation scavenging activity was carried out by the procedure described by Delgado-Andrade *et al* [19]. ABTS^{•+} was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and the mixture was left undisturbed in the dark at room temperature for 12-16 hrs. The ABTS solution (stable for 2 days) was diluted with 5 mM phosphate-buffered saline (pH 7.4) to an absorbance of 0.70±0.02 at 730 nm. After the addition of methanol extract of varying concentrations (20-120 µg/mL) to 1 mL of diluted ABTS^{•+} solution, the absorbance was measured after 10 min. The ABTS^{•+} radical-scavenging activity of the samples was expressed as

$$\% \text{ of ABTS}^{\bullet+} \text{ radical cation inhibition} = [(\text{Control} - \text{Sample}) / \text{Control}] * 100$$

Hydroxyl radical (OH[•]) scavenging activity

The hydroxyl radical scavenging capacity of chloroform extract of *Salvia officinalis* was evaluated by the method described by Olabinri *et al* [20]. Various concentrations (20-120 µg/mL) of extract were added with 1.0 mL of iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%), and 1.0 mL of dimethyl sulphoxide (DMSO) (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). Initially, 0.5 mL of ascorbic acid (0.22%) was added and incubated at 80-90°C for 15 min in a water bath. After incubation, 1.0 mL of ice-cold TCA (17.5% w/v) was

added in the end. Three mL of Nash reagent (75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid, and 2 mL of acetyl acetone) was added and made up to 1 L with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The absorbance was measured spectroscopically at 412 nm. Ascorbic acid was used as the reference standard. The percentage of inhibition was calculated using the following formula:

$$\% \text{ of OH}^{\bullet} \text{ radical inhibition} = [(\text{Control} - \text{Sample}) / \text{Control}] * 100$$

Phosphomolybdenum reduction assay

The antioxidant capacity of the chloroform extract of *Salvia officinalis* was assessed as described by Prieto *et al* [21]. The essential oils with concentrations ranging from 20 to 120 µg/mL was combined with reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). The reaction mixture was incubated in water bath at 90°C for 90 min. The absorbance of the coloured complex was measured at 695 nm. Ascorbic acid was used as standard reference.

Ferric (Fe³⁺) reducing power assay

The reducing power of chloroform extract of *Salvia officinalis* was determined by slightly modified method of Yen and Chen, 1995 [22]. One mL of plant extract of different concentrations (10 - 60 µg/mL) was mixed with phosphate buffer (1 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (1 mL, 1 %). The mixtures were then incubated at 50°C for 20 min. One mL of trichloroacetic acid (10 %) was added to each mixture. Then to the mixture 1 mL of FeCl₃ (0.1 %) was added and the absorbance was measured at 700 nm using Spectrophotometer. Ascorbic acid was used as the standard reference.

Thin layer chromatography

Thin layer chromatography (TLC) was carried out for the chloroform extract of *Salvia officinalis* on Merck TLC aluminium sheets, silica gel 60 F254 (20 x 20 cm), preloaded plates [23, 24]. The extract was spotted at 0.3 mm above from the bottom of the TLC plate. Development of a chromatogram was seen in a mixture of suitable solvent system. The spots were visualized with UV light at 356 nm. The R_f values of the coloured spots were recorded. The ratio in which distinct bands appeared was optimized and R_f values were calculated.

Calculation of R_f value

R_f value = Distance travelled by the solute / Distance travelled by the solvent

Gas chromatography–Mass Spectrometry (GC–MS)

The samples were injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness), Agilent technologies 6890 N JEOL GC Mate II GC-MS model. Following chromatographic conditions were used: Helium as carrier gas, flow rate of 1 mL/min; and the injector was operated at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Following MS conditions were used: ionization voltage of 70 eV; ion source temperature of 250°C; interface temperature of 250°C; mass range of 50-600 mass units.

Identification of components

The database of National Institute Standard and Technology (NIST) having more than 62,000 patterns was used for the

interpretation on mass spectrum of GC-MS. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

Result and Discussion

Phytochemical screening

The phytochemical analysis was carried out according to the standard procedure. The results showed the presence of alkaloids, terpenoids, phenolic compounds and flavonoids.

Table 1: Qualitative analysis of chloroform extract of *S. officinalis*

S. No	Phytochemical Constituents	Test	Result
1	Alkaloids	1. Mayer's test 2. Wagner's test	+
2.	Terpenoids	Salkowski test	+
3.	Phenolic Compounds	Ferric chloride test	+
4.	Flavonoids	NaOH test	+

DPPH[•] radical scavenging assay

The ability of chloroform extract of *Salvia officinalis* to scavenge free radicals formed was assessed using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH). The maximum DPPH[•] radical scavenging activity was 75.37% at 30 µg/mL. Chloroform extract of *Salvia officinalis* demonstrated high capacity for scavenging free radicals by reducing the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picrylhydrazine and the reducing capacity increased with increasing concentration of the extract. The IC₅₀ value was found to be 10.47 µg/mL concentration (Figure 2) and was compared with standard (Ascorbic acid, IC₅₀ = 11.98 µg/mL concentration).

ABTS^{•+} radical cation scavenging assay

ABTS^{•+} is a blue chromophore produced by the reaction between ABTS and potassium persulfate and in the presence of the plant extract or ascorbic acid, preformed cation radical gets reduced and the remaining radical cation concentration was then quantified. The maximum ABTS^{•+} radical cation scavenging activity was 79.37% at 30 µg/mL concentration. The experiment demonstrated high antioxidant activity the IC₅₀ of 10.32 µg/mL concentration (Figure 2) and was compared with standard Ascorbic acid (IC₅₀ = 4.21 µg/mL concentration).

Hydroxyl (OH[•]) radical scavenging activity

The hydroxyl radical is a highly reactive free radical formed in biological systems and reacts with a wide range of molecules found in living cells, such as sugars, amino acids, lipids and nucleotides. Scavenging of hydroxyl radical is an important antioxidant activity. The maximum hydroxyl radical scavenging activity was 73.82% at 30 µg/mL concentration. This experiment also showed reliable antioxidant activity with IC₅₀ of 68.27 µg/mL concentration (Figure 2) and was compared with standard Ascorbic acid (IC₅₀ = 3.26 µg/mL concentration). The chloroform extract of *Salvia officinalis* significantly inhibited generation of OH[•] radicals in a dose-dependent manner.

Phosphomolybdenum reduction assay activity

The total antioxidant activity of chloroform extract of *Salvia officinalis* was measured spectrophotometrically by phosphomolybdenum reduction method which is based on the reduction of Mo (VI) to Mo (V) by the formation of green phosphate/Mo (V) complex at acidic pH, with a maximum

absorption at 695 nm. The maximum absorbance was 0.975 at 120 µg/mL concentration (Figure 3). It was compared with the standard (0.359) Ascorbic acid.

Ferric (Fe³⁺) reducing power activity

The reducing power of Fe³⁺ to Fe²⁺ by the chloroform extract of *Salvia officinalis* was studied and showed reduction ability in a dose dependent manner (Figure 3). The maximum absorbance was 0.777 at 120 µg/mL and was compared with the standard (0.289) Ascorbic acid.

Table 2: DPPH[•] radical, ABTS^{•+} radical cation and OH[•] radical scavenging activities of chloroform extract of *S. officinalis*

S. No	Concentration (µg/mL)	% of inhibition		
		DPPH [•]	ABTS ^{•+}	OH [•]
1	5	34.65±2.42	44.35±3.12	24.27 ± 1.69
2	10	47.72±3.34	48.44±3.39	27.34 ±1.91
3	15	58.96±4.12	53.68±3.75	38.28 ±2.67
4	20	65.34±4.57	70.37±4.92	58.59 ±4.10
5	25	71.42±4.99	78.88±5.52	68.40 ±4.78
6	30	75.37±5.27	79.37±5.55	73.82 ±5.16

Table 3: Phosphomolybdenum reduction and Fe³⁺ reducing power assays of chloroform extract of *S. officinalis*

S. No	Concentration (µg/mL)	Phosphomolybdenum Reduction Assay Absorbance @ 695 nm	Fe ³⁺ Reducing Power Assay Absorbance @ 700 nm
1	20	0.148±0.010	0.031±0.002
2	40	0.367±0.025	0.088±0.006
3	60	0.542±0.037	0.215±0.015
4	80	0.630±0.044	0.387±0.027
5	100	0.868±0.060	0.584±0.040
6	120	0.949±0.066	0.669±0.046

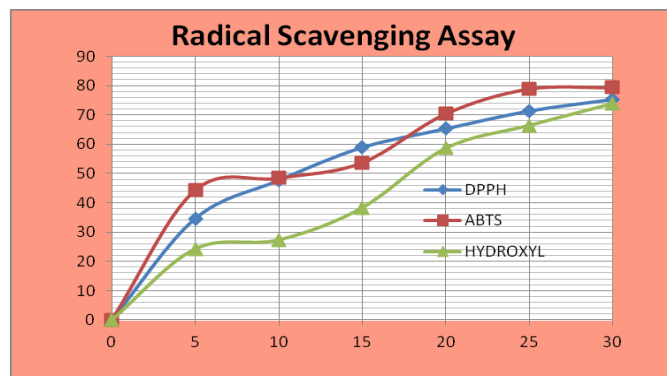


Fig 2: Radical scavenging assay

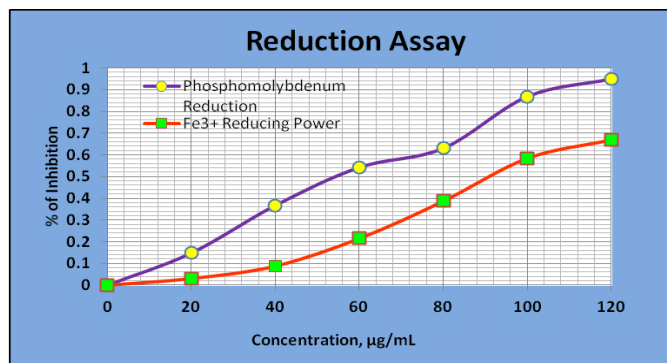


Fig 3: Reduction Assay

Thin layer chromatography

Thin layer chromatography analysis was carried out in the

solvent system of Toluene: Ethyl Acetate with the ratio of 1:1. The separated compounds in TLC were showed in Figure 5.

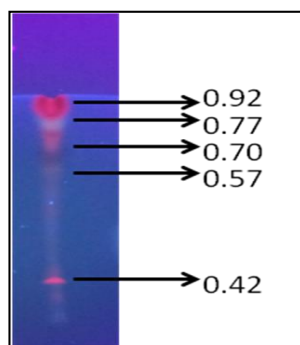


Fig 4: Compounds separated by Thin Layer Chromatography

Table 4: R_f values of compounds separated by thin layer chromatography

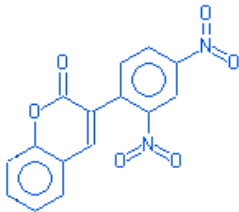
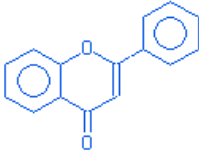
Spot	R _f
1	0.92
2	0.77
3	0.70
4	0.57
5	0.42

GC-MS analysis

GC-MS analysis was carried out for the chloroform extract of *S. officinalis* and the eluted compounds were showed in Table 5. Phytol compound (3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol) was eluted by GC-MS which is a potential antioxidant compound and could be one of the reasons for the antioxidant property of the extract.

Table 5: Active compounds identified in chloroform extract of *Salvia officinalis* by GC-MS analysis

Structure	IUPAC name	Molecular weight G/mol	Molecular formula	Retention time
	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	154.2493	C ₁₀ H ₁₈ O	14.17
	8H-Azecino(5,4-b)indol-8-one, 5-ethylidene-1,2,3,4,5,6,7,9-octahydro-6-(2-hydroxyethyl)-3-methyl-, (S-(E))-	326.4374	C ₂₀ H ₂₆ N ₂ O ₂	20.83
	4H-Benzo[de][1,3]benzodioxolo[5,6-g]quinoline,5,6,6a,7-tetrahydro-1,2,3-trimethoxy-6-methyl-[S],	369.16	C ₂₁ H ₂₃ NO ₅	25.77
	Bicyclo[3.3.1]non-6-ene-2-carboxylic acid, ethyl ester	194.13	C ₁₂ H ₁₈ O ₂	16.12
	Tricosan-2-ol	340.63	C ₂₃ H ₄₈ O	27.57
	3,7,11,15-tetramethyl-2-hexadecen-1-ol	296.53	C ₂₀ H ₄₀ O	18.8
	4H-1-Benzopyran-4-one, 5,7-dihydroxy-3-phenyl-	254.06	C ₁₅ H ₁₀ O ₄	17.78

	3-[2,4-dinitrophenyl]- Coumarine,	312.04	C ₁₅ H ₈ N ₂ O ₆	19.63
	2-Phenyl-4H-chromen-4-one	222.24	C ₁₅ H ₁₀ O ₂	17.07

Reference

- Alizadeh A. Essential oil constituents, antioxidant and antimicrobial activities of *Salvia virgata* Jacq. from Iran. *J Essen Oil Bearing Plants*. 2013; 16(2):172-182.
- Reza Sepahvand, *et al.* *Asian Pac J Trop Biomed*. 2015; 5(1):935.
- Al-Qudah MA, Al-Jaber HI, Abu Zarga MH, Abu Orabi ST. Flavonoid and phenolic compounds from *Salvia palaestina* L. growing wild in Jordan and their antioxidant activities. *Phytochemistry*. 2014; 99:115-120.
- Topçu G, Öztürk M, Kuşman T, Babla Demirköz AA, Kolak U, Ulubelen A. Terpenoids, essential oil composition, fatty acid profile, and biological activities of Anatolian *Salvia fruticosa* Mill. *Turk J Chem*. 2013; 37(4):619-632.
- Usano-Aleman J, Palá-Pául J, Rodríguez MS, Herraiz Peñalver D. Chemical description and essential oil yield variability of different accessions of *Salvia lavandulifolia*. *Nat Prod Commun*. 2014; 9(2):273-276.
- Abdallah M, Abu-Dahab R, Afifi F. Composition of the essential oils from *Salvia dominica* L. and *Salvia hormium* L. grown in Jordan. *Jordan J Pharm Sci*. 2013; 6(1):40-47.
- Ulukanli Z, Karabörklü S, Cenet M, Sagdic O, Ozturk I, Balcilar M. Essential oil composition, insecticidal and antibacterial activities of *Salvia tomentosa* Miller. *Med Chem Res*. 2013; 22(2):832-840.
- Valifard M, Mohsenzadeh S, Kholdebarin B, Rowshan V. Effects of salt stress on volatile compounds, total phenolic content and antioxidant activities of *Salvia mirzayanii*. *S Afr J Bot*. 2014; 93:92-97.
- Al-Mariri A, Safi M. The antibacterial activity of selected Labiatae (Lamiaceae) essential oils against *Brucella melitensis*. *Iran J Med Sci*. 2013; 38(1):44-50.
- Al-Tawaha A, Al-Karaki G, Massadeh A. Antioxidant activity, total phenols and variation of chemical composition from essential oil in sage (*Salvia officinalis* L.) grown under protected soilless condition and open field conditions. *Adv Environ Biol* 2013; 7(5):894-901.
- El Abdouni Khiyari M, Kasrati A, Jamali CA, Zeroual S, Markouk M, Bekkouche K, *et al.* Chemical composition, antioxidant and insecticidal properties of essential oils from wild and cultivated *Salvia aucheri* subsp. *blancoana* (Webb. & Helder), an endemic, threatened medicinal plant in Morocco. *Ind Crops Prod* 2014; 57:106-109.
- Hayet E, Fatma B, Souhir I, Waheb FA, Abderaouf K, Mahjoub A, *et al.* Antibacterial and cytotoxic activity of the acetone extract of the flowers of *Salvia sclarea* and some natural products. *Pak J Pharm Sci*. 2007; 20(2):146-148.
- Salehi P, Sonboli A, Moghadam SE. Essential oil composition and antioxidant activity of *Salvia staminea* Benth. Extracts. *J Essen Oil Bearing Plants*. 2013; 16(5):582-587.
- Akhondzadeh S, Noroozian M, Mohammadi M, Ohadinia S, Jamshidi AH, Khani M. *Salvia officinalis* extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomized and placebo-controlled trial. *J Clin Pharm Ther*. 2003; 28:53-9.
- Romvari M. *Book of Spices*, third ed, Konyve Kiado, Mezogazdasagi, Budapest. 1976, 91.
- Trease GE, Evans WC. *Textbook of Pharmacognosy*. 12th edn. Balliere Tindall and Company Publisher, London. 1983, 343-383.
- Harborne JB. *Phytochemical Methods*. Chapman and Hall Ltd., London. 1973, 49-188.
- Raaman N. *Phytochemical techniques*. New India Publishing Agency, New Delhi. 2006, 306.
- Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*. 1958; 29:1199-1200.
- Delgado-Andrade C, Morales FJ. Unraveling the contribution of melanoidins to the antioxidant activity of coffee brews. *J Agric Food Chem*. 2005; 53:1403-1407.
- Olabinri BM, Odedire OO, Olaleye MT, Adekunle AS, Ehigie LO, Olabinri PF. *In vitro* evaluation of hydroxyl and nitric oxide radical scavenging activities of artemether. *Research Journal of Biological Science*. 2010; 5:102-105.
- Prieto P, Pineda M, Anguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a Phosphomolybdenum Complex: Specific application to the determination of Vitamin E. *Anal. Biochem*. 1999; 269:337-341.
- Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity. *J Agri. Food Chem*. 1995; 43:27-32.
- Gennaro AR. *Remington- The Science and Practice of Pharmacy*. 20th Edition, Lippincott Williams and Wilkins, Maryland, U.S.A. 2000; 1:606-609.
- Stahl E. *Thin Layer Chromatography*, 2nd ed., Springer Pvt. Ltd., New Delhi. 2005, 85.