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**Original Article**

# Anti-inflammatory, Antimicrobial and Cytotoxic Activities of Essential Oil Extracted from *Salvia officinalis* L.

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## ABSTRACT

The aim of the study was to evaluate antimicrobial, antiinflammatory and cytotoxic activities of essential oil extracted from *Salvia officinalis* using chloroform as the solvent by Soxhlet apparatus. Total phenols and flavonoids were estimated by Folin-Ciocalteu reagent and $AlCl_3$ reagent methods. The antimicrobial activity was carried out by well diffusion method while the antiinflammatory activity was performed by albumin as well as BSA denaturation inhibition assay method. The cytotoxic activity was carried out on HeLa cell lines by MTT assay method.

**Keywords:** *Salvia officinalis*, phenolic compounds, antimicrobial, antiinflammatory, HeLa, MTT.

## INTRODUCTION

*Salvia officinalis* belongs to Lamiaceae family and resides mostly in the Mediterranean region, however, it has naturalized in many regions throughout the world. It has a long history of medicinal and culinary uses [1]. *S. officinalis* is one of the essential herbs with a savory and slightly peppery flavor. The oil from sage contains several compounds including cineole, borneol, and thujone. Its leaf contains oleic acid, ursonic acid, tannic acid, ursolic acid, falvones, flavonoid, niacin, nicotinamide, glycosides, cornsole, cornsolic acid, fumaric acid, chlorogenic acid, caffeic acid, and estrogenic substances [2]. Investigations have taken place into using sage as a treatment for hyperlipidemia and Alzheimer's disease [2, 3]. The properties of *S. officinalis* were depression and to mitigate aging symptoms [4]. Moreover, it has a lot of cosmetic uses such as skin and hair care [5]. *S. officinalis* is considered to have the highest amount of this oil when compared to the other species within the genus Salvia [6]. Sage is usually used for medicine for several aims such as carminative, diuretic, antiheroic, analgesic, expectorant, disinfectant, gargle etc. Sage has no side effects in recommended doses. The objectives of the present study was to evaluate the antiinflammatory activity by albumin and BSA denaturation method, antimicrobial activity by well diffusion method and cytotoxic activity by MTT assay method against HeLa cell line.

**Figure 1: *Salvia officinalis* (Sage)**

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## MATERIALS AND METHODS

***Collection and Preparation of crude extract:*** The plant species of *Salvia officinalis* was collected from the market in Chennai, Tamil Nadu, India. The essential oil was extracted by Soxhlet apparatus using chloroform as the solvent. The supernatant was filtered and condensed in a hot plate at 45ºC.

***Quantitative estimations***

***Determination of total phenolic content:*** Folin-Ciocalteau reagent method was used to estimate total phenolic compounds as described by Djeridane et al [7] with slight modifications. 100 µL chloroform extract of *S. officinalis* (from 1mg/mL solution) was mixed 900 µL of methanol and 1 mL of Folin Ciocalteau reagent (1:10 diluted with distilled water). After 5 min, 1 mL of aqueous solution of $Na_2CO_3$ (20%) was added. The mixture was then allowed to stand for 30 min incubation in dark at room temperature. The absorbance was measured by UV-VIS spectrophotometer at 765 nm. The total phenolic content was expressed in terms of Gallic acid.

***Determination of total flavonoid content:*** The total flavonoid content of chloroform extract of *S. officinalis* was determined using the aluminium chloride colorimetric method with slight modifications [8]. 100 µL of chloroform extract (from 1mg/mL solution) was mixed with 900 µL of methanol and 0.5 mL of 5% sodium nitrite and incubated for 5 min at RT. Then, 0.5 mL 10% aluminium chloride solution was added and after 5 min incubation at RT, 1 mL of NaOH solution (1 M) was added. The total volume was made up to 5 mL with distilled water. Absorbance was measured at 510 nm using the spectrophotometer. The result was expressed as quercetin equivalent (µg/mg of dry mass), which is a common reference compound.

***Antioxidant Activity***

***Screening of antioxidant activity by dot-blot DPPH staining method:*** Drops of DPPH solution in methanol (1, 1- diphenyl 2-picrylhydrazyl; 0.4 mM) were loaded onto a 5 cm x 5 cm TLC plate (silica gel 60 F254; Merck) in each column and allowed to dry for 3 min. DPPH was stained in the first row of TLC plate and was considered as control. Chloroform extract of *S. officinalis* of various concentrations was carefully loaded onto the DPPH spot in a second row. The third row of TLC plate was considered as the standard reference, where ascorbic acid was carefully loaded onto the DPPH spot. The staining of the silica plate was based on the procedure of Soler- Rivas et al., 2000 [9]. A purple background with yellow to white spots at the location where radical scavenging capacity observed was revealed on the stained silica gel layer. The intensity of disappearance of purple colour depends upon the amount and nature of radical scavenger present in the essential oil containing chloroform extract of *S. officinalis*.

***Antimicrobial Activity***

***Microorganisms:*** Microorganisms such as *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, Shigella flexneri,* and *Proteus vulgaris* were stored in a refrigerator at the microbiology lab.

***Reference and control:*** Tetracycline was chosen as the reference compound for bacteria. The control consists of solidifying agar onto which was solvent, and the test extract was soluble in it.

***Aseptic conditions:*** The aseptic chamber which consists of a wooden box (1.3m x 1.6m x 0.6m) with a door was cleaned with 70% ethanol and irradiated with short wave UV light from lamp.

***Agar preparation:*** Nutrient Agar was used to make up the medium for bacteria.

***Nutrient Agar (Bacteria):*** Nutrient agar with the composition of peptone- 1.25 g; yeast extract- 0.75 g; NaCl- 1.25 g; agar- 5 g were suspended in 250 mL of distilled water in a 500 mL flask, stirred, boiled to dissolve and then autoclave at 15 lbs and at 121ºC for 15 minutes. The plates were poured in a sterile environment and covered. As soon as the agar was partial solidified, the plates were inverted and left them for 15 min. Under aseptic conditions, the microorganisms were streaked onto the solidified plates and the wells were made by using 8 mm cork borer that was sterilized with alcohol and flame. The essential oil of chloroform extract was poured into the different wells of microorganism streaked plates in sterilized environment at different volumes (25, 50, 75, 100 µL). They were labeled and placed in an incubator at 37ºC for 24 hr for bacterial growth [10].

***Antiinflammatory Activity***

***Albumin Denaturation:*** The inhibition of albumin denaturation was carried out by making slight modifications as described in Sree et al., 2015 [11]. 5ml of the reaction mixture was comprised of 0.2ml of eggs albumin (from hen's egg), 0.8ml of phosphate buffered saline (PBS, pH 6.4) and varying concentration of extract (0-30 µg/mL). The solution was made up to 2mL by adding phosphate buffer. Then the mixture was incubated at 37ºC for about 15 mins and then heated at 70ºC for 15 mins. After cooling, their absorbance was measured at 660 nm by using pure blank.

***Protein Denaturation:*** The procedure was followed as mentioned in Sree et al., 2015 [11] in which the reaction mixture consisted of 0.50 ml

bovine serum albumin (5% aqueous solution) and chloroform extract of *S. officinalis* at different concentrations (0-30 µg/mL) and made up to 1.5mL by adding phosphate buffer saline (pH 6.3). The samples were incubated at 37ºC for 30 min. After cooling the samples, 0.5 mL phosphate buffer was added to each tube. Turbidity was measured spectrophotometrically at 660 nm.

***Cytotoxic activity of chloroform extract of S. officinalis on HeLa (human cervical cancer) cell line:*** Cell viability was measured with the conventional MTT reduction assay method as described Mossman et al., 1983 [12] with slight modifications. Briefly, HeLa cells were seeded at a density of 5×103 cells/well in 96-well plates for 24 h, in 200 µL of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations (0.781-100 µg/mL) of essential oil containing chloroform extract was added and incubated for 48 h. After treatment cells were incubated with MTT (10 µL from 5 mg/mL solution) at 37°C for 4 h and then with DMSO at room temperature for 1h. The plates were read at 595nm on a scanning multi-well Spectrophotometer.

***Statistical analysis***

All the experiments were conducted in triplicates and data given in tables were an average of the three replicates. All data were reported as the mean ± standard deviation of three replicates.

## RESULTS AND DISCUSSION

***Quantitative analysis of total phenol and flavonoid content:*** The total phenolic content of chloroform extract of essential oil was 375.8± 1.26 µg/mg of GAE and the flavonoid content was 102.77± 2.17 µg/mg of QE (Table1). The phenolic and flavonoid compounds quantified in the extracts seemed to be responsible for the antioxidant activity. Phenolic acids and flavonoids are the most commonly found polyphenolic compounds in plant extracts. Phenolic compounds possess effective antifungal, antiviral and antibacterial activity. Plant phenolic compounds are widely known for their antioxidant properties and the aromatic structure of polyphenol is a significant feature in oxidative stress in preventing the formation and scavenging of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Flavonoids act on reactive oxygen species; cell signal transduction pathways related to cellular proliferation, apoptosis, and angiogenesis and hence inhibit carcinogenesis.

**Table 1: Quantitative Estimations of Phenols and Flavonoids**

| Phytochemical constituents | Amount (µg/mg) |
|---|---|
| Phenols | 375.80±1.26 |
| Flavonoids | 102.77±2.17 |

***Antioxidant Activity***

***Dot-blot assay for rapid radical scavenging activity:*** The results of dot-blot assay showed coloured spots where the aliquots of the chloroform extract of *S. officinalis* were placed in a row. The purple zone on the plate indicates no (free radical scavenging) antioxidant activity and the colourless zone indicates antioxidant activity. The intense colourless zone indicates the effective antioxidant activity (Figure 2). The result showed that the chloroform extract of *S. officinalis* has significant antioxidant activity when compared to standard ascorbic acid, by H donating ability of essential oil extracted from *S. officinalis* which neutralizes the free radicals.

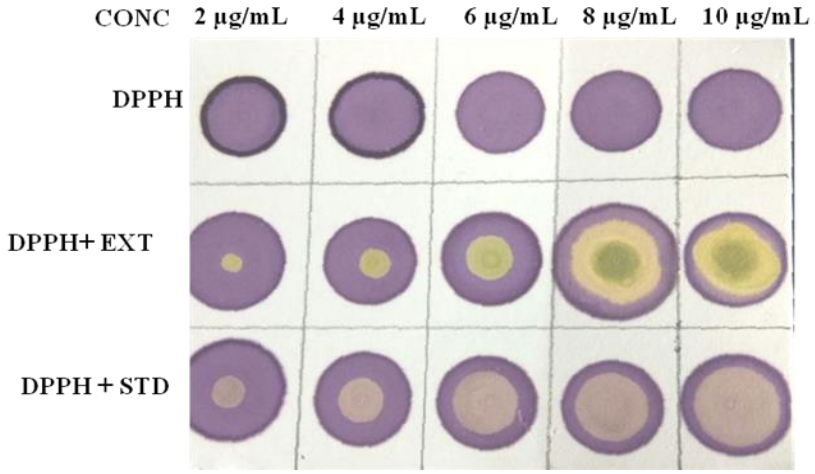


**Figure 2: Dot-Blot Assay of Chloroform extract of *S. officinalis* in DPPH Radical Scavenging Activity**
CONC - Concentration; DPPH - 1, 1-Diphenyl-2-picryl hydrazyl; EXT- Extract (*S. officinalis)*; STD - Standard (Ascorbic acid).

***Antimicrobial Activity:*** The growths of all microorganisms were inhibited with values ranging from 25, 50, and 75 µg/mL concentrations of chloroform extract of *S. officinalis.* The activity may be due to the sensitivity of the crude extract associated with the different cell wall structures of bacteria and fungi, while the cell walls of bacteria contain murein and fungal contain chitin. At 75 µg/mL concentration of extract, the maximum zone of inhibition was found to be 23 mm for *Proteus vulgaris* (Table 2 and Figure 3).

**Table 2: Antimicrobial activity of Chloroform extract of *S. officinalis* against Bacterial strains**

| Organism | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | | Chloroform extract | | |
| | Standard (20 µg) | 125 µg | 250 µg | 375 µg |
| *Klebsiella pneumonia* | 20 | 11 | 14 | 16 |
| *Escherichia coli* | 19 | 12 | 14 | 18 |
| *Bacillus subtilis* | 20 | 15 | 18 | 21 |
| *Proteus vulgaris* | 21 | 17 | 20 | **23** |
| *Shigella flexneri* | 18 | 12 | 14 | 18 |
| *Staphylococcus aureus* | 21 | 14 | 18 | 22 |

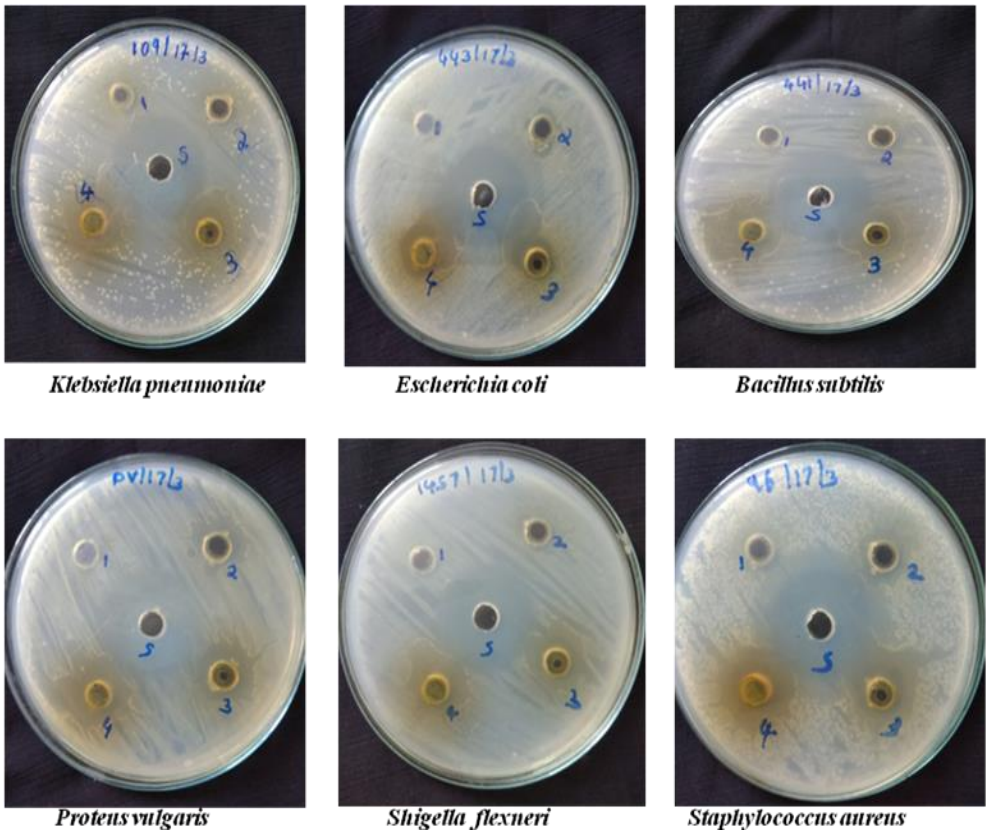


**Figure 3: Antimicrobial activity of chloroform extract of *S. officinalis* against bacterial strains**
(S- Standard (Tetracycline); C-Control (Chloroform); 1-25µL, 2-50µL, 3-75µL)

***Antiinflammatory Activity:*** The maximum albumin denaturation inhibition was 88.48% while the protein (BSA) denaturation inhibition was 55.35% at 30 µg/mL concentration (Figure 4). The $IC_{50}$ values were calculated as 16.28 µg/mL concentration for albumin and 25.76 µg/mL concentrations for protein (BSA) to inhibit the denaturation. The denaturation of proteins as one of the causes for inflammation is well documented. Production of auto-antigens in certain rheumatic diseases may be due to *in vivo* denaturation of proteins. The antiinflammatory activity was evaluated by inhibition of albumin and protein (BSA) denaturation assay methods. By application of external stress or compound such as strong acid or base, a concentrated inorganic salt, an organic

solvent or heat, protein denaturation is a process in which proteins lose their tertiary structure and secondary structure. When denatured most biological proteins lose their biological functions. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the antiinflammatory activity, ability of plant extract to inhibit protein denaturation was studied and was effective in inhibiting heat induced albumin denaturation.

***Cytotoxic activity of chloroform extract of S. officinalis on HeLa (human cervical cancer) cell line:*** Cytotoxic activity was carried out for chloroform extract of *S. officinalis* by MTT assay method. The morphology of HeLa cells progressively changed from 12.5 µg/mL to 250 µg/mL concentration of the extract and was compared with control. The maximum cell death was 87.12% at 250 µg/mL concentrations and the $IC_{50}$ value was showed 24.75 µg/mL concentrations. (Table 4 and Figure 5, 6).

**Table 3: Antiinflammatory Activity of Chloroform Extract of *S. officinalis***

| S. No | Concentration (µg/mL) | % of inhibition | |
|---|---|---|---|
| | | Albumin(egg) denaturation | Protein (BSA) denaturation |
| 1 | 5 | 7.88 ± 0.55 | 3.08 ± 0.21 |
| 2 | 10 | 30.44 ± 2.13 | 11.98 ± 0.83 |
| 3 | 15 | 46.05 ± 3.22 | 24.77 ± 1.73 |
| 4 | 20 | 56.62 ± 3.96 | 31.84 ± 2.22 |
| 5 | 25 | 77.12 ± 5.39 | 48.51 ± 3.39 |
| 6 | 30 | 88.48 ± 6.19 | 55.36 ± 3.87 |

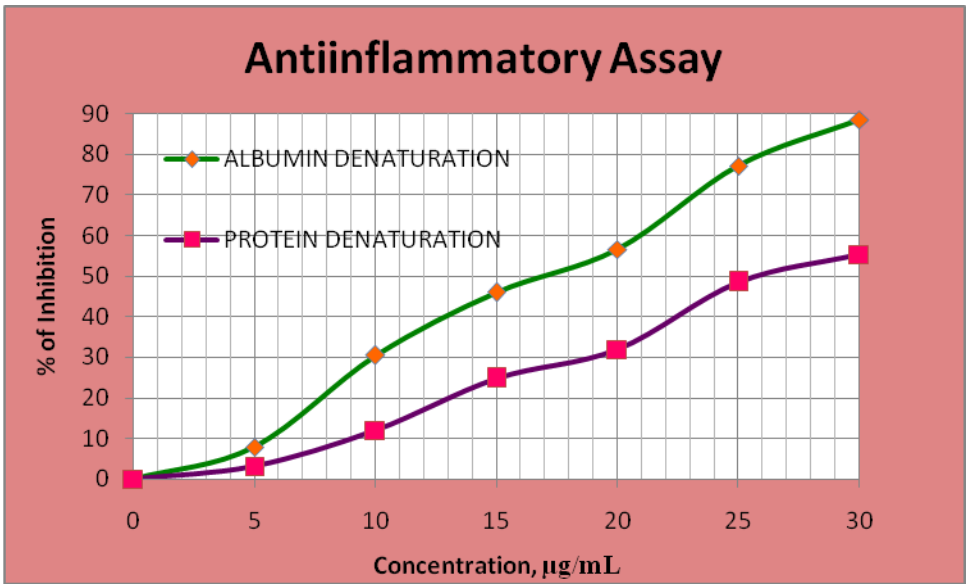


**Figure 4: Graph showing Antiinflammatory Activity of Chloroform Extract of *S. officinalis***

**Table 4: Cytotoxic activity of Chloroform Extract of *S. officinalis***

| S. No | Concentration (µg/mL) | % of cell death |
|---|---|---|
| 1 | 0.781 | 10.42 ± 8.52 |
| 2 | 1.562 | 18.49 ± 5.92 |
| 3 | 3.125 | 25.81 ± 11.96 |
| 4 | 6.25 | 32.10 ± 2.13 |
| 5 | 12.5 | 40.46 ± 4.70 |

| | | |
|---|---|---|
| 6 | 25 | 50.49 ± 3.58 |
| 7 | 50 | 66.45 ± 8.11 |
| 8 | 100 | 74.38 ± 1.71 |
| 9 | 250 | 87.12 ± 0.71 |

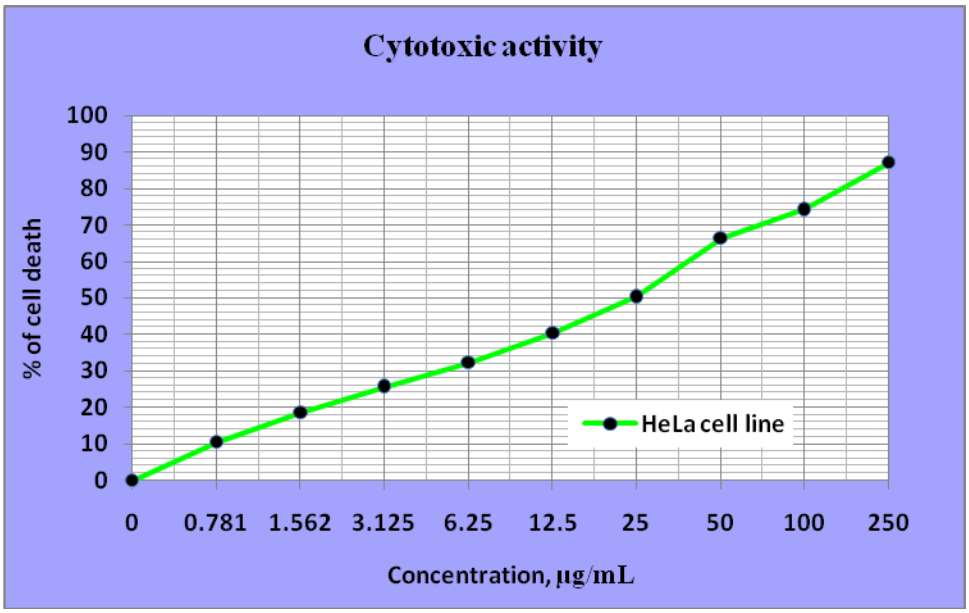


**Figure 5: Graph showing cytotoxic activity of Chloroform extract of *S. officinalis***

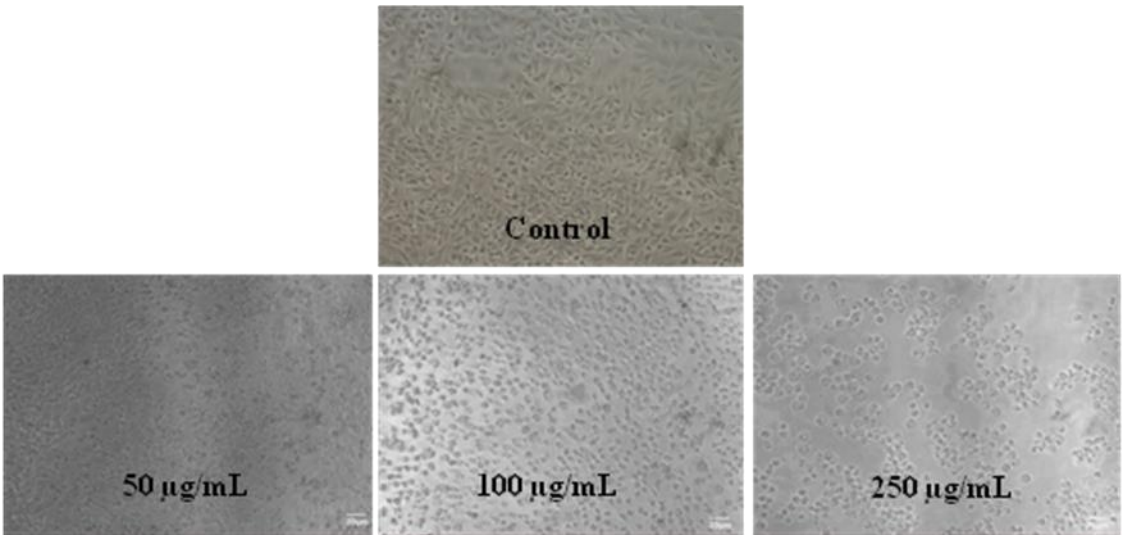


**Figure 6: Cytotoxic Activity of Chloroform Extract of *S. officinalis* on HeLa cell line**

## CONCLUSION

Even though a large number of synthetic drugs are being available throughout the world but still no such medicine can solve the entire health problem. Hence new therapeutic drugs from natural sources are needed for human welfare. Sage is one of the unexplored plant species for their biological activities with scientific evidence. *S. officinalis* showed significant antiinflammatory, antimicrobial and cytotoxic activities. So the essential oil contains potential secondary metabolites to arrest the mutation of the cells which causes inflammation and cancer.

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