



Bioactivity Analysis of Lavender oil

Keerthana Balaji*

B.D.S 1st year, Saveetha Dental College, 162, P.H. Road, Chennai, Tamilnadu, India.

*Corresponding author's E-mail: bjithana@gmail.com

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ABSTRACT

Lavender oil is used as complementary medicine and possesses anti-inflammatory, anti-microbial and anti-oxidant properties. It has potential therapeutic benefits due to its active constituent. The present study was aimed to find out the potentiality of lavender oil which could be the alternative approach of many chronic diseases such as cancer. Bioactivity of Lavender oil was examined with various enzymatic inhibition assay. The antioxidant activity of lavender oil was evaluated by enzymatic inhibition assays such as Thin Layer Chromatography, Folin-Ciocalteu's method for Total phenolic content estimation and DPPH assay. The antioxidant activity of lavender essential oil was evaluated by DPPH assay.

Keywords: Lavender oil, Anti-oxidant, Enzymatic assay, Therapeutic benefits, Cancer.

INTRODUCTION

Lavender oil is one of the important essential oils. Essential oils and other plant extracts have evoked interest as sources of natural products. Essential oils also called as volatile oils, are aromatic oily liquids obtained from plant materials such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots¹. Some essential oils have been used in cancer treatment². The Lavenders are a genus of about 25-30 species of flowering plants in the mint family, Lamiaceae, native to the Mediterranean region south to tropical Africa and to many regions of Asia. The genus includes annuals, herbaceous plants, sub shrubs, and small shrubs. Lavender has been used for centuries as an herbal remedy. Lavender yields a highly effective essential oil with very sweet over tones, and can be used in balms, salves, perfumes, cosmetics, and topical applications³. In food manufacturing, lavender essential oil is employed in flavouring beverages, ice-cream, candy, baked goods and chewing gums⁴. The increasing recognition and importance of bacterial and fungal infections, the difficulties encountered in their treatment and the increase in resistance to antibacterial and antifungal agents have stimulated the search for therapeutic alternatives⁵. Lavender essential oil is traditionally believed to be antibacterial, antifungal, carminative, sedative, antidepressive and effective for burns and insect bites⁶. Their volatile and non-volatile compounds are used by local population to treat wounds, coughs, diabetes, cold, vaginal infection, asthma, respiratory and digestive diseases. They also have antilithiasic, hypoglycaemic, anti-rheumatism, anti-inflammatory and antioxidant activities⁷. Among many different sources of materials with antiseptic activity, essential plants acts as natural source of agents with multifunctional properties including antimicrobial activity⁸. They show fungistatic effects against *Candida albicans*, *Microsporum canis*,

Aspergillus fumigates, *Fusarium oxysporum* etc.⁹ A great advantage of essential oils is that their use is not associated with long-term genotoxic risk. Some of them show an antimutagenic activity that could be linked to an anticarcinogenic activity¹⁰. Several studies investigated the effects of different constituents of essential oils, such as alpha-pipine, alpha-terpinene, alpha-terpineol, terpin-4-ol, linalyl acetate and linalool. These studies concluded that the compounds present in the lavender essential oil may have direct or indirect anti-inflammatory or antinociceptive activities¹¹. The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defences which counteract the harmful effects of free radicals and other oxidants. Free radicals are responsible for causing large number of diseases including cancer, Alzheimer's disease etc.¹² The essential oils from plants have been known to have potent antimicrobial and antioxidant activities. Furthermore, the antioxidant activity of Lavender oil has also attracted the interest of many researchers¹³. This study was conducted to analyse the bioactivity of Lavender oil.

MATERIALS AND METHODS

Materials

Lavender oil used in this study was obtained from Cyprus Enterprises, Arugambakkam, and Chennai, India. The reagents required for the procedure was procured by Himedia.

Methods

Thin layer chromatography

Thin-layer chromatography (TLC) is the simplest and cheapest method of detecting plant constituents since the method is easy to run, reproducible and requires little equipment (Marston et al., 1997). TLC is an important method for the isolation, purification and confirmation of



natural products. Thin Layer Chromatography (TLC) is a solid-liquid type in which the two phases are a solid (stationary phase) and a liquid (moving phase). In our experiments thin layer chromatography (usually 5 µl of a 100 mg extract/ml solution) is loaded on Merck TLC F254 plate.

The distance from the starting point to the center of the spot on the TLC plate was measured as (Distance- a).

The distance from the starting point to the solvent front was measured as (distance -b).

The Retention Factor was calculated using this formula;

$$Rf = \frac{a}{b}$$

The sample was loaded on TLC sheet of dimensions 10 x 5 cm. The mobile phase (Toluene: Ethyl acetate; 7:3) was added in the TLC chamber mixed well and allowed to saturate for 10 minutes. The TLC sheet was placed on the chamber and the chamber was closed. After the run, the sheet was observed in visible light, short UV range, long UV range and Vanillin dye to locate the compounds.

Estimation of Total phenolic content (TPC)

Total phenolic content of essential oils was assessed according to the Folin-Ciocalteu's method (Slinkard & Singleton, 1977) with some modifications. Briefly, Different concentrations of oils (200, 400 and 600 µg), made to 2 ml with distilled water and 1 ml of Folin–Ciocalteu's reagent were seeded in a tube, and then 1 ml of 100 g/l sodium carbonate was added. The reaction mixture was incubated at 25°C for 2 h and the absorbance of the mixture was read at 765 nm. A calibration curve with six data points for catechol was obtained. The results were compared to a catechol calibration curve and the total phenolic content of extracts was expressed as mg of catechol equivalents per gram of extract.

Antioxidant Assay

DPPH Assay

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhydrazyl (DPPH) free radical according to the method described by Brand-Williams et al. with slight modifications. 1ml of 0.1mM DPPH solution in methanol was mixed with 1ml of essential oil solution of varying concentrations (200, 400 and 600µg). Corresponding blank sample were prepared and L-Ascorbic acid (1-100 µg/ml) was used as reference standard. Mixer of 1ml methanol and 1ml DPPH solution was used as control. The decrease in absorbance was measured at 517nm after 30 minutes in dark using UV-Vis spectrophotometer. The inhibition % was calculated using the following formula.

% of Inhibition = (A of control – A of Test)/A of control * 100

RESULTS AND DISCUSSION

In this research antioxidant effect of Lavender essential oil was measured by in vitro methods such as Thin Layer Chromatography, Total Phenolic Content and DPPH assay.

Thin Layer Chromatography:

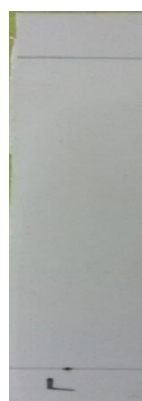


Figure: A



Figure: B

(Figure showing the TLC sheet in visible light and Short UV)



Figure:C



Figure:D

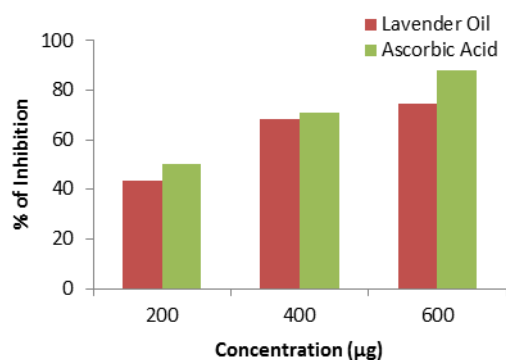
(Figure showing the TLC sheet in Long UV and vanillin dyed)

The presence of phenol was qualitatively confirmed using TLC. The presence of Phenol was identified using short UV, long UV and Vanillin dye (fig B,C,D). The presence of phenol was detected by all the methods. Thus, the presence of phenol was confirmed qualitatively by TLC.

Total Phenolic Content

Table 1: Total Phenolic Content

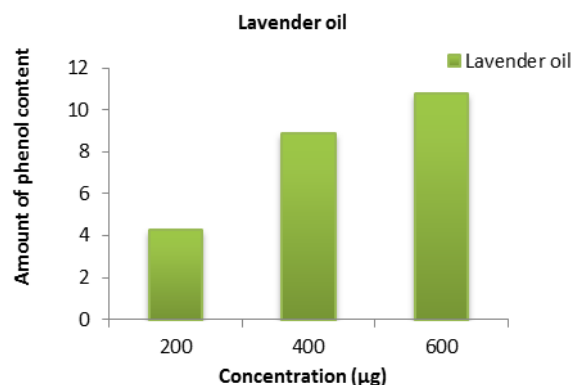
| Total phenolic Content | | | |
|------------------------|--------------|----------|--------------------------------|
| Concentration (µg) | Lavender oil | Catechol | Phenol Content of Lavender Oil |
| 200 | 0.0394 | 1.844 | 4.273318872 |
| 400 | 0.0791 | 3.565 | 8.875175316 |
| 600 | 0.0996 | 5.552 | 10.76368876 |



Graph 1: Total Phenolic Content

As the presence of Phenolic Content in Lavender oil was confirmed by Thin Layer Chromatography which is the qualitative analysis, the phenol content was measured by Folin Ciocalteus method. (Table 1) From the result, the Phenol content in 200µg of lavender oil was found to be 4.27µg whereas the 600µg of oil showed 10.7µg of phenol as compared with the standard catechol (graph-1). Radical Scavenging activity of Lavender oil was studied.

From the result (Table:2), 200µg of lavender oil showed 43.25% inhibition which was almost equal to the inhibition expressed by standard ascorbic acid (50.37%). As the concentration of lavender oil increased the radical scavenging activity also increased linearly (graph-2). This exhibited the antioxidant property of lavender oil.



Graph 2: DPPH Scavenging assay

DPPH Assay

Table 2: DPPH Scavenging assay

| DPPH Scavenging Assay | | | | | |
|-----------------------|---------|--------------|-----------------|---------------|-----------------|
| Concentration (µg) | Control | Lavender Oil | % of Inhibition | Ascorbic Acid | % of Inhibition |
| 200 | 1.068 | 0.606 | 43.25842697 | 0.53 | 50.374532 |
| 400 | 1.068 | 0.337 | 68.44569288 | 0.31 | 70.973783 |
| 600 | 1.068 | 0.271 | 74.62546816 | 0.13 | 87.827715 |

CONCLUSION

From this study, it is evident that the Lavender Oil possesses antioxidant activity. The lavender oil is used for relieving stress, anxiety, headache, diarrhoea etc. Which are the common problems faced by all. It also possesses antifungal, antimicrobial, antibacterial properties in addition to that of antioxidant activity. The flavonoids are a group of plant metabolites containing phenol. From this research, it has been proved that lavender oil contains a significant quantity of phenol and the antioxidant activity of the oil was also explored. Apart from Lavender essential oil being used in aromatherapies and in food industries as flavouring agent, it can also be used as an anticancer drug in future because of the antioxidant activity. As a whole, Lavender essential oil plays a major role in almost all the fields. Since, it has a great potential in medicinal field it can be used as an alternative medicine in our future prospect of life.

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