

Comparative Acetylcholinesterase Inhibitory Potential of Citrus Essential Oils

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Received: 16 Aug 2026 | Revised: 25 Aug 2026 | Accepted: 10 Sept 2026 | Published: 12 Sept 2026

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ABSTRACT–Evaluation of the in vitro acetylcholinesterase (AChE) inhibitory activity of lemon (*Citrus limon*) and sweet orange (*Citrus sinensis*) essential oils (EOs) from peels was carried out. EO were extracted by hydrodistillation. EOs were tested at concentrations ranging from 100 to 1000 µg/mL using the Ellman colorimetric assay. Both oils exhibited concentration-dependent AChE inhibition, with orange oil showing higher activity than lemon oil. Orange oil exhibited 39–87% inhibition, with a calculated IC_{50} of 147.43 µg/mL, whereas lemon oil showed 10–54% inhibition with an IC_{50} of 902.01 µg/mL. These findings indicate greater apparent AChE-inhibitory potential of orange essential oil.

Keywords: Citrus limon, Citrus sinensis, hydrodistillation, essential oil

1. INTRODUCTION

Acetylcholinesterase (AChE; EC 3.1.1.7) is a key enzyme in cholinergic neurotransmission, which converts the acetylcholine neurotransmitter into two products namely choline and acetate [1]. AChE activity may also lead to a decrease of AChE levels and consequently cholinergic neurotransmission, rendering the AChE an important pharmacological target, especially in cognitive and neurodegenerative disorders [2,3]. As a result, there has been a great deal of scientific attention to discover AChE inhibitors that are naturally occurring [4]. EOs derived from medicinal and scented plants are mixtures of volatile secondary metabolites mainly comprised of monoterpenes, sesquiterpenes and oxygenated terpenoids. Limonene, α -pinene, β -pinene, linalool and 1,8-cineole are examples of EOs and its compounds that have shown AChE-inhibitory activity [5]. The inhibitory properties of EOs owes to its combination or synergic effect of multiple components rather than a single compound [6]. Citrus essential oils are especially noteworthy as they tend to contain relatively high levels of monoterpenes, particularly limonene [7]. The essential oils of lemons (*Citrus limon*) and sweet oranges (*Citrus sinensis*) are well studied and have a many biological activities such as antioxidant, antimicrobial and enzyme inhibitory properties. Lemon essential oil (LEO) and other preparations obtained these citrus plants have shown to bear AChE-inhibitory activity [8,9]; however, the strength of such activity depends on assay conditions, extraction methods, geographical origin and composition [10]. Thus, the present study was designed to comparatively investigate the in vitro AChE inhibitory activity of EOs of lemon and orange at various concentrations and calculate their IC_{50} values. The concentration-response analysis was used to determine the IC_{50} values for orange and lemon essential oil.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Material

Fresh, healthy fruits of lemon (*Citrus limon*) and sweet orange (*Citrus sinensis*) were collected from Gadchandur during March. The fruits were cleaned with distilled water for removing the adhering dirt and contaminants. The fruit were peeled manually and cut into small pieces before essential-oil extraction.

2.2 EOs extraction from orange and lemon peels

EOs were obtained from fresh citrus peels by hydrodistillation. The apparatus used for this process was a Clevenger-type apparatus [11]. A known quantity of freshly prepared lemon and orange peels (100 g) was weighed in the round-bottom flask containing sufficient distilled water. Then it was subjected to hydrodistillation for approximately [3-4 h] after boiling commenced. The volatile components were co-distilled with water and subsequently condensed in the Clevenger apparatus. The layer of EO was then separated from the aqueous phase and collected carefully. The residual moisture was removed by sodium sulfate and transferred into clean, airtight amber-coloured glass vials. The samples kept transferred to refrigerator at 4°C until further analysis. The extraction yield was calculated as the percentage volume of essential oil obtained which was relative to fresh plant material weight used.

2.3 Preparation of Essential-Oil Solutions

The extracted lemon and orange essential oils were diluted in an appropriate solvent to prepare the required concentrations for the AChE inhibition assay. A range of concentrations ranging from 100 to 1000 $\mu\text{g/mL}$ was used for the experiment. The same solvent concentration was maintained in all treatment groups and controls to minimize solvent-related effects.

2.4 Acetylcholinesterase Inhibition Assay

The AChE inhibitory potential of lemon and orange EOs was studied using the Ellman colorimetric method. Some minor changes were also incorporated [12]. The assay was based on the acetylthiocholine iodide (ATCI) hydrolysis which produces thiocholine, enzymatically, thiocholine then combines with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to generate a yellow-coloured product. The increase in OD was monitored at 412 nm using UV-vis spectrophotometer (1780), Shimadzu, Japan. The reaction mixture contained EO, AChE enzyme and phosphate buffer with pH 8.0 and concentration 100 mM. After pre-incubation for 15 min, the reaction was started by addition of ATCI and DTNB. OD was measured at 412 nm after 10 min.

2.5 Determination of IC_{50}

The percentage inhibition values obtained at different concentrations of lemon and orange essential oils were plotted against the corresponding concentrations. A logarithmic concentration-response regression was employed to calculate the concentration essential to inhibit 50% of AChE activity (IC_{50}). Lower IC_{50} values were interpreted as indicating greater AChE-inhibitory potency.

3. RESULTS & DISCUSSION

The acetylcholinesterase (AChE) inhibitory activity of lemon (*Citrus limon*) and orange (*Citrus sinensis*) essential oils were determined at increasing concentrations. Both essential oils were inhibitory toward AChE and the degree of inhibition was higher in one concentration than in the other concentration for each EO. The inhibitory effect of orange EO was highest over the range of different concentrations than was the lemon essential oil. For orange EO, AChE inhibition ranged from ~39% to 87% between concentrations of 100 $\mu\text{g/mL}$ to 1000 $\mu\text{g/mL}$, respectively. Concentration-response relationship showed highly logarithmic relationship with the equation $y = 20.377 \ln(x) - 51.75$ with R^2 value of 0.9876 (Fig 1).

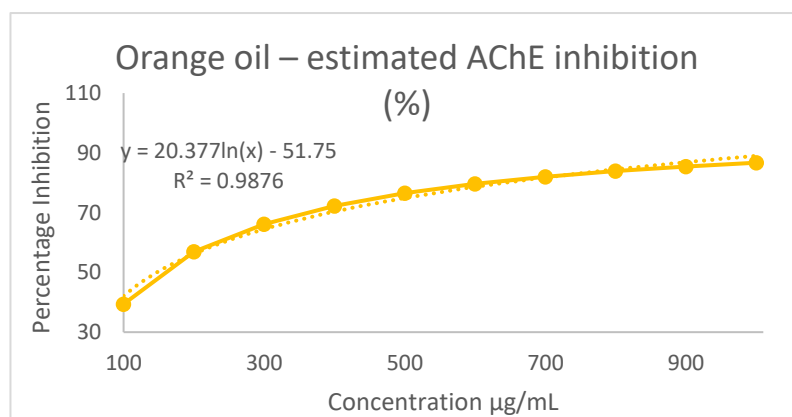


Figure 1 Dose dependent estimation of AChE inhibition by orange essential oil

The IC_{50} value of OEO was calculated, using this regression equation, as 147.43 $\mu\text{g/mL}$ (Fig 3). The AChE inhibitory activities of LEO also showed rise as the concentration becomes higher. Inhibition was 10 % at 100 $\mu\text{g/mL}$ and 54 % at 1000 $\mu\text{g/mL}$. The logarithmic regression equation was $y = 19.552 \ln(x) - 83.044$, with an R^2 value of 0.9819 (Fig 2).

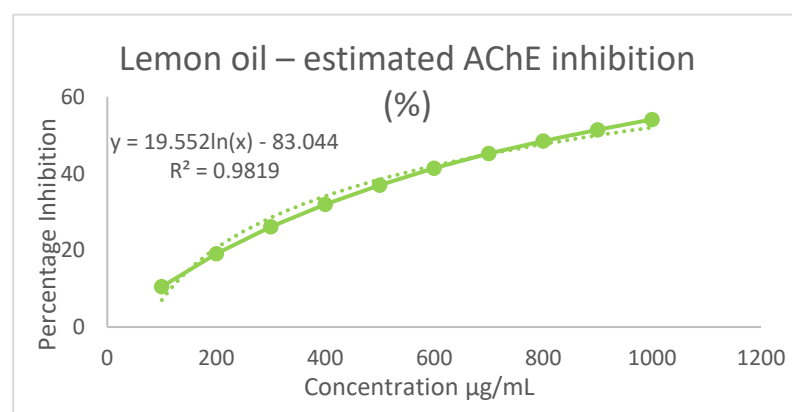


Figure 2 Dose dependent estimation of AChE inhibition by LEO

The calculated IC_{50} was 902.01 $\mu\text{g/mL}$ (Fig 3).

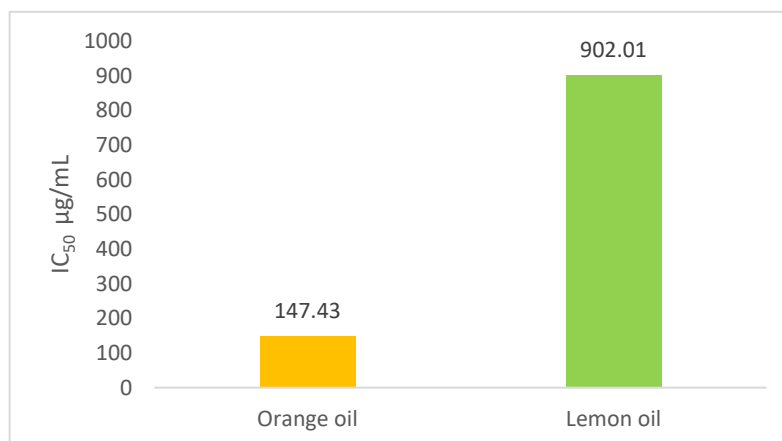


Figure 3 IC_{50} value for AChE inhibition of citrus essential oils

The lower IC_{50} values of orange oil compared to lemon oil suggest that under the experimental conditions there is a significant difference in apparent AChE-inhibitory potency. Lemon oil was around 6.1 times higher as compared to the IC_{50} of orange oil. A significantly lower concentration of orange oil was needed to obtain 50 % inhibition based on regression graph. The difference in activity may be due to the difference in chemical composition of the two essential oils. The monoterpenes and oxygenated terpenoids of citrus EOs possess limonene, α -pinene, β -pinene, linalool and other compounds [13]. Lemon oil and orange oil often contain high concentrations of limonene but the biological potential of a whole essential oil is not always because of limonene. The effects of minor constituents and possible synergistic interaction among components may have an effect on enzyme inhibition. Citrus essential oils (CEOs) have been reported as having AChE inhibitory activity along with a number of other plant essential oils, indicating the possible involvement of volatile terpenoid components in the inhibition of AChE [15,8]. The obtained high coefficients of determination ($R^2 = 0.9876$ and 0.9819) show that there is a strong association between the observed inhibition and concentration of essential-oils in the present data sets. The IC_{50} values given here should be referred to as calculated IC_{50} values based upon a logarithmic regression. A wider range of concentrations and a nonlinear sigmoidal dose response analysis would be more useful for definitive pharmacological comparison to obtain a better estimate of IC_{50} . The apparent AChE-inhibitory potential of the OEO was comparatively higher in comparison with the LEO, indicating that the constituents of the orange essential oil may be more effective to inhibit the AChE as revealed in the present *in-vitro* assay.

4. CONCLUSION

The present *in vitro* study revealed the acetylcholinesterase (AChE) inhibitory activity of both lemon (*Citrus limon*) and orange (*Citrus sinensis*) essential oils, which increased with the increasing concentration of the essential oils. The inhibitory activity of orange essential oil (OEO) was significantly higher than that of lemon essential oil (LEO) in the range of 39–87% at the concentrations of 100–1000 $\mu\text{g/mL}$, and the IC_{50} was calculated as 147.43 $\mu\text{g/mL}$. The same concentration range of LEO showed 10–54% inhibition with a calculated IC_{50} value of 902.01 $\mu\text{g/mL}$. Therefore, the apparent AChE-inhibitory potency of LEO was slightly lower than that of OEO, with the calculated IC_{50} being around 6.1-fold higher than that of OEO under the experimental conditions. The concentration-dependent inhibition is supported by the strong logarithmic concentration–response relationships ($R^2 = 0.9876$ for OEO and 0.9819 for LEO). The difference between these two essential oils may be related to compositional differences, as well as to possible synergistic interactions between the major and minor volatiles. The reported IC_{50} values are regression-based values; however, a wider range of concentrations and sigmoidal dose–response analysis are recommended in order to make a more definitive determination of AChE-inhibitory potency.

5. ACKNOWLEDGEMENTS

Author would like to thank Dr. Utkarsh Moon, Assistant Professor, Mahatma Gandhi College of Science, Gadchandur, India for providing facilities for carrying out these experiments.

6. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

7. FUNDING

This research received no external funding.

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