Anti-cholinesterase Activity of Volatile Constituents of *Plumeria alba* L. and *Plumeria rubra* L. Flowers obtained by Head Space and Hydrodistillation

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PG-06

Introduction

Plumeria is a genus of flowering plants in the family Apocynaceae, characterized by shrubs and small flowering trees. The genus is indigenous to South America but are grown as ornamentals in tropical and sub-tropical areas worldwide. *Plumeria alba* L. and *P. rubra* L. are two famous Plumeria species widely recognized for their fragrant flowers [1]. *P. alba* flower essential oil was reported to have moderate antioxidant [2], antibacterial, antifungal [3] [4], and antipyretic [3] activities. Decoction of *P. rubra* flowers was reported to have antidiabetic effect [5] and essential oil of the flowers was prescribed to treat toothache [6] and used as birth control [7].

Alzheimer's disease (AD) is the most common type of dementia, which affects more than 55 million individuals worldwide, making it the seventh leading cause of death globally [8]. As medication currently used only slows the progression of AD rather than reversing degeneration, early diagnosis of the condition is crucial.

From these findings, the present study will be the first article aimed to evaluate the anti-cholinesterase activities of essential oils derived from the flowers of *P. alba* L. and *P. rubra* L. cultivated in Egypt; in relation to their chemical compositions using GC/MS, machine learning, and chemometric analysis.

Materials and Methods

Plant material

Plumeria alba L. and *Plumeria rubra* L. flowers were collected from a private garden in Alexandria in August 2023 (https://maps.app.goo.gl/erFyXDxa35DHUgHH7). Voucher specimens (# 23.9.23-I and 23.9.23-II) have been preserved in the herbarium of the Faculty of Pharmacy, Cairo University, Cairo, Egypt (https://goo.gl/maps/RvzjwPmjJfzHNKc97).The plants' name was verified with the plant list (http://www.theplantlist.org/). The plants were authenticated by MS Therese Labib, Botanical Specialist and Consultant at Orman Botanical Garden, Giza, Egypt.

Essential oil extraction

The steam distillation method was used to prepare the essential oils of *P. alba* L. and *P. rubra* L. fresh flowers (350 g) in 3L of distilled water using a Clevenger-type apparatus for 5 h. The oily layer was separated and dehydrated over sodium sulphate anhydrous. Oils obtained were stored in sealed, airtight glass vials at 4 °C until use.

GC/MS analysis

In this study GC-MS analysis was performed for EOs prepared by steam distillation and headspace of *P. alba* L. and *P. rubra* L. flowers. In the case of steam distillated samples, we follow the method reported by Ali. et al (Ali, Abd el-Aziz, et al., 2023) as the following: The Shimadzu GCMS-QP2010 (Koyoto, Japan) with a Rtx-5MS fused bonded column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) and a split-splitless injector was used to record the GC/MS spectra. The starting column temperature was maintained at 45 °C for two minutes (isothermal), then it was programmed to rise to 300 °C at a rate of 5 °C per minute and stayed there for five minutes (isothermal). The temperature of the injector was kept at 250 °C. The flow rate of helium carrier gas was 1.41 mL/min. The following parameters were used to record all of the mass spectra: ionisation voltage of 70 eV; ion source temperature of 200°C; filament emission current of 60 mA (equipment current). Split mode (split ratio 1: 15) was used to inject diluted samples (1% v/v), with an injected volume of 1 µL.

In case of Headspace collected samples, we used the same previous equipment with the following modification; the GC column was replaced with Rtx-1MS fused bonded column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) (Restek, USA) [Fig.1] .

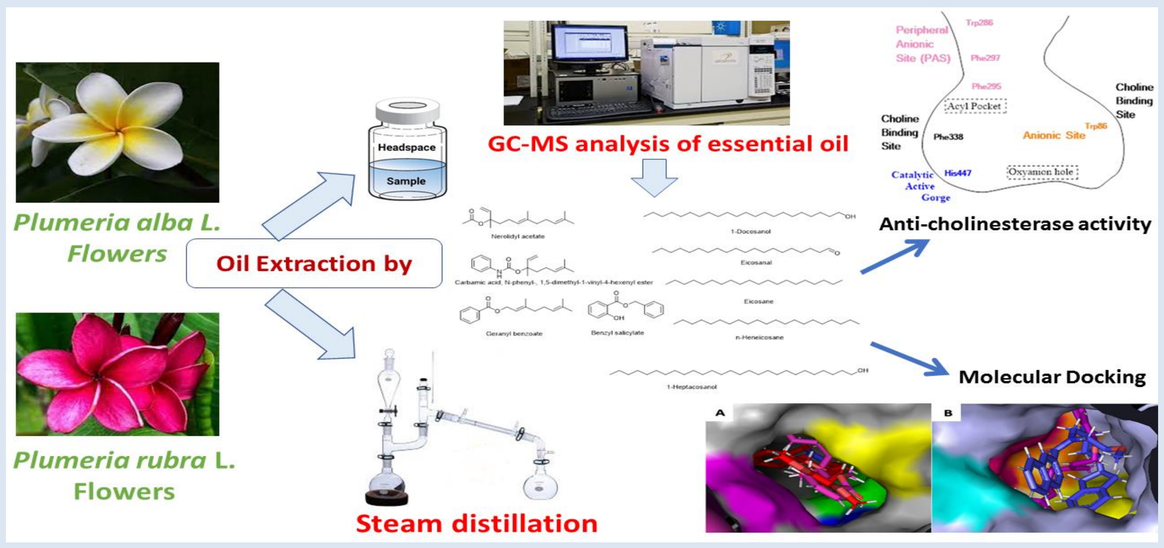


Fig. 1. Scheme outlines procedures followed in this study

Results

Chemical profile and GC–MS analysis of the EOs prepared from the flowers of P.alba and P. rubra

Hydrodistillated samples

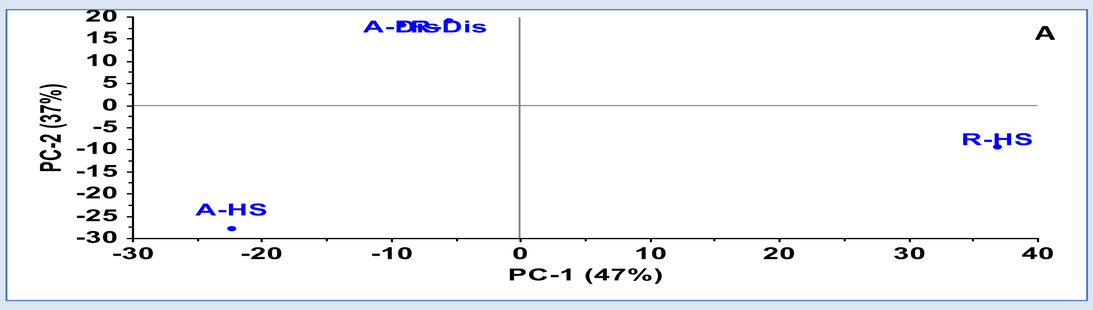
Essential oil analysis of *P.alba and P. rubra* using GC-MS showing that fifteen and eighteen compounds (representing 88.11%, and 90.07% of the total oil) were identified in the steam distilled EOs prepared from the flowers of *P. alba* L**.** and *P. rubra* L., respectively. The major identified compounds of steam distillated EO of *P. rubra* were 1-heptacosanol (23.86%), geranyl benzoate (13.45%), 1-docosanol (9.46%), eicosanal (9.19%), *n*-heneicosane (8.4%), benzyl salicylate (6.97%), and pentatriacontane (3.06%). Furthermore, the major identified components of steam distillated sample of *P. alba* L. were geranyl benzoate (27.55%), benzoic acid, 2-hydroxy-, phenylmethyl ester (15.41%), eicosane (10.12%), nerolidyl acetate (8.04%), carbamic acid, N-phenyl-, 1,5-dimethyl-1-vinyl-4-hexenyl ester (7.65%), farnesyl alcohol (4.87%), eicosanal (3.37%), hexatriacontane (3.22%), and benzyl benzoate (3.1%).

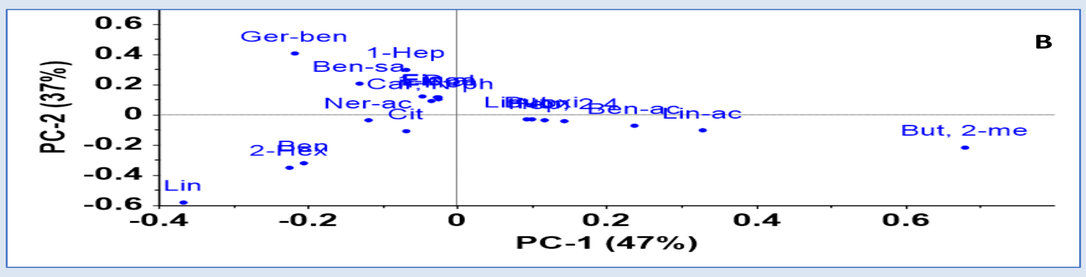
Headspace collected samples

Thirteen and ten components (representing 100%, and 96.5% of *P.alba and P. rubra, respectively*) were identified in the EOs collected by headspace. The major identified components of *P. rubra* L., oil collected by headspace were butyl aldoxime, 2-methyl-, syn- (36.38%), linalyl acetate (17.54%), benzene acetaldehyde (12.73%), 2,4-dimethylheptane (7.72%), nonanal (6.28%), linalool oxidebutanoic acid (5.32%), 3-methyl-2-oxo-, methyl ester (4.97%), and 2-hexenal (3.64). Additionally, the major identified components of *P. alba* L. oil collected by headspace are linalool (32.52%), 2-hexenal (19.88%), benzaldehyde (18.16%), nerolidyl acetate (7.28%), and citral (6.03%).

Cholinesterase inhibitory activity

The main finding of the present study was that *Plumeria* oils inhibit AChE, and BChE. *P. rubra* L. essential oils showed more promising results than P. alba L. (5.07±0.04 µg/mL vs. 11.56±0.69 µg/mL) and (7.21±0.14 µg/mL vs. 10.78±0.54 µg/mL) for AChE, and BChE, respectively.





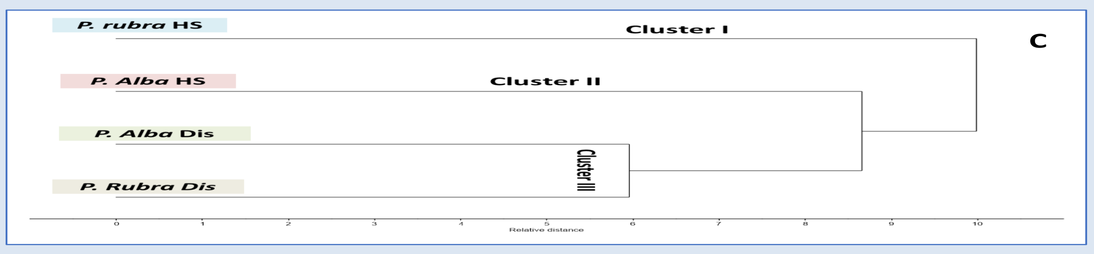


Fig 2. A: PCA score plot, **B:** loading plot and **C:** HCA of GC–MS analysis of essential oil of *Plumeria alba L. and Plumeria rubra L. Flowers* cultivated in Egypt.

* *P.alba*- Dis: *Plumeria alba* L. flower essential oil prepared by hydro-distillation.
P.rubra- Dis: *Plumeria rubra* L. flower essential oil prepared by hydro-distillation.
P.alba- HS: *Plumeria alba* L. flower essential oil prepared by headspace.
P.rubra- HS: *Plumeria rubra* L. flower essential oil prepared by headspace.

Conclusions

In conclusion, this article is considered the first study on the essential oil of *P. alba* L. and *P. rubra* L flowers cultivated in Egypt. Also, the first study that presented a comparison between chemical profiling of the oil composition of P. alba L. and P. rubra L prepared by different techniques (steam distillation & headspace). Additionally, the tested essential oils unveiled a dual anti-cholinesterase activity with geranyl benzoate being the most suitable for clinical trials among the species studied thus far.

References

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