Chemical profiling of different plant parts of two Bougainvillea species using UPLC-MS/MS spectrometry and in-vitro anti-inflammatory activity

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Introduction

Bougainvillea (family Nyctaginaceae) is a widespread genus all over the world. It contains about 18 species and it is native to South America (Abarca-Vargas and Petricevich, 2018). Plants of this genus have flowers that bloom all over the year, and possess different colors, depending on the species, cultivars, or hybrids (Abarca-Vargas and Petricevich, 2018).

Bougainvillea flowers are used as a source of a wide range of environment-friendly safe pigments, namely anthocyanins, that are used in cosmetic, textile, and pharmaceutical industries, providing higher safety than synthetic coloring agents (Rasool et al., 2023; Rodríguez-Herrera et al., 2023).

This study is concerned with two *Bougainvillea* species; *B. spectabilis* Willd (great bougainvillea) and *B. glabra* Choisy (lesser bougainvillea) that are famous for their traditional use in many cases of inflammation. Regarding *B. glabra,* it is used for inflammatory conditions, asthma, cough, pertussis, bronchitis, ulcers, microbial infections, and diarrhea. In Panama, flowers are used for hypotension, while in India, different parts are utilized to treat many ailments, such as stomach acidity, hepatitis, diarrhea, cough, blood vessels problems, and sour throat. Moreover, the decoctions of *B. glabra* are used in Nigeria to mitigate many cases of inflammation, intestinal disorders, and pain (Abarca-Vargas and Petricevich, 2018; Saleem et al., 2021).

Materials and Methods

Collection of *Bougainvillea* species:

Fresh flowers and leaves of *B. glabra and B. spectabilis* were gathered from Antoniades Garden, Alexandria, Egypt in March 2023. The plant's authenticity was assured via Dr. Therese Labib, plant identification specialist in El Orman Garden, Cairo, Egypt. Species voucher specimens (BG1 and BS1) were placed at the herbarium belonging to the Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University. The gathered plant materials were dried at room temperature prior to phytochemical analysis.

2.3. Preparation of *Bougainvillea* species extracts:

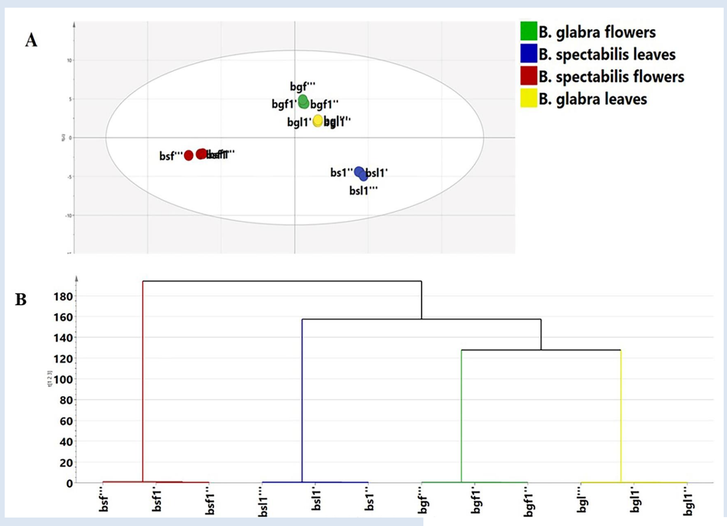
Each species was divided into flowers samples (3 samples from each species) and leaves samples (3 samples from each species). The net samples number was 12 samples. Every sample (100 g) was extracted individually via sonication in 200 mL of 95% ethanol utilizing an ultrasonic bath 28 kHz/1100 W at 35ºC for 30 min. After that, filtration of each extract was done. Each extract dryness was carried out hiring rotary evaporator, under reduced pressure, at 45ºC.

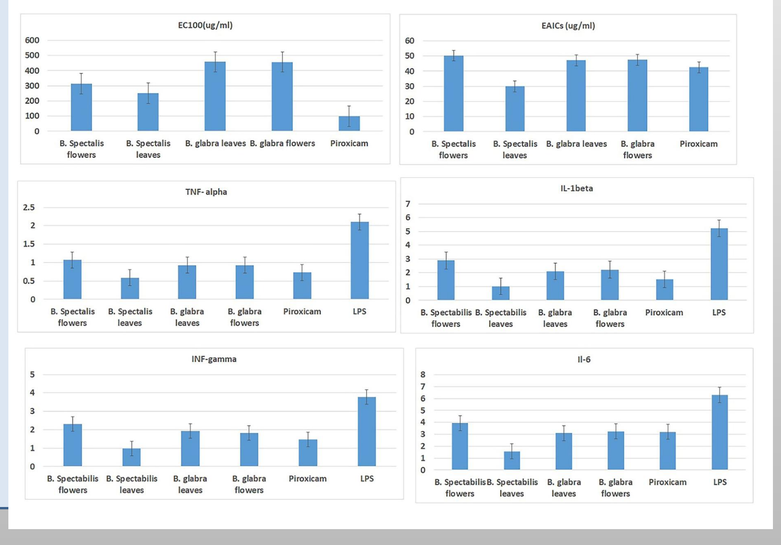
2.4. Analysis of *Bougainvillea* species extracts via UPLC-MS/MS:

2.5. Cytotoxicity and anti-inflammatory potential of tested *Bougainvillea* extracts:

2.6. Multivariate statistical analysis:

Semiquantitative analysis and biological activity testing were statistically analyzed via ANOVA (one-way analysis of the variance) hiring SPSS 26.0 program (SPSS Inc., Chicago, IL. USA) and Metaboanalyst 4.0 (http://www.metaboanalyst.ca) which is a web-based tool for processing metabolomics data to construct hierarchical cluster analysis (HCA) heat maps. Moreover, SIMCA-P ver 14.0 software (Umetrics, Sweden) was hired for chemometric analysis, enclosing unsupervised pattern recognition; PCA (principal component analysis) and supervised pattern recognition; OPLS (orthogonal projection to latent structures analysis).





Results

Forty-four constituents were totally detected in the tested extracts. The identified compounds were quantified using external It was obvious that the major identified classes were phenolic acids, flavonoids and anthocyanins in addition to mono-di, and triterpenes.

Supervised pattern recognition analysis (OPLS-DA) of the tested *Bougainvillea* extracts:

To obtain comprehensive patterns showing the inter-group differences of *Bougainvillea* samples and to clearly identify the secondary metabolites accounting for chemical differences in the metabolic profiles, OPLS-DA model was constructed. OPLS-DA model was able to discriminate the samples into two main clusters along two orthogonal PCs, with an entire variance of 74% within samples. The constructed model proved covered variance and good predictive power as reflected by its computed parameters "R2 (0.998)" and "Q2 (0.986)", respectively.

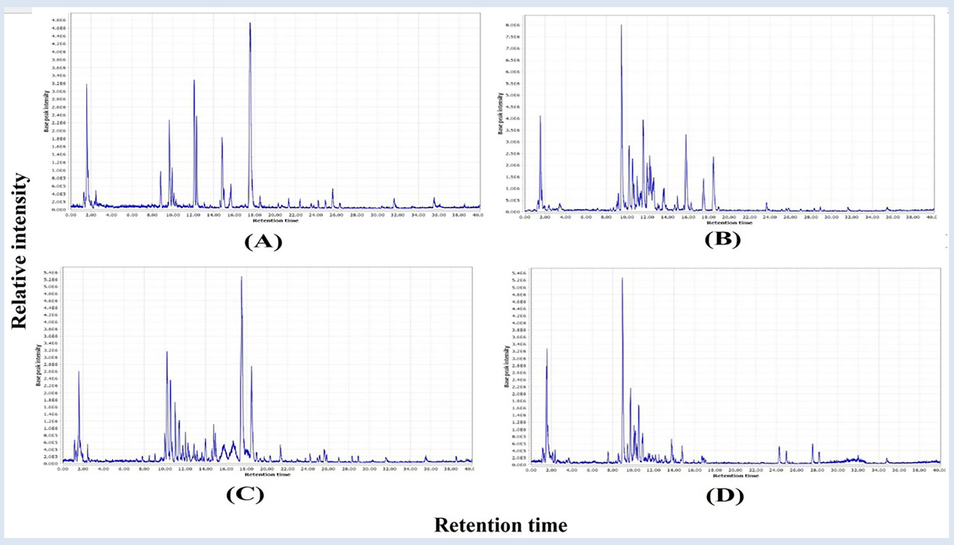
Assessment of *In vitro* cytotoxicity and anti-inflammatory activity of *Bougainvillea* species extracts:

All the tested extracts showed EC100 values higher than that of piroxicam (100 µg/mL) indicating their higher safety (Figure 6).

Afterwards, effective anti-inflammatory concentrations (EAICs) of piroxicam and the tested extracts were determined. It was found that B. glabra flowers and leaves in addition to B. spectabilis flowers extracts showed EAICs values of 47.4, 47.5, and 50.25 µg/mL, respectively, that were comparable to that of piroxicam (42 µg/mL) indicating their potential anti-inflammatory effectiveness. It was surprisingly noticed that B. spectabilis leaves extracts showed EAIC (29.9 µg/mL) lower than that of piroxicam proving its superior inflammation inhibitory efficiency to that exhibited by piroxicam.

The effect of Bougainvillea extracts on the gene expression of four pro-inflammatory markers (TNF-α, IL-1β, IFN-γ, IL-6) was carried out by real time polymerase chain reaction (PCR) in normal WBCs and the WBCs treated with lipopolysaccharide (LPS) in order to determine the anti-inflammatory mechanisms of each tested extract. Lipopolysaccharides are protein components of gram-negative bacteria cell wall which degraded into O-antigen, Core protein in addition to Lipid-A which possess high immunogenic and pro-inflammatory effects (Darwish et al., 2020). Cytokines such as, TNF-α, IL-1β, IFN-γ, IL-6 are greatly contributed to inflammatory process as vasodilatation and edema as well as their important role in cases of chronic inflammation and autoimmune ailments (Muhl et al., 2003).

The genes of TNF-α, INF-γ, IL6, and IL-1β were upregulated by LPS by 2.1, 3.78, 6.3, and 5.23 folds, respectively. It was found that all tested extracts significantly decreased such genes upregulation to levels comparable to that shown by piroxicam . Moreover, it was noticed that the extract of B. spectabilis leaves was the most active one as it decreased the upregulation of all studied genes to more effective levels than those of piroxicam.



Conclusions

Metabolomic study of *B. glabra* and *B. spectabilis* leaves and flowers extracts unveiled 44 compounds covering different classes and categorized different *Bougainvillea* species and organs based on their chemical profiles. Coupling UPLC-MS results to multivariate analysis showed that species type rather than organ type had a significant effect on the chemical profiles of *B. glabra* samples as both leaves and flowers samples segregated together. On the other hand, organ type had a more pronounced effect than species type on the segregation of leaves and flowers samples of *B. spectabilis* as both organ samples were distantly clustered indicating the great variation in their chemical profiles. All tested extracts decreased TNF-α, IL-1β, IL-6, and INF-γ gene upregulation to levels comparable to those exhibited by piroxicam, but *B. spectabilis* leaves showed the highest anti-inflammatory potential regarding all tested cytokines due to its enrichment of bioactive metabolites that positively contributed to the anti-inflammatory activity. This study revealed the importance of *Bougainvillea* plants as a source of bioactive compounds having promising anti-inflammatory potential besides their industrial importance. Isolation of the exact bioactive markers from the complex matrix of *Bougainvillea* extracts utilizing various isolation techniques followed by biological testing of isolated chemicals would be the next step to afford more conclusive and comprehensive therapeutic approaches.

References

Abarca-Vargas, R., Petricevich, V.L., 2018. *Bougainvillea* genus: a review on phytochemistry, pharmacology, and toxicology. Evid. -Based Complement. Altern. Med. 2018, 9070927 https://doi.org/10.1155/2018/9070927. Athar, M.T., Tamboli, E.T., Ansari, S.H., Ahmad, S., 2013.

Darwish, R.S., Hammoda, H.M., Ghareeb, D.A., Abdelhamid, A.S.A., Bellah El Naggar, E. M., Harraz, F.M., Shawky, E., 2020. Efficacy-directed discrimination of the essential oils of three Juniperus species based on their in-vitro antimicrobial and anti-inflammatory activities. J. Ethnopharmacol. https://doi.org/10.1016/j. jep.2020.112971.

Muhl, H., Pfeilschifter, J., Mühl, H., Pfeilschifter, J., 2003. Anti-inflammatory properties of pro-inflammatory interferon-gamma. Int Immunopharmacol. 3, 1247–1255. Muvhulawa, N., Dludla, P.V., Ziqubu, K., Mthembu, S.X.H., Mthiyane, F., Nkambule,

Rasool, W., Adeel, S., Batool, F., Ahmad, S.A., Mumtaz, S., Hussaan, M., 2023. Environmental friendly silk and cotton dyeing using natural colorant of Bougainvillea (Bougainvillea glabra) flowers: the sustainable approach towards textile industry. Environ. Sci. Pollut. Res. 30, 21863–21871. https://doi.org/ 10.1007/s11356-022-23417-7.

Rodríguez-Herrera, V.V., García-Cruz, L., Valle-Guadarrama, S., 2023. Aqueous two-phase extraction: a non-thermal technique to separate and concentrate betalains from Bougainvillea glabra Choisy bracts. Ind. Crops Prod. 193, 116245 https://doi. org/10.1016/j.indcrop.2023.116245.

Saleem, H., Usman, A., Mahomoodally, M.F., Ahemad, N., 2021. Bougainvillea glabra (choisy): a comprehensive review on botany, traditional uses, phytochemistry, pharmacology and toxicity. J. Ethnopharmacol. 266, 113356 https://doi.org/ 10.1016/j.jep.2020.113356.