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Analyzing Cymbopogon sp methanolic extracts via liquid chromatography

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ABSTRACT

Cymbopogon species can be utilized in various ways. It is applied in cooking, and aromatherapy, among others. Essential oil is also used extensively in traditional medicine. From the literature, it possesses various classes of compounds, for example, terpenoids and flavonoids. In this study, an unidentified *Cymbopogon* methanolic was subjected to a Reversed Phased-High Performance Liquid Chromatography (RP-HPLC). The mobile phase was adapted from the national herbal monograph, comprising 0.3% formic acid and acetonitrile. The HPLC chromatograms were recorded, and two major peaks were observed. Both signals were apparent in the stepwise gradient when compared to the fast mode. In the absence of the standard monoterpenoids, geraniol, geranial and neral could be further investigated from this sample. In conclusion, a specimen of *C. citratus* could be involved in the experimental procedures.

Keywords: chromatography, *Cymbopogon*, serai makan, lemongrass, monograph

INTRODUCTION

The genus *Cymbopogon* encompasses approximately 55 species, native to tropical and semi-tropical regions of Asia. These species are also cultivated in South and Central America, Africa, and other tropical countries (Shah et al., 2011). *Cymbopogon* is commonly known as lemongrass or "serai" in Malay. Among its species, *C. citratus* is particularly popular, often referred to as "serai makan." The chemical composition of *Cymbopogon* species, including the essential oil and its citral content, can vary

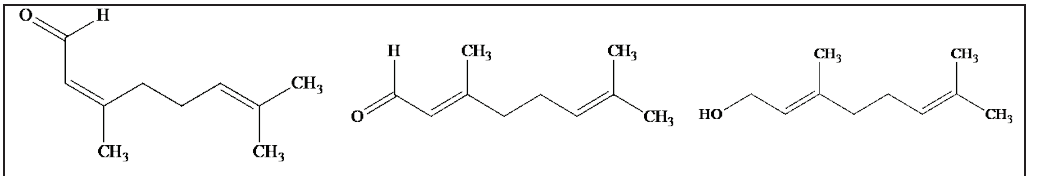
significantly depending on several factors, such as geographic origin, plant part used, maturity stage, genetic variations, extraction method, and harvest season (Méabed et al., 2018). Therefore, optimal collection at the correct stage of maturity is crucial for ensuring high-quality essential oil and minimizing production costs (Tajidin et al., 2012).

The natural bioactive compounds in *Cymbopogon* include terpenoids (Figure 1), saponins, tannins, alkaloids, phenols, flavonoids, and anthraquinones. Notable constituents include citral, geranial, geraniol, myrcene, limonene, citronellol, borneol, neral, nerol, α-terpineol, elemicin, kaempferol, apigenin, caffeic acid, quercetin, luteolin, geranyl acetate, and chlorogenic acid, along with other unidentified compounds (Shah et al., 2011).

Various analytical techniques have been employed to study *Cymbopogon* essential oils. For example, an assay was developed to quantify citral in *C. citratus* volatile oil using normal-phase High-Performance Liquid Chromatography (HPLC) combined with UV spectroscopic detection (Rauber et al., 2005). Additionally, gas chromatography-mass spectrometry (GC-MS) has been used to analyze these oils (Tajidin et al., 2012; Madivoli et al., 2012). For phenolic compound quantification, Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) has been successfully employed (Figueirinha et al., 2008; Costa et al., 2015; Méabed et al., 2018).

In urban areas of Sabah, *C. citratus* and *C. nardus* are commercially traded as medicinal plants (Foo et al., 2016a, 2016b), with Sabah Tea products available online in various forms, including pot bags and loose tea (Sabah Tea Resort, 2020). *C. schoenanthus* (camel grass) has also been used in Iranian traditional medicine for its purported ability to reduce fatigue (Alembagheri et al., 2023). Despite these uses, *Cymbopogon* species still lack comprehensive phytochemical characterization and standardization.

Recent studies have explored advanced techniques such as Nuclear Magnetic Resonance (NMR) to elucidate the flavonoid profiles of *C. giganteus*, a wild lemongrass species (Bationo et al., 2022). Additionally, a comprehensive NMR metabolic profiling study of five *Cymbopogon* species was conducted (Otify et al., 2022), where RP-HPLC was used to analyze an unidentified *Cymbopogon* species. This chromatographic approach may prove useful for identifying and distinguishing species based on the pattern of their alcoholic extract, especially given the current lack of studies focused on RP-HPLC analysis of *Cymbopogon* species.



Neral	Geranial	Geraniol
(*Z*)-3,7-dimethylocta-2,6-dienal	(*E*)-3,7-dimethylocta-2,6-dienal	(*E*)-3,7-dimethylocta-2,6-dien-1-ol
$C_{10}H_{16}O$	$C_{10}H_{16}O$	$C_{10}H_{18}O$

Figure 1 Structures of monoterpenes from the *Cymbopogon* extract.

MATERIALS AND METHODS

Sample preparation

Aerial portions of *Cymbopogon* species were procured from a local market in Puncak Alam, Selangor. The plant materials were mechanically fragmented using a mortar and pestle to enhance surface area (Tajidin *et al*., 2012). Dried and powdered samples were subjected to ultrasonic extraction using 100% methanol prior to chromatographic analysis. The extract was filtered through a 0.45 µm Millipore filter, into the vials. Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC) using an Agilent 1200 system was employed to profile the extract (Globinmed, 2021a; 2021b).

A 10 µL aliquot of the sample was injected into the HPLC system. The separation was achieved using a Thermo C-18 column (250 mm × 4.6 mm, 5 µm particle size) as the stationary phase. Two approaches were employed to compare the extract profiles between a rapid and a non-isocratic, stepwise gradient (Table 1, Figure 2) elutions in RP-HPLC methods. The mobile phase consisted of various compositions of 0.3% formic acid in water and acetonitrile, with a flow rate of 1 ml/min. A diode array detector (DAD) monitored the eluent at 254 and 360 nm and the column temperature was maintained at 36°C throughout the experiment. The running time was set at 30 and 50 minutes for the rapid and stepwise gradient modes, respectively. The data acquisition was performed by using ChemStation software.

Table 1 The solvents for the stepwise gradient elution.

Time (minutes)	Aceto-nitrile (%)	0.3% Formic Acid in Water (%)
0	10	90
5	10	90
20	40	60
22	40	60
30	70	30
32	70	30
40	90	10
45	90	10
47	10	90
50	10	90

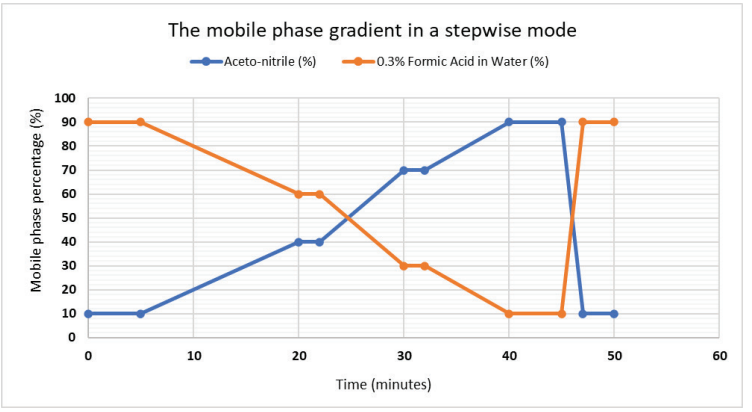


Figure 2 The composition of the mobile phase for the HPLC run (stepwise gradient mode).

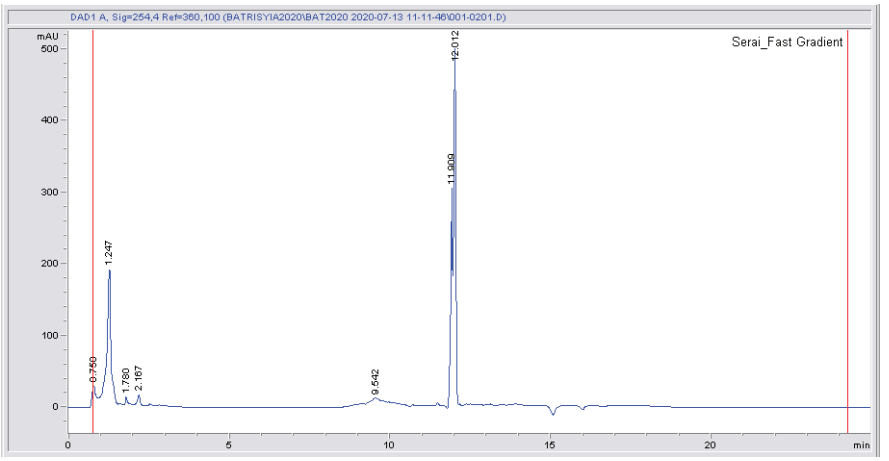


Figure 3 The chromatogram of *Cymbopogon* methanolic extract (fast gradient).

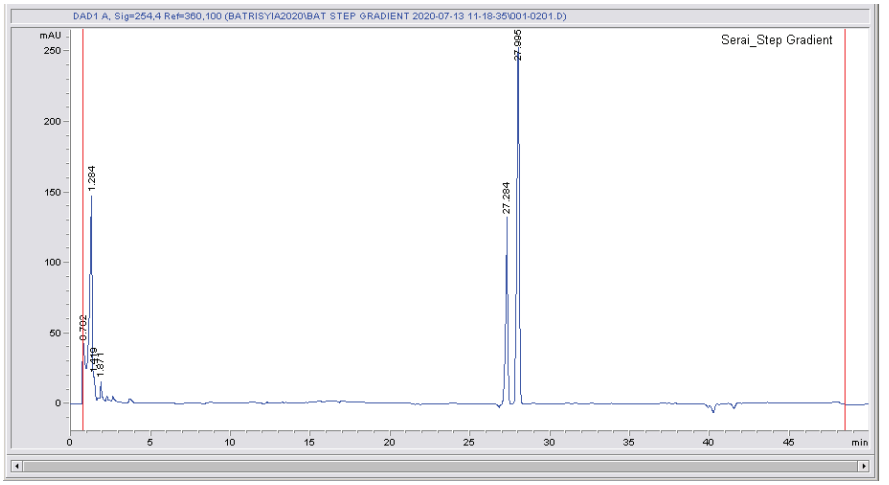


Figure 4 The chromatogram of *Cymbopogon* methanolic extract (stepwise gradient).

RESULTS AND DISCUSSION

The lipid- and essential oil-free infusion of *Cymbopogon* can be prepared by adding boiling water to the plant material (Figueirinha et al., 2008; Costa et al., 2015). In contrast, the essential oil can be extracted using methanol in this study. If a more nonpolar solvent, such as n-hexane, is employed for extraction (Rauber et al., 2005), normal-phase HPLC would be suitable for detecting compounds in the volatile oil. Figure 2 shows the chromatogram of the methanol extract under a fast gradient, while Figure 4 presents the chromatogram using a stepwise gradient. In the fast-gradient method, the compounds elute as two overlapping peaks at retention times (tR) of 11.909 and 12.012 minutes for peak 1 and peak 2, respectively (Figure 2). In the stepwise gradient mode, the peaks are better resolved, with tR values of 27.284 and 27.995 minutes for peak 1 and peak 2, respectively (Figure 3).

Baseline separation was achieved when the mobile phase composition approached a 50:50 ratio of acetonitrile and 0.3% formic acid. This setup is consistent with previously published data, which used an acetonitrile (70:30) system in isocratic flow at 1 mL/min for RP-HPLC. In that system, the order of elution was neral (tR = 7.276 minutes), followed by geranial (tR = 7.689 minutes). While *Cymbopogon* species can contain geraniol, it is typically present in trace amounts (Gaonkar et al., 2016).

In this study, the inclusion of formic acid increases the acidity of the acetonitrile mixture, which in turn lengthens the retention time. The protonated solvent interacts with the monoterpene aldehydes (Figure 2), helping to retain these molecules on the C-18 column. In the stepwise gradient mode (Figure 3), both compounds are retained longer in the column due to the gradual decrease in the mobile phase's polarity.

Optimization of the RP-HPLC mobile phase was conducted using acetonitrile and water, though the specific 50:50 acetonitrile
mixture was not previously reported (Gaonkar et al., 2016). As expected, both compounds eluted at retention times above 12 minutes, as shown in this experiment (Figures 2 and 3). It was found that acetonitrile (70:30) provided the best resolution for separating the neral and geranial peaks, along with other citral components (Gaonkar et al., 2016). Using a higher acetonitrile concentration (e.g., 90:10) resulted in faster elution and lower peak resolution (tR around 4.5 minutes), as seen in Figure 3. For *C. citratus*, a more complex RP-HPLC method has been described, using ammonium acetate and 0.1% formic acid in water as the first solvent, and methanol as the second mobile phase (Globinmed, 2021a). The elution order remained similar to that in Figures 3 and 4, with neral (tR = 16.850 minutes) eluting before geranial (tR = 19.006 minutes). A simpler RP-HPLC method for *C. nardus* has also been documented (Globinmed, 2021b), using a mobile phase of 0.1% formic acid in water and acetonitrile, with a major peak corresponding to geraniol (tR = 11.2 minutes).

In comparison, *C. citratus* has been found to be particularly enriched in neral and geranial, which are the primary characteristic metabolites of this species (Otify et al., 2022). This is in contrast to other *Cymbopogon* species such as *C. flexuosus*, *C. procerus*, *C. martini*, and *C. nardus* (locally known as "serai wangi"). Additionally, Kaur et al. (2021)

reported that the essential oil of *C. nardus* is primarily composed of citronellal, citronellol, and geraniol. The chemical composition of *Cymbopogon* essential oils can vary based on factors such as geographic origin, environmental conditions, developmental stages, harvest time, and genetic differences (Kaur et al., 2021). Furthermore, an HPLC study of *Cymbopogon* ethanolic extracts from Pakistan revealed additional phytochemicals, including flavonoids and phenolics (Lin et al., 2021). Given these variations, the *C. citratus* specimen selected for this study can be sourced from retail vendors (Globinmed, 2021a).

CONCLUSION

The identification of monoterpenoids was suggested by comparing the retention times and HPLC data of each compound of the peaks in this random *Cymbopogon* sample to those of the published chromatogram. In short, geraniol, geranial and neral could possibly be introduced in this chemical investigation due to the absence of any standard compounds.

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REFERENCES

Alembagheri, A., Hajimehdipoor, H., Choopani, R., Esmaeili, S. (2023). The role of selected medicinal plants from Iranian traditional medicine for the treatment of fatigue in metabolic syndrome. *Tradit Med Res*., *8*(4), 23. doi: 10.53388/TMR20220706001

Bationo, R. K., Dabiré, C. M., Hema, A., Nébié, R. H. Ch., Palé, E., & Nacro, M. (2022). HPTLC/HPLC-mass spectrometry identification and NMR characterization of major flavonoids of wild lemongrass (*Cymbopogon giganteus*) collected in Burkina Faso, *Heliyon*, *8*, e10103.

Costa, G., Nunes, F., Vitorino, C., Sousa, J. J., Figueredo, I. V. & Batista, M. T. (2015). Validation of a RP-HPLC Method for Quantitation of Phenolic Compounds in three Different Extracts from *Cymbopogon citratus*. *Research Journal of Medicinal Plant,* 1-9.

Figueirinha, A., Paranhos, A., Pérez-Alonso, J. J., Santos-Buelga, C. & Batista, M. T. (2008). *Cymbopogon citratus* leaves: Characterization of flavonoids by HPLC–PDA–ESI/MS/MS and an approach to their potential as a source of bioactive polyphenols, *Food Chem.*, *110*, 718–728.

Foo, J., Mohamad, A. L., Omar, M. & Amir, A. A. (2016a). Ethnobotanical Survey of Medicinal Plants Traded at Tamu in Sabah Urban Area. *International Journal of the Malay World and Civilisation (Iman)*, *4* (Special Issue 1), 79 – 87.

Foo, J., Mohamad, A. L., Omar, M. & Amir, A. A. (2016b). Utilitarian tumbuhan ubatan di Tamu Pantai Barat Sabah. *Geografia Online™, Malaysian Journal of Society and Space*, *12* (12), 99-112.

Gaonkar, R., Yallappa, S., Dhananjaya, B. L. & Hegde, G. (2016). Development and validation of reverse phase high performance liquid chromatography for citral analysis from essential oils. *J Chromatogr B*, *1036*, 50-56.

Globinmed (2021a). *Cymbopogon citratus* (DC.) Stapf, Accessed on 25th August 2022, https://www.globinmed.com/medicinal_herbs/cymbopogon-citratus-dc-stapf-105681/

Globinmed (2021b). *Cymbopogon nardus (L.)* Rendle, Accessed on 25th August 2022, https://www.globinmed.com/medicinal_herbs/cymbopogon-nardus-l-rendle-2/

Kaur et al. (2021). *Cymbopogon nardus* essential oil: a comprehensive review on its chemistry and bioactivity, *Journal of Essential Oil Research*, 33:3, 205-220, DOI: 10.1080/10412905.2021.1871976.

Lin, X., Rafique, A., Fayyaz, T., Bashir, W., Luqman, M., Zahid, F.M. and Zhou, K. (2021) Appraisal of *Cymbopogon Citratus* (Lemon grass) for antibacterial activity against Uropathogens. *Pak Vet J*, *41*(1): 122-126. http://dx.doi.org/10.29261/pakvetj/2020.040

Madi et al. (2022). Morphological and Anatomical Characterization of *Cymbopogon citratus* (DC.) Stapf Cultivated in Egypt," Bulletin of Faculty of Pharmacy Cairo University: Vol. 60: Issue 1, Article 6. Available at: https://doi.org/10.54634/2090-9101.1032

Madivoli, E. S., Gitu, L. & Gumba, E. (2012). Isolation and Identification of Essential Oils from *Cymbopogan Citratus* (Stapf) DC Using GC-MS and FT-IR. *Chemistry and Materials Research*, *2* (4), 13 – 22.

Méabed, E. M. H., Abou-Sreea, A. I. B. & Roby, M. H. H. (2018). Chemical analysis and giardicidal effectiveness of the aqueous extract of *Cymbopogon citratus* Stapf, *Parasitology Research*, *117* (6), 1745-1755.

Mohamad, T. A. S. T., Islahudin, F., Jasamai, M., Jamal, J. A. (2022). Traditional Practitioner's Knowledge of Malay Post-Partum Herbal Remedies in Malaysia. *Arch Pharm Pract*., *13* (2), 11-6. https://doi.org/10.51847/ani1usVKCvhttps://doi.org/

Otify, A. M., Serag, A., Porzel, A., Wessjohann, L. A. & Farag, M. A. (2022). NMR Metabolome-Based Classification of *Cymbopogon* Species: A Prospect for Phyto-equivalency of its Different Accessions Using Chemometric Tools. *Food Analytical Methods*, *15*, 2095–2106.

Rauber, C. da S., Guterres, S. S. & Schapoval, E. E. S. (2005). LC determination of citral in *Cymbopogon citratus* volatile oil. *J. of Pharm. and Biomed. Analysis*, *37*, 597–601.

Sabah Tea Resort - Kota Kinabalu (2020). Accessed on 6th January 2023, via https://www.sabahtea-eshop.com/products/borneo-exotic-series-lemongrass

Shah, G., Shri, R., Panchal, V., Sharma, N., Singh, B., & Mann, A. S. (2011). Scientific basis for the therapeutic use of *Cymbopogon citratus*, Stapf (lemon grass). *J Adv Pharm Technol Res.*, *2* (1), 3–8.

Tajidin, N. E., Ahmad, S. H., Rosenani, A. B., Azimah, H. & Munirah, M. (2012). Chemical composition and citral content in lemongrass (*Cymbopogon citratus*) essential oil at three maturity stages. *African Journal of Biotechnology*, *11* (11), 2685-2693.