



PROFILING OF METABOLITES IN *Chromolaena odorata* USING GAS CHROMATOGRAPHY- MASS SPECTROMETRY (GC-MS) AND ATTENUATED TOTAL REFLECTANCE-FOURIER TRANSFORM INFRARED SPECTROSCOPY (ATR-FTIR)

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ABSTRACT

Chromolaena odorata, also known as the *Kapal Terbang* plant is commonly identified in open areas like bushes and forests. This study employs Attenuated Total Reflectance Fourier-Transform Infrared (ATR-FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS) analyses to examine the metabolite profiles of *C. odorata* leaves, using acetone extraction. ATR-FTIR analysis detected the presence of functional groups associated with active compounds, including -CH (aromatic), -CH (alkane), and C=C (aromatic) at 2,925.95 cm⁻¹, 2,855.24 cm⁻¹, 1,601.77 cm⁻¹, and 1,458.21 cm⁻¹, respectively. Significant peaks at 1,205.37 cm⁻¹, 1,089.67 cm⁻¹, and 836.83 cm⁻¹ correspond to C-Si (bending), -CO (aliphatic ether), and C-Si (stretching), respectively. GC-MS analysis further confirmed these findings by identifying bioactive compounds, including 1,4-bis(trimethylsilyl)benzene, 2,2-dimethoxybutane and decane. Early research suggests that 2,2-dimethoxybutane exhibits anti-inflammatory properties by inhibiting pro-inflammatory cytokines and chemokines, indicating its potential for treating inflammatory disorders. Decane, owing to its lipophilic nature, aids in the dissolution and stabilisation of antimicrobial agents such as certain antibiotics and antiseptics. The strong correlation between ATR-FTIR and GC-MS data highlights the medicinal potential of *C. odorata*, warranting further investigation to expand its applications in both traditional and modern medicine.

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Introduction

Chromolaena odorata, commonly known as “Siam weed” is a perennial shrub that can reach three metres to seven metres in height (Jumaat *et al.*, 2017). Native to Central and South America, this plant has spread extensively to Southeast Asia, Africa, and the Pacific Islands (Tanhan *et al.*, 2007; Kouamé *et al.*, 2013). In Malaysia, it is referred to as “Pokok Kapal Terbang” due to its leaf shape, which locals believe resembles an airplane. Gradually, it has evolved into

a significant pest, rapidly colonising areas such as forestry, pastures, and plantations of economically vital crops like cashew, coconut, coffee, cocoa, and rubber (Akinmoladun *et al.*, 2007). Nonetheless, the aggressive nature of its spread poses challenges to local ecosystems, agriculture, and biodiversity.

However, despite its notoriety as an invasive species, *C. odorata* possesses several attributes that could be leveraged for sustainable use.

The plant has been traditionally utilised for its fragrant and medicinal properties, particularly in Asia and West Africa. Specifically, ethnopharmacological applications include treatments for malaria, wounds, diarrhea, skin infections, toothaches, dysentery, stomachaches, sore throats, convulsions, piles, coughs, and colds (Omokhua *et al.*, 2016; Okwakpam *et al.*, 2023). These traditional uses suggest the potential to integrate *C. odorata* into modern healthcare systems, providing a sustainable alternative to synthetic drugs and contributing to the well-being of local communities (Nina *et al.*, 2024).

From a sustainability perspective, the management and utilisation of *C. odorata* could turn its invasive characteristics into an opportunity for resource efficiency. By exploiting its bioactive compounds for medicinal purposes, regions affected by its spread could benefit economically while mitigating its negative impacts on agriculture and biodiversity. Therefore, this approach would promote sustainable development by reducing the reliance on imported medicinal products and encouraging the use of locally available resources (Mugwedi, 2020). Additionally, the controlled cultivation and harvesting of *C. odorata* could provide communities with a livelihood, turning an ecological problem into a source of income (Masri *et al.*, 2022).

Metabolite profiling of *C. odorata* is crucial for identifying the specific bioactive compounds responsible for its medicinal properties. Attenuated Total Reflectance Fourier-Transform Infrared (ATR-FTIR) spectroscopy is a widely used analytical technique that provides information about the functional groups present in a compound. Notably, ATR-FTIR is a rapid, non-destructive method that identifies molecular vibrations, allowing researchers to determine the chemical bonds of active compounds within plant extracts. This technique is beneficial for detecting functional groups such as alkanes, aromatics, ethers, and other bioactive constituents. Thus, by analysing absorption peaks at specific wavenumbers, ATR-FTIR aids in characterising the chemical nature of

plant metabolites, forming a crucial first step in phytochemical investigations.

Following ATR-FTIR analysis, Gas Chromatography-Mass Spectrometry (GC-MS) is employed to provide a more detailed identification of individual bioactive compounds present in *C. odorata*. GC-MS is a powerful analytical technique capable of separating, identifying, and quantifying metabolites with high specificity. It provides fragmentation patterns that help differentiate between structurally similar compounds such as isomers and offers deeper insights into the complex chemical makeup of plant extracts (Taduri *et al.*, 2022; Khoo *et al.*, 2024). Moreover, GC-MS data complement ATR-FTIR findings by confirming the presence of key compounds and offering a molecular-level understanding of their structures. The combination of ATR-FTIR and GC-MS provides comprehensive characterisation of the plant's bioactive constituents, thereby improving the reliability of its metabolite profiling.

Conversely, integrating *C. odorata* into sustainable practices involves harnessing its medicinal potential while managing its presence to reduce its ecological footprint. By promoting research into its metabolite composition and exploring its full range of uses, the researcher can develop strategies to control its spread and utilise its properties for the benefit of both human health and the environment. Hence, this aligns with broader sustainability goals, which emphasise the need to balance economic development with the preservation of natural ecosystems (Adhikari *et al.*, 2023).

Furthermore, profiling metabolites extracted from *C. odorata* enables a detailed study of its physiological and biochemical properties. This information can guide sustainable harvesting practices that do not deplete natural populations or disrupt local ecosystems. It also opens the possibility of cultivating the plant in controlled environments, where its growth can be managed to prevent it from becoming a pest while still providing valuable resources for medicinal and industrial applications (Skubel *et al.*, 2020).

Note that *C. odorata* is often viewed as a problematic invasive species. Nevertheless, its potential for contributing to sustainability should not be overlooked. By focusing on its medicinal properties and employing advanced techniques like ATR-FTIR and GC-MS for metabolite profiling, this plant can be transformed from a pest into a resource (Kato-Noguchi & Kato, 2023). Therefore, this research focuses on the detection of compounds using both ATR-FTIR and GC-MS. This approach addresses the ecological challenges posed by its spread and offers economic and health benefits to the communities affected by its presence. Through sustainable management and innovative research, *C. odorata* can become an integral part of efforts to balance ecological conservation with human development.

Materials and Methods

Sample Preparation

The method used in this research was adopted from Meela *et al.* (2019). Fresh leaves of *C. odorata* were harvested from bushes near the lake at Kolej PERMATA Insan. Consequently, the samples were brought to the Ar-Razi Halal Action Lab at Kolej PERMATA Insan. *C. odorata* was dried for 14 days on a laboratory bench.

Subsequently, it was ground into a powder using a mortar and pestle. 10 mL of powdered *C. odorata*, approximately 1 g was placed in another Falcon tube and 10 mL of acetone was added. It was vigorously shaken for about 30 minutes at high speed using a Vortex Mixer to mix *C. odorata* with the solvent.

The mixture was then transferred into smaller polyester centrifuge tubes. Correspondingly, these tubes were centrifuged at 3,500 rpm for 10 minutes, separating the extracts from their supernatants. The supernatant was transferred to another Falcon tube using a pipette and the extraction was repeated three more times into two beakers containing 100 μ l of the prepared supernatant. The sample is now ready for GC-MS analysis. The results mark one successful

sample. Therefore, to achieve accurate findings, this process was repeated twice more.

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR) Analysis

Metabolite functional groups in the samples were identified by ATR-FTIR, with frequency measurements recorded as wave numbers ranging from 4,000 to 650 cm^{-1} . In the analysis, the samples were placed on the clean surface of an Agilent Cary 630 instrument equipped with a diamond ATR accessory and analysed using Micro-Lab software.

GC-MS Analysis of *Chromolaena odorata*

The compounds in the sample were detected using an AGILENT INTUVO 9000 GC-MS with an ionisation voltage of 70 eV. Notably, Gas Chromatography (GC) was conducted in temperature-programming mode using a Restek column (0.25 mm, 60 m, XTI-5).

Furthermore, the initial column temperature was 80°C for 1 minute, then, increased linearly at 70°C min^{-1} to 220°C, held for 3 minutes, followed by a linear increase at 10°C min^{-1} to 290°C for 10 minutes. The injection port temperature was 290°C and the GC-MS interface was maintained at the same temperature. The sample was introduced via an all-glass injector operating in split mode, with a helium carrier gas flow rate of 1.2 mL min^{-1} (Raju & Kumar, 2020).

Results and Discussion

ATR-FTIR analysis detected the functional groups of the active compounds. Notably, these include -CH (aromatic), -CH (alkane), and C=C (aromatic) at 2,925.95 cm^{-1} , 2,855.24 cm^{-1} , 1,601.77 cm^{-1} , and 1,458.21 cm^{-1} , respectively.

Additionally, the ATR-FTIR analysis exhibits significant peaks at 1,205.37 cm^{-1} , 1,089.67 cm^{-1} , and 836.83 cm^{-1} , which correspond to C-Si (bending), -CO (aliphatic ether), and C-Si (stretching), respectively. The ATR-FTIR findings have been validated by the

GC-MS spectrum peak (Figure 1). Hence, this implies that three phytocompounds are present in the acetone extract of *C. odorata* leaf. The compounds in every peak and the structures of the suggested phytocompounds are tabulated in Table 1.

Decane is a straight-chain alkane hydrocarbon with 10 carbon atoms and a molecular formula of $C_{10}H_{22}$. It is typically discovered in plant-based sources as a natural product. Note that its applications in the medical and pharmaceutical fields (Gyanaranjan *et al.*, 2023) primarily focus on its extraction, purification, and potential therapeutic properties (Chaachouay & Zidane, 2024).

Decane can be identified in trace amounts in various plants, both terrestrial and aquatic. Its presence is often due to the natural synthesis of hydrocarbons by these plants. Furthermore, decane is a nonpolar solvent, meaning it can dissolve both nonpolar and lipophilic (fat-soluble) molecules. Many antimicrobial compounds, including certain antibiotics and

antiseptics are lipophilic. Therefore, decane can act as a reliable transporter for these substances, assisting in their dissolution and stability.

Moreover, 2,2-dimethoxybutane can be employed as an additive in cryopreservation, where cells, tissues, or medications must be preserved at extremely low temperatures. Its capacity to preserve biological materials from freezing damage has applications in tissue engineering and regenerative medicine. Note that 2,2-Dimethoxybutane can be utilised in the synthesis and stabilisation of some chemical reagents used in medical research. It can help extend the shelf life and stability of specific reagents, resulting in more reliable experimental results (Cock & Kalt, 2012).

On the other hand, 1,4-bis(trimethylsilyl) benzene can be used as a building block in organic chemical synthesis. It could be utilised in the pharmaceutical industry to generate compounds with specific structural characteristics of functional groups relevant to medication development (Huo *et al.*, 2024). In addition,

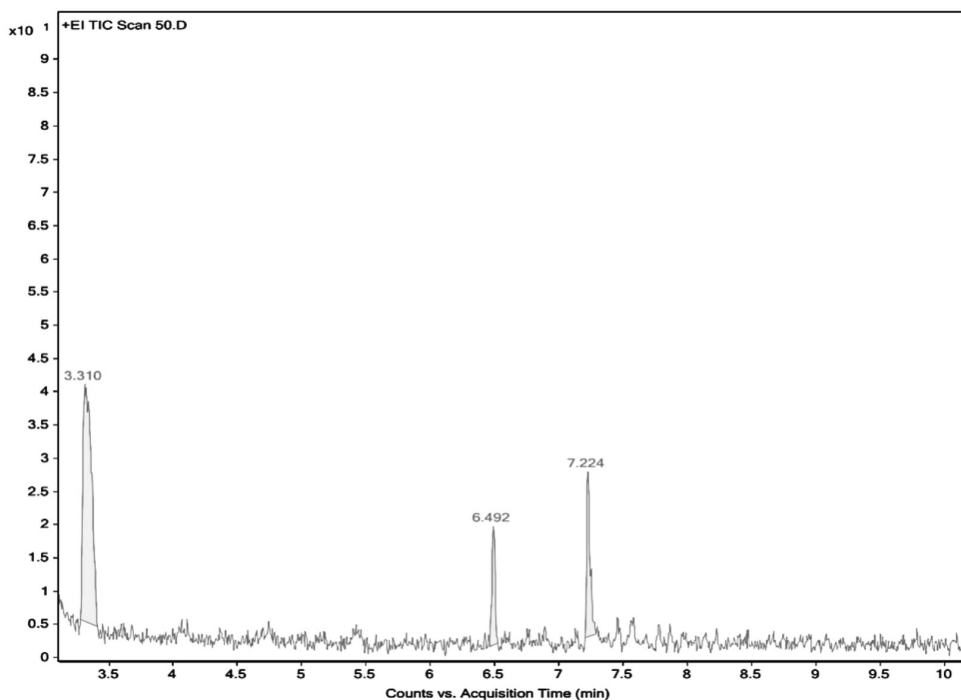
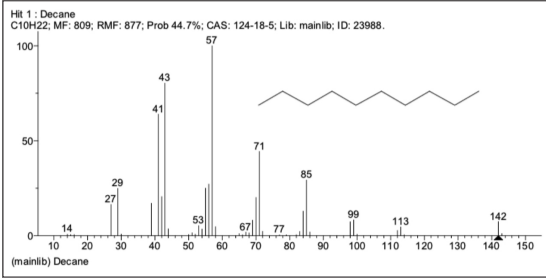
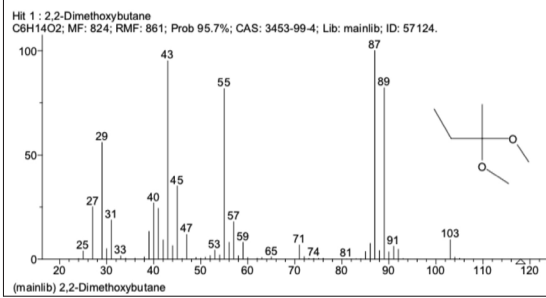
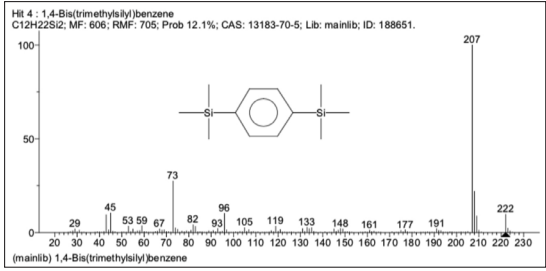


Figure 1: Results in GC-MS

Table 1: The main components present in the *Chromolaena odorata*

Compound	Chemical Structure
Decane	 <p>Hit 1 : Decane C₁₀H₂₂; MF: 809; RMF: 877; Prob 44.7%; CAS: 124-18-5; Lib: mainlib; ID: 23988.</p>
2,2-Dimethoxybutane	 <p>Hit 1 : 2,2-Dimethoxybutane C₆H₁₄O₂; MF: 824; RMF: 861; Prob 95.7%; CAS: 3453-99-4; Lib: mainlib; ID: 57124.</p>
1,4-Bis(trimethylsilyl) benzene	 <p>Hit 4 : 1,4-Bis(trimethylsilyl)benzene C₁₂H₂₂Si₂; MF: 606; RMF: 705; Prob 12.1%; CAS: 13183-70-5; Lib: mainlib; ID: 188651.</p>

1,4-bis(trimethylsilyl)benzene is a metabolite discovered in the plant *Willughbeia edulis* in Malaysia. This finding has been supported by Sami (2022). *W. edulis* is a climbing shrub native to Bangladesh, India, Laos, Cambodia, Thailand, Malaysia, and Myanmar. Traditionally, its stem has been used to treat yaws, dysentery, and liver discomfort while its root has been used to treat heartburn, jaundice, and diarrhoea.

The chemical compounds highlighted in this text, including decane, 2,2-dimethoxybutane and 1,4-bis(trimethylsilyl)benzene have a wide range of potential uses across various sectors

of the medical and pharmaceutical industries. Decane's role as a nonpolar solvent emphasises its importance in the delivery of lipophilic drugs (Preeti *et al.*, 2023) while 2,2-dimethoxybutane's application in cryopreservation demonstrates promise in regenerative medicine.

Lastly, 4-bis-trimethylsilyl-benzene underscores the importance of chemical innovation in drug discovery, particularly in the synthesis of heterocyclic compounds that are critical to many pharmaceutical developments (Li Petri *et al.*, 2023).

Conclusions

The *C. odorata* contains many distinct chemicals. The bioactivities of these chemicals have been emphasised. The exploration of these compounds enriches the understanding of their individual capabilities. It underscores the importance of chemical research in advancing medical and pharmaceutical advancements. Consequently, these compounds may well serve as the foundation for next-generation treatments and therapeutic strategies, reflecting the profound impact of chemistry on the future of healthcare.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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