Phytochemical Analysis and Antioxidant Activity Assessment of Methanolic Extract from Jasmine Flowers

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ABSTRACT

This study offers a comprehensive analysis of the phytochemical composition and antioxidant properties of the methanol extracts from Jasmine flowers. Employing a combination of advanced techniques, including Gas-Chromatography Mass-Spectrometry (GC-MS), Fourier Transform Infrared Spectroscopy (FTIR),

Scanning Electron Microscopy (SEM), and antioxidant activity assays, the research uncovered key insights into the bioactive potential of Jasmine. The GC-MS analysis identified nine distinct compounds, including major constituents, such as 2-Phenylthiolane (44.12%), Cyclohexene, 3-ethenyl- (25.88%), Acetaldehyde (12.70%), and N-Methylallylamine (10.31%) among others. The FTIR spectra revealed significant functional groups, including O-H and C-C stretches, suggesting the presence of phenolic compounds. The SEM imaging highlighted the morphological changes in the Jasmine flower powder, showing expanded oil glands post-pre-treatment, which enhanced the oil extraction process. The methanol extract exhibited a strong antioxidant activity, as evidenced by the DPPH radical scavenging assay. These findings position Jasmine flowers as a promising natural source of phytochemicals, particularly antioxidants, with potential for further pharmacological and industrial applications. Future studies could focus on isolating and evaluating additional bioactive compounds for their therapeutic potential.

2-phenylthiolane; microwave-assisted hydrodistillation; Keywords-jasmine flower; Chromatography-Mass Spectroscopy (GC-MS); Fourier Transform Infrared Spectroscopy (FTIR); DPPH scavenging activity

INTRODUCTION

Essential oil extraction is the process of obtaining aromatic liquids from plants through distillation [1]. These oils are characterized by their volatile nature, which arises from compounds such as esters, alcohols, aldehydes, ketones, hydrocarbons, and phenols [2]. Essential oils derived from various plant sources are readily available on the market and have been extensively studied for their biological activities, even in small quantities [3, 4].

The growing global mortality caused by infectious diseases is closely linked to the emergence of antibiotic-resistant bacteria, posing a critical challenge. Addressing this issue requires the urgent development of innovative antimicrobial drugs [5]. Medicinal plants, abundant in bioactive compounds, play a vital role in this effort and hold significant socio-cultural value, particularly in countries like Malaysia. The biocompounds derived from plant components, such as flowers, bark, seeds, roots, and leaves, are crucial in basic healthcare systems, especially in resource-limited regions [6, 7]. Identifying these plant-derived bioactive chemicals has advanced the development of novel pharmaceuticals for conditions like cancer [8] and Alzheimer's disease [9]. These active compounds exhibit antibacterial, antioxidant, and antifungal properties. Recent studies have demonstrated that essential oils from plants contain significant levels of phenols, which possess strong antioxidant properties and a high capacity for neutralizing free radicals [10]. Additionally, plant leaves are rich in phenolic compounds and flavonoids [11]. Modern techniques for analyzing and identifying active plant components are essential for establishing consistent standards in herbal preparations. The GC-MS is frequently employed to identify bioactive compounds, including hydrocarbons, esters, ethers, alcohols, and acids [12, 13].

The Jasmine plant, originally native to tropical regions, such as Southeast Asia, Australia, and Africa, is now cultivated worldwide. This study examined the essential oils derived from indigenous Malaysian Jasmine blossoms, specifically the Melati and Melur varieties. Jasmine essential oils are known for their distinctive taste and fragrance, which are attributed to their specific glycerides and hydrocarbons. These oils are widely used as expectorants for dry skin treatment, as well as for their antiseptic, antispasmodic, and antidepressant properties. Furthermore, Jasmine essential oils address various issues, such as melancholy, fatigue, delicate skin, migraines, and respiratory irritations.

Microwave-assisted Hydro-distillation (MAHD) emerged as a preferred method for extracting biological substances due to its advantages, including selective solvent removal, reduced extraction time, and controlled heating. This eco-friendly process emits lower levels of carbon dioxide (CO₂) [14] and is more efficient than traditional steam distillation [15,16] and liquid-liquid extraction methods [17]. MAHD has proven to be effective in extracting different bioactive constituents from plants, with its efficiency being influenced by the dielectric constants of both the sample and the solvent [18]. Unlike traditional methods, which are timeconsuming and lack precise control over heating, MAHD offers a more efficient and regulated extraction procedure [19]. Numerous studies have highlighted the use of microwave technology to extract essential oils from medicinal plants [20]. This study explores the utilization of MAHD to extract bioactive compounds from Jasmine flowers, focusing on their potential application as antioxidants.

MATERIALS AND METHODS

A. Chemicals and Materials

Jasmine blossoms were collected from Kuantan, Pahang, Malaysia. To remove contaminants, the flowers underwent thorough water washing. The cleaned flowers were then dehydrated in an oven at 90 °C for 1 hour. After drying, they were ground into a fine powder to maximize the surface area for the solvent interaction. A particle size of 80 µm was achieved using mechanical grinding and sifting with a sieve shaker. Methanol, supplied by Fisher Scientific, was utilized as the solvent, while dichloromethane, also from Fisher Scientific, was employed for the extraction of essential oils from the

B. Microwave-Assisted Hydro-Distillation

The Milestone MWS Ethos E Solvent Extraction System was utilized for the MAHD process, with specifications of 2.5 kW power, 230 V-60 Hz voltage, and 2450 MHz frequency. The microwave oven was modified to accommodate the MAHD operations. A total of 35 grams of Jasmine flowers were placed in a 1-liter flask containing 280 milliliters of methanol. The flask was heated in the modified microwave oven at 400 W for 120 minutes [20]. During the process,

volatile oils evaporated and were directed through a condenser using a Clevenger apparatus. The resulting mixture of methanol and essential oil was collected and transferred to a separation funnel containing dichloromethane for phase separation. The essential oil yield, calculated as 0.89% (v/w), was determined based on the dry weight of the sample.

C. Analysis Using Gas Chromatography-Mass Spectrometry

The chemical analysis was conducted using the Agilent 5975C Series GC/MSD system equipped with a fused silica column (DB-WAX, 30 m \times 0.25 mm ID \times 2.5 µm) and an 100% dimethyl polysiloxane stationary phase. The oven temperature was initially set at 60 °C for 10 minutes, then increased by 20 °C per minute until reaching 250 °C, where it was held for an additional 10 minutes [21]. Helium was used as the carrier gas at a flow velocity of 30 cm/s. The spectrum of compounds was analyzed using the National Institute of Standards and Technology (NIST) database. The component identification was performed based on the NIST library.

D. Analysis Using Fourier Transform Infrared Spectroscopy

The FTIR analysis was conducted using the Thermo Scientific Nicolet iS5 FT-IR Spectrometer. Spectra were recorded across wavenumbers ranging from 4000 cm⁻¹ to 400 cm⁻¹, providing insights into the functional groups present in the sample.

E. Scanning Electron Microscopy Analysis

SEM was employed to analyze the surface morphology of the powdered Jasmine blossoms [22]. Both untreated dehydrated Jasmine flower powder and pre-soaked powder samples were examined to observe morphological changes. Essential oils were extracted using the MAHD for a duration of 120 minutes before the SEM analysis. The SEM analysis was performed using the Tabletop Microscope TM3030 Plus. To prevent electrical discharge during imaging, the samples were sputter-coated prior to examination. The testing was conducted in a high-vacuum environment with an applied voltage between 5 and 15 kV, a magnification range of 15 to 60,000, and an analytical working distance of 11.3 mm.

F. DPPH Radical Scavenging Test

The antioxidant properties of the methanolic extract of Jasmine flowers were evaluated using the DPPH radical scavenging assay. This method determines the sample's ability to neutralize free radicals. A DPPH solution was prepared by dissolving 0.004% (w/v) DPPH in 95% methanol. Test tubes containing five different concentrations of the sample were prepared. Subsequently, 1 ml of freshly prepared DPPH reagent was added to each test tube, and the mixtures were incubated in darkness for 10 minutes. The absorbance of the solutions was measured at 517 nm using a Systronics UV-Visible Spectrophotometer (USA). Ascorbic acid was utilized as the positive control. The percentage of DPPH free radical scavenging activity was calculated using:

Inhibition (%) =
$$\left(\frac{A_0 - A_1}{A_0}\right) * 100$$
 (1)

where A_0 represents the absorbance of control and A_1 refers to the absorbance of the test sample.

III. RESULTS AND DISCUSSION

A. Compositional Analysis of Chemical Compounds in Jasmine Flower Oil extracted via Microwave-assisted Hydro-Distillation

The essential oil extracted from Jasmine blossoms was analyzed using GC-MS, which identified several components through mass spectrometry. The findings are summarized in Table I, with chemical identities being determined using the NIST Database. The primary compounds identified, and their respective percentages are: 2-Phenylthiolane (44.12%), Cyclohexene, 3-ethenyl- (25.88%), Acetaldehyde (12.70%), N-Methyl allylamine (10.31%), Propanamide (6.89%), Phthalic acid, bis(7-methyloctyl) ester (5.32%), 10-Methylnonadecane (2.47%),1H-Tetrazol-5-amine (0.38%),1,2-Benzenedicarboxylic acid diisooctyl ester (0.26%). The Retention Times (RTs) for these nine primary components were 6.253, 33.024, 40.653, 45.587, 50.119, 51.250, 55.012, 57.501, and 61.462, as can be seen in Figure 1.

TABLE I. CHEMICAL COMPONENTS OFJASMINE FLOWER ESSENTIAL OIL PORDUCED BY MADH PROCESS

No	Compound	Molecular Formula	MW	RT	Area%
1	Acetaldehyde	CH ₃ CHO	44.05	6.253	12.70
2	2-Phenylthiolane	$C_{10}H_{12}S$	164.26	33.024	44.12
3	Propanamide	C ₃ H ₇ NO	73.09	40.653	6.89
4	Cyclohexene, 3- ethenyl	C ₄ H ₉ N	108.18	45.587	25.88
5	N-Methylallylamine	C_8H_{12}	71.12	50.119	10.31
6	1H-Tetrazol-5-amine	C ₄ H ₉ N	390.55	51.250	0.38
7	1,2- Benzenedicarboxylic acid, diisooctyl ester	C ₈ H ₁₂ O	124.18	55.012	0.26
8	10-Methylnonadecane	$C_{24}H_{38}O_4$	282.54	57.501	2.47
9	Phthalic acid, bis(7- methyloctyl) ester	$C_{20}H_{42}$	418.61	61.462	5.32

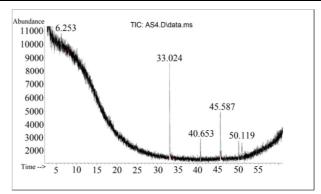


Fig. 1. The GC-MS chromatogram of volatile compounds extracted from Jasmine flowers.

B. Structural Analysis of Essential Oil

The functional groups present in the essential oil were analyzed using FTIR. Absorption bands at 3286.39 cm⁻¹ and 2920.65 cm⁻¹, which represent O-H stretching in phenols and C-C stretching, respectively, were visible in the FT-IR spectrum. The presence of a C=O bond in aldehydes was revealed by an absorption band at 1627.08 cm⁻¹. Ring stretching was identified as the cause of the peak at 1304.70

cm⁻¹, whilst C-O stretching vibrations were linked to the peak at 1151.75 cm⁻¹. Furthermore, the vibrational absorption of the C-H group in the benzene ring was correlated with the peak at 767.11 cm⁻¹. Table II provides a summary of the major peaks and the functional groupings that correlate to them, while Figure 2 illustrates them.

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TABLE II. FTIR SPECTRAL ANALYSIS AND FUNCTIONAL GROUP CALCULATIONS BASED ON THEORETICAL **STUDIES**

No	Vibration assignment (v) (cm ⁻¹)	Absorption band
1	3286.39	О-Н
2	2920.65	C-C stretching
3	1627.08	C=O stretching
4	1304.70	Ring stretching
5	1151.75	C-O stretching
6	1016.30	C-OH deformation vibration
7	767.11	C=H vibration of benzene ring
8	518.76	C=C vibration of benzene ring

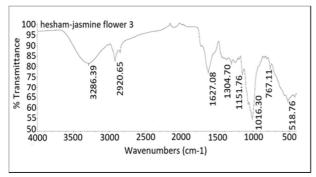
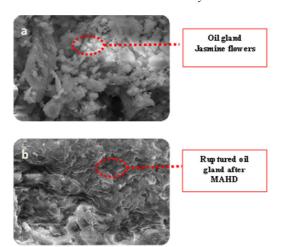


Fig. 2. FTIR spectrum of essential oil extracted from Jasmine flower.

C. Morphological Alterations of Jasmine Flowers Following Extraction with Microwave-Assisted Hydro-Distillation



SEM images of oil cell glands in Jasmine flowers: (a) SEM image of glands after pretreatment by soaking for 60 min, (b) SEM image of oil glands after being subjected to MAHD extraction for 120 minutes.

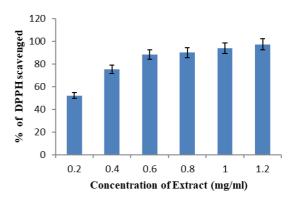
The morphological changes in the Jasmine flower powder during the extraction process were investigated using SEM. Figure 3a shows the impact of the soaking process on the oil glands, causing them to expand and enlarge. This expansion facilitates a smoother and more efficient release of essential oil during the extraction process. Conversely, in the absence of soaking, the oil glands appear contracted, which would require greater pressure for an effective oil release. Following the MAHD process, the SEM image, observed in Figure 3b, reveals the ruptured and deformed structure of the oil glands, indicating the successful extraction of essential oil from the Jasmine flower powder.

D. DPPH Radical Scavenging Test

The DPPH assay is widely recognized for assessing the antioxidant properties of the plant extracts, including those derived from the Jasmine flowers. The results of the DPPH scavenging activity are summarized in Table III. Figure 4 portrays the DPPH radical scavenging activity of the Jasmine flower essential oil and methanol extract after a 10-minute incubation period. Absorbance was measured at 517 nm, showing a concentration-dependent increase in antioxidant activity. Notably, the scavenging effect of the methanol extract from Jasmine flowers closely parallels that of the ascorbic acid, which served as a positive control [24]. Compared to previous studies [25], the Jasmine flower extract demonstrated superior antioxidant activity. This enhanced activity may be attributed to the presence of unique bioactive compounds, such as N-Methylallylamine, underscoring Jasmine's considerable therapeutic potential.

DPPH ASSAY RESULTS FOR METHANOL TABLE III. EXTRACT OF JASMINE FLOWER

No	Extract concentration (mg/ml)	% of DPPH scavenged
1	0.2	52.2
2	0.4	75.1
3	0.6	88.3
4	0.8	90
5	1.0	94
6	1.2	97.3
7	Ascorbic acid (5mg/ml)	99.5



4. DPPH radical scavenging activity of essential oil and methanol extract from jasmine flowers.

IV. CONCLUSIONS

This study presents a detailed examination of the bioactive components in the methanol extract of Jasmine flowers, utilizing Gas Chromatography-Mass Spectrometry (GC-MS) to

identify nine specific compounds, including major constituents, such as 2-Phenylthiolane and Cyclohexene, 3-ethenyl-. Unlike previous studies that mainly focused on the antioxidant and aromatic qualities of Jasmine, this research highlights individual bioactive properties, particularly its significant antioxidant activity. The identification of unique bioactive compounds, such as N-Methylallylamine, Propanamide, and 1H-Tetrazol-5-amine, suggests their potential for synergistic effects, enhancing antioxidant formulations and broadening their therapeutic and industrial applications. This study not only provides a valuable chemical baseline for Jasmine flowers, but also expands on previous research by investigating its antibacterial properties and alternative extraction methods. These findings pave the way for optimizing the extraction and application of Jasmine's bioactive compounds. Furthermore, the use of Microwave-Assisted Hydrodistillation (MAHD) highlights an efficient, environmentally friendly extraction method that could be employed in both small-scale and industrial applications. The research underscores Jasmine's medicinal potential, positioning it as a promising source for the development of bioactive compounds with diverse therapeutic benefits. Overall, this study makes a significant contribution to medicinal plant research, providing new insights into Jasmine's bioactive profile and its potential in pharmaceutical, nutraceutical, and cosmetic industries. Further studies, including clinical evaluations and synergistic compound analysis, are recommended to fully explore and capitalize on the therapeutic potential of Jasmine flowers.

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