Comparative Analysis of Vegetable Chemical Compounds from Orange Peel Extract and Thuja Plants Using Gas Chromatography Mass Spectrometry Technique

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Abstract: The present study was carried out to investigate the chemical composition of the alcoholic thuja and oranges peels extract using Gas Chromatography – Mass Spectrometry (GC-Ms). Fifty-five compounds were determined for Thuja leaves extract, most notably the D-Limonne, Ferrgoinol, and Terpine-4-IL, which are known as its antioxidant and microbial properties. In the orange peel extract, it was determined 78 compounds, including nootkatone, Squalene and D-Limonne, known as its role in the pharmaceutical industries and cosmetics. The current study analyzes indicated that the use of methanol alcohol in the Soxhlet system increased the efficiency of extracting semi -polar compounds such as phenols and glycosides and flavonoids. The current study also emphasized the sensitivity of the GC-MS technology in detecting volatile and non-polar compounds, such as D-Limonne, which appeared in both extractors. The study emphasized the importance of choosing the technique of extraction and the type of solvent in enhancing the efficiency and quality of the extracted vehicles. the present study also offers a valuable chemical database for the optimal exploitation of these plants in the medical, agricultural, and industrial fields, in line with the principles of environmental sustainability.

1 INTRODUCTION

Plant extracts are natural treasures rich in various chemical compounds that play a vital role in various fields, medical, industry and agriculture [1]. These compounds are derived from the secondary metabolism of the plant, such as phenols, rectaloids, alkaloids, and flavonoids, which show distinctive biological activities such as antioxidants, antimicrobials, and anti -inflammatory properties. It is also alternatives that can be applied as natural insecticides, which seemed industrial, due to the diversity of the active chemical compounds that contain and subject to biological decomposition, which reduces the harmful environmental effects [2]. Thuja is an important plant in proportion to scientists because it contains phenolic compounds with anti - bacterial and antioxidants [3]. Because of its association with proteins, it is used in industries and also on compounds that prevent the growth of germs and fungi with high efficiency [4]. Orange peels, which are taken from orange fruit, are widely used because they contain volatile compounds such as lemon, flavonoids and glycosides, which work to promote heart health and reduce oxidative stress [5]. Essential oils extracted from orange scales - rich in chemical compounds - can be used as effective natural pesticides, which helps protect soil and water from chemical pollution caused by artificial pesticides [6]. The active oils extracted from the leaves of the thuja plant and orange peels are one of sustainable products to reduce the harmful environmental effects as their ability to manage agricultural pests effectively due to the chemical components in both extractors. The GC-MS is the separation and identification of volatile and semi-volatile chemical compounds that make it a very accurate analytical tool for the detection of chemical compounds in plant extracts [7]. This technique has been successfully used to analyze secondary compounds in different plants, providing visions about its chemical diversity and potential applications [8]. This study is compared chemical compounds for the extract of the leaves of Thuja plant and orange peel using gas- chromatography technique. This analysis is important for understanding the differences in phenolic, triny and aromatic compounds, which may explain the contrast in its biological activity. These results contribute to enhancing our understanding of how these plants are optimally used in industrial or medicinal environments due to their effective chemical properties.

2 MATERIALS AND METHODS

The fruits of orange plants (family Rutaceae.) were harvested in the Bohriz, Baquba district, Diyala Governorate, Iraq, from November to February for its orange peels and the leaves of the thuja plant (cypress family).

2.1 Preparing the Sample

Thuja and orange peels were washed to remove dust and dirt using sterile distilled water, then it was dried with air at room temperature ((25°C ± 2°C)) in a dark place to avoid the decomposition of active compounds, according to standard protocols to prepare plant samples [6].

Thuja leaves and orange peels were left to dry in room temperature. After drying, the two plants were ground using an electric grinder to obtain the powder of thuja leaves and orange peel to increase the contact area with the solvent as shown in Figures 1 and 2.

Figure 1: Steps for preparing ground thuja leaves.

Figure 2: Steps for preparing ground orange peels.

Figure 3: Steps for extracting thuja leaves using a Soxhlet.

Figure 4: Steps for preparing orange peel extract using the Soxhlet device.

2.2 Soxhlet Preparation

The samples were weighed using a sensitive balance. Fifty grams of the dried samples were put inside a Whatman no1 filter paper funnel and sealed tightly. The Soxhlet was used with 400 mL of methanol for 6 hours. The sample extract was cooled and transferred to a rotary evaporator for 7 minutes to concentrate the sample to 50 mL, as shown in Figures 3 and 4.

2.3 Chemical Analysis

The chemical analysis of the thuja leaf and orange peel extracts was performed. Chemical compounds were identified using gas chromatography-mass spectrometry (GCMS), using Agilent Technologies A GC System GC-MS 7890, manufactured in the USA, from the Basra Oil Company - Laboratory Division. After starting at 40°C, the temperature in the column was raised by 10°C every minute until it reached 300°C. The helium carrier gas flow rate was 1 ml per minute. One microliter of the extract was injected, and peaks were identified using the National Institute of Standards and Technology (NIST) database and the device's memory list [9].

3 RESULTS AND DISCUSSION

3.1 Results

The chemical composition of the plant extract was analyzed using GC-MS, an advanced technology characterized by its high efficiency, specificity, and sensitivity in analytical chemistry applications. As indicated in Table 1 and 2, this technique was used to investigate the compounds found in the alcoholic extract of orange peels and thuja leaves. For the chemical compounds determined in thuja extract, the fifty five peaks obtained are displayed in the gas chromatography-mass spectrometry diagram in Figure 5. D-limonene, terpinen-4-ol, p-cymene, ferruginol, gamma-terpinene, trans-4-(hexyloxy) chalcone, citronellol, alpha-bisabolol, 2-tridecanone, and myo-inositol 4-C-methyl-were the most effective chemical compounds (Fig. 5). The most ten bioactive chemical compounds were identified for extract of thuja (Table 1).

The most ten effective chemical compounds identified were ibuprofen, nootkatone, D-Limonene, squalene, 1-Methyl-5-fluorouracil, lactose, megastigmatrienone, fercomin, 2-Methoxy-4-vinylphenol, and thymine (Table 2). A total of 78 peaks were obtained for the chemical compounds used, which are displayed in the gas chromatography-mass spectrometry diagram shown in Figure 6.

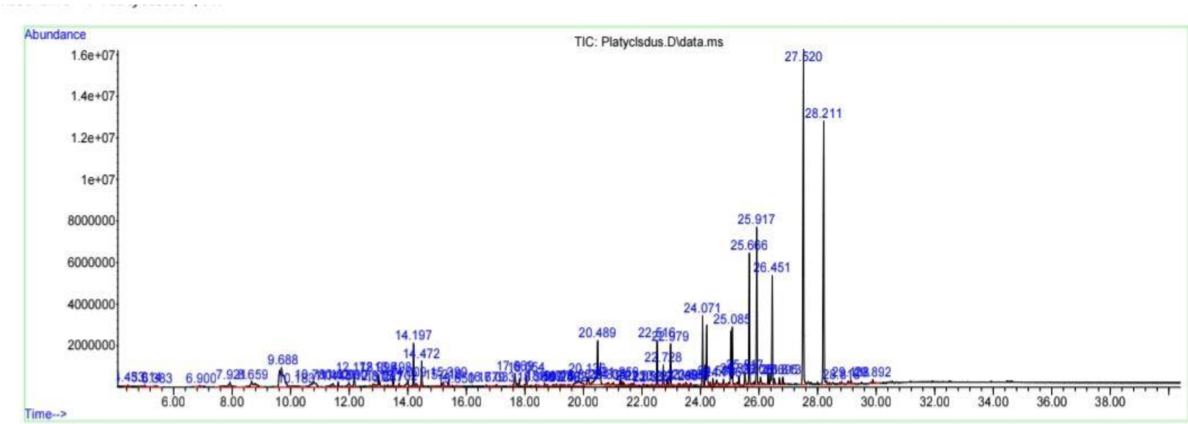


Figure 5: Gas chromatography-mass spectrometry of the alcoholic extract of Thuja leaves.

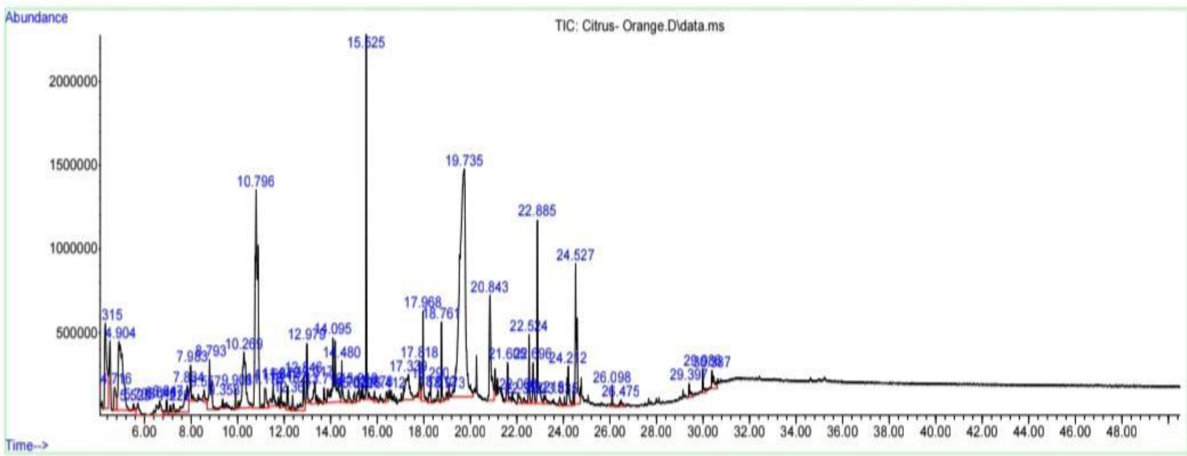


Figure 6: Gas chromatography-mass spectrometry of the alcoholic extract of orange peels.

Table 1: Phytochemical compounds identified in the thuja leaf extract.

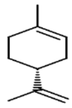
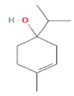
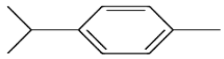
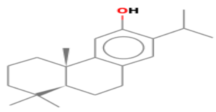
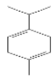
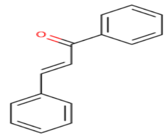
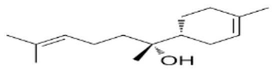
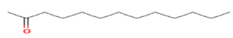
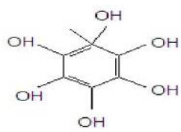
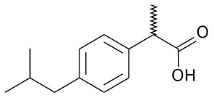
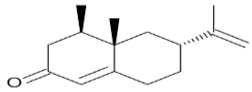
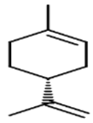
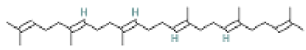
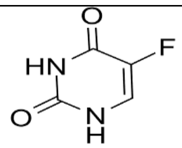
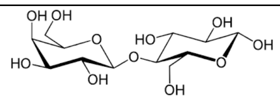
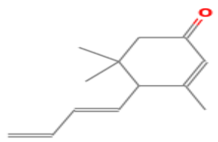
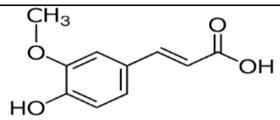
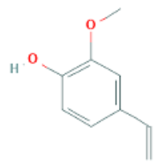
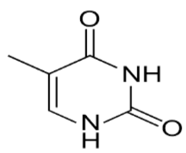
Number	Name	Formula	M.W	CAS ID	Area	RT	Formula for structure
1	D-Limonene	$C_{10}H_{16}$	136	5989-27-5	932689	10.768	
2	Terpinen-4-ol	$C_{10}H_{18}O$	154	562-74-3	987614	13.5	
3	p-Cymene	$C_{10}H_{14}$	134	99-87-6	611922	10.802	
4	Ferruginol	$C_{20}H_{30}O$	286	514-62-5	8430109	26.448	
5	. gamma. -Terpinene	$C_{10}H_{16}$	136	99-85-4	872086	11.447	
6	trans-4-(Hexyloxy)chalcone	$C_{21}H_{24}O_2$	308	99259-59-3	12150399	25.919	
7	Citronellol	$C_{10}H_{20}O$	156	106-22-9	3538529	14.198	
8	. alpha. -Bisabolol	$C_{15}H_{26}O$	222	515-69-5	855408	20.134	
9	2-Tridecanone	$C_{13}H_{26}O$	198	593-08-8	540903	17.807	
10	Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$	194	472-95-7	3127481	20.518	

Table 2: Phytochemical compounds identified in orange peel extract.

#	Name	Formula	M.W	CAS ID	Area	RT	Formula for structure
1	Ibuprofen	C13H18O2	206	15687-27-1	169070	19.367	
2	Nootkatone	C15H22O	218	4674-50-4	223622	21.607	
3	D-Limonene	$C_{10}H_{16}$	136	5989-27-5	6801365	10.794	
4	Squalene	C30H50	410	111-02-4	189969	29.986	
5	1-Methyl-5-fluorouracil	C5H5FN2O2	140	1000427-92-0	1113101	12.977	
6	Lactose	C12H22O11	342	63-42-3	607049	22.696	
7	Megastigmatrienone	C13H18O	190	38818-55-2	249880	19.539	
8	Fercomin	C23H30O5	254	104758-20-5	163536	26.095	
9	2-Methoxy-4-vinylphenol	C9H10O2	150	7786-61-0	3541874	15.527	
10	Thymine	C5H6N2O2	126	65-71-4	310941	11.869	

4 DISCUSSION

Advanced analytical methods, such as GC-MS, help identify chemical compounds in plant extracts and understand their different biological properties better [10]. Thuja leaf extract analysis was performed in GC-MS, which had determined numerous chemical compounds, including D-Limonne, Terpine-4-WOL and Ferruginol. These results correspond to Sharma study and his colleagues [11] . They demonstrated that the Thuja extract contained a lot of turbine components, especially the D-Limonne, which constitutes 15-25 % of the total essential oil. Moreover the results of [12] supported what was obtained from the current results that terpinene-4-IL is one of the main components of Thuja extracts, as they confirmed its presence in concentrations ranging from 8 to 12%. The presence of these compounds has biological importance, as Wang and others [13] reported that the presence of virogenel enhances a high capacity against free radicals and infection-causing pathogens. Furthermore, in accordance with the extraction technique, the study pointed out that the concentration of virogenol is greater in the alcoholic in comparision with the aqueous extracts. In a study conducted by Naguin and others [14], they were reported that gamma-terpinene had antimicrobial activity against both gram-positive and gram-negative bacteria, which enhanced the bioactive properties of the extract as a natural antibiotic. The analysis of orange peel extract showed 78 chemical compounds, the most important chemical compounds were D-Limonne, Squalene and Nootkatone. The presence of D-Limonne is compatible with Liu et al. [15] who they reported this substance makes 68-98 % of the essence of orange peel therapeutic oil. The presence of D-limonene is directly correlated with the quality of the extract and its antioxidant efficacy. The finding that orange peel extract contains squalene is significant because of its various health benefits. Recently, Martinez-Rodriguez et al. [16] suggested that squalene had numerous biological effects such as anti-inflammatory and antioxidant action and proposed that it should be used to improve heart health. The study also stated that using plant products like orange peels to extract squalene is more environmentally friendly than using animal sources. The findings from the study have confirmed the presence of a phenolic compound called 2-methoxy-4-vinylphenol in orange peel extract, which is recognized for its antioxidant properties. This aligns with the essay conducted by Kim and his team [17], where they found this powerful phenolic antioxidant in various plant extracts, essay serving in protect against oxidative stress. In examining Warumuh Zimat's extractors, it was observed that both contained D-Limonene, highlighting the significance of this compound as a key element in aromatic plants. This observation Supported by Mahato and colleagues [18], who noted that D-Limonene is a common component in many plant extracts, particularly those from the Citrus and Pine families. Despite similarities in presence numerous compounds , there is considerable variation in the composition of extracts from different sources. For instance, fruit extracts tend to have higher levels of certain compounds like Terpine-4-OL and GAMMA-TERPINENE, while orange peel extracts are rich in squawen, nootkatone, and other unique compounds. These variations are further explored in a recent study by Chen et al. [19], which highlighted that the differences in chemical composition of plant extracts arise from genetic factors, environmental influences, and the extraction methods used. The striking similarity notwithstanding, there exists great diversity in the composition in the extracts of the different extractors. For one, the fruit extracts contain higher amounts of turbine compounds such as Terpine-4-IL and GAMMA-TERPINENE, whereas orange peel extracts contain squawen, nootkatone, and other compounds. These differences provide scope to a recent study conducted by Chen et al [19] [20] that pointed out that the differences in the chemical composition of plant extracts are the resultant of inheritance, influences of the surroundings, and also the techniques and methods of extraction that employed.

5 CONCLUSIONS

This research has effectively showcased the chemical abundance and phytochemical variety of extracts from Thuja leaves and orange peels via GC-MS analysis. This study confirms that Thuja occidentalis (Thuja) leaf and Citrus sinensis (orange) peel extracts contain bioactive phytochemicals with promising applications in medicine, agriculture, and industry. Methanol-based Soxhlet extraction demonstrated high efficiency in isolating these bioactive compounds. GC-MS analysis identified several key constituents—including D-limonene, ferruginol, terpine-4-ol, nootkatone, and squalene—which exhibit properties relevant to pharmaceuticals (e.g., antimicrobial and anti-inflammatory agents), cosmetics (fragrances and skin-care formulations), and eco-friendly agrochemicals (biopesticides). Which confirms the efficiency of this technology. In the Thuja extract, 55 phytochemicals were identified, while 78 compounds were found in the orange peel extract. Essential components like D limonene, terpinen-4-ol, ferruginol, nootkatone, and squalene demonstrate well-established biological effects, featuring antimicrobial, antioxidant, and anti-inflammatory characteristics. The existence of overlying compounds like D-limonene in both extracts indicates a common therapeutic potential, whereas distinctive compounds such as squalene in orange peel and ferruginol in Thuja emphasize plant-specific benefits.

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