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Extraction, Characterization, and Biological Activity of Volatile and Non-volatile Components of Grapefruit

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ABSTRACT

Grapefruit (*Citrus X paradisi)* is among the biggest and most popular fruit crops grown for profit, worldwide. It is not only economically essential but also remains a popular human dietary ingredient. There are varieties of citrus, all coming with vibrant colors. Both processed and fresh forms are consumed. Half of the fruit, including seeds, peels, and pith remnants are inedible and wasted after processing. Pectin, essential oils, citric acid, flavonoids, and other significant phytochemicals derived from citrus are currently widely studied in food, industrial, and synthetic chemistry. This study examines the properties of flavonoids derived from citrus leaves, their extraction process, and their usage with respect to potential health advantages. Using GC-MS/MS, the flavonoids content of grapefruit leaf extracts was determined. It was found that the primary flavonoid present in grapefruit pulp, oven-dried leaves, and fresh leaves was isonaringin. In new experiments, fresh and dried leaves of grapefruit (dried at 45°C) showed a potent cytoprotective effect on neuroblastomas SH-SY5Y. According to the data, bioactive flavonoids are naturally found in fruit leaves that can be used as remedy in many therapeutic and antibacterial activities.

Keywords: antioxidant activity, citrus, essential oils (EO), DPPH assay, GC-MS analysis, physic-chemicals

1. INTRODUCTION

Because of its importance to the global economy, citrus is one of the most extensively produced genera, worldwide. Citrus essential oils are volatile, odorous, and naturally occurring oils found in synthetic or epidermal cells, as well as in non-forest portions of aromatic plants, such as fruits, buds, flowers, leaves, stems, seeds, roots, and twigs. The primary product of the genus citrus is citrus essential oil, which is typically extracted

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by solvent extraction or distillation [1]. It is a complex substance made up of over 400 chemicals, which contains 85–99% volatile components [2]. Citrus essential oils also contain some aromatic compounds, alcohols, esters, acids, aldehydes, and aliphatic hydrocarbons as additional organic components [3]. According to Svoboda and Greenaway [4], limonene is the main chemical component of citrus essential oils and it ranges from 32-98%. Citrus essential oils include beneficial phenolic chemicals, flavonoids, and glycosides, such as hesperidin and naringin which are responsible for reducing food intake. Moreover, citrus essential oils function as natural antioxidants. Since lemon peel essential oils are high in terpenes, flavonoids, and carotene which show antibacterial activity, they have a wide range of biological effects and are highly significant in medicine [5]. Hence, citrus essential oils are widely used in pharmaceutical industry as antimicrobial, anti-oxidant, anti-diabetic, insect repellent, antiviral, carminative, and anti-hepatotoxic agents [6]. So, the steadily increasing significance of essential oils derives from the fact that citrus is used in pharmaceutical products, perfume, flavor, food, and aroma.

These factors provide an opportunity to produce high quality essential oils obtained from citrus fruits. Therefore, focusing on essential oil extraction as a solid waste management strategy and strengthening the economy is critically needed [7–10]. Hence, this study examines the antioxidant properties, phytochemical constituents, and antibacterial activity of five citrus species and the C-paradisi (L) grapefruit.

2. MATERIALS AND METHODS

2.1. Collection of Sample

The samples were collected from various parts of the cities of Kasur and Lahore situated in Punjab, Pakistan. The samples comprised fresh leaves, pulp, and dried leaves [11–14]. The samples were dried, crushed, and then mixed into different solvents.

2.2. Extraction of Grapefruit Pulp

A 5 kg sample of grapefruit was taken and the pulpy parts of the fruit was separated to be analyzed further by using different solvents to get the extract [15–17]. Firstly, the pulp was treated with ethanol. For this purpose, the pulp was put in the magnetic stirrer apparatus. Then, 100 mL of ethanol and 50 mL of water were added in the ratio of 2:1 and the mixture was stirred for 4-5 hours. Afterwards, the solution was filtered. The residue was further treated with methanol. For this process, 100 mL methanol and 50 mL water were added to the pulp which was placed in the apparatus and stirred for 4 hours. Then, the solution was again filtered. The filtrate was obtained and the residue was treated with another solvent (ethyl acetate). Then, 100 mL ethyl acetate and 50 mL water were added with continuous stirring for another 4 hours. The solution was again filtered. Afterwards, the filtrate obtained by using three different solvents was collected in a china dish and placed in the hot plate to evaporate the water contents of the extract (filtrate). After removing the water, a thick sticky material was obtained that was put in the round bottom flask. Then, more water was added and distillation was carried out on water bath to evaporate the solvent.

2.3. Extraction of Grapefruit Fresh Leaves

Firstly, fresh leaves of grapefruit were dried, crushed, and grinded. The powder obtained was then treated with ethanol. For this purpose, leaf powder was put in the magnetic stirrer apparatus. Then, 100 mL of ethanol and 50 mL of water were added in the ratio of 2:1 and the mixture was stirred for at least 4 hours. Afterwards, the solution was filtered. The filtrate was obtained and the residue was further treated with methanol as solvent. For this process, 100 mL methanol and 50 mL water were added. The leaf powder placed in the apparatus was grinded and stirred for 4 hours. Then, the solution was again filtered. The filtrate was obtained and the residue was treated with another solvent (ethyl acetate). Then, 100 mL ethyl acetate and 50 mL water were added with the help of stirring. Again, the filtrate was obtained by using the filter paper. Afterwards, the filtrate obtained by using three different solvents was collected and placed in the rotary evaporator for evaporation.

3. RESULTS AND DISCUSSION

3.1. Extraction of Oil

The percentage of oil matters a lot due to health reasons and the number of calories consumed. Oil is used in food products either as a main ingredient for the preparation of these products or for frying purpose. So, the extraction of essential oil was done by DPPH and Soxhlet method. The value showed that the maximum percentage of oil was formed.

3.2. DPPH Radical Scavenging Assay

The impact of processing on grapefruit leaf extracts, antioxidant activities, and total polyphenol index was observed. Using the DPPH test, the effects of drying procedures on the antioxidant activity of grapefruit leaf extracts was assessed [2, 18]. It's interesting to observe that in every instance, dried leaves increased antioxidant activity. Grapefruit fresh leaves exhibited considerably higher levels of antioxidant capacity that were substantial ($p < 0.05$) in the DPPH experiment, as compared to dry leaves. Fresh leaf extracts showed values of 31.47 and 25.07 mg BHT/g DW, whereas pink grapefruit leaves reported 121.83 and 111.99 mg trolox/g DW. According to earlier studies [19], this process is linked to a large synthesis of redox-active metabolites which are crucial for adsorbing and neutralizing free radicals and breaking down peroxides. The DPPH values of pink grapefruit leaves after drying out were notably higher than those of extracts made from fresh leaves. The increased antioxidant activity is indicated by these increased values. These findings are consistent with the results published for other citrus kinds treated to similar heat treatments. They most likely resulted from the interaction between the temperature at which the grapefruit leaves were exposed and the mechanism that breaks down antioxidant chemicals.

3.3. Antioxidant Activity

According to antioxidant research, all citrus leaf oils have a high capacity to convert DPPH radicals to DP H-H (83 to 91%) [20]. C. raticulata var. was found to have the highest antioxidant activity, followed by C. reticulate var. Tangerine (88%), and C. paradisi (87%). The increasing order of the efficiency of citrus leaf oils antioxidant is as follows: Grapefruit > Grapefruit Pulp > Fresh leaves > Grapefruit Dry leaves. These outcomes are consistent with the research that showed maximum antioxidant potential, whereas dry leaves of grapefruit showed minimum antioxidant potential, as shown in Figure 1. Accordingly, limonene, a significant component of citrus oils, has antioxidant capacity comparable to that of stronger antioxidants.

Two batches of citrus species were used in the hydro-distillation method to extract citrus oils in order to calculate the average productivity of extracted oils (Table 1).

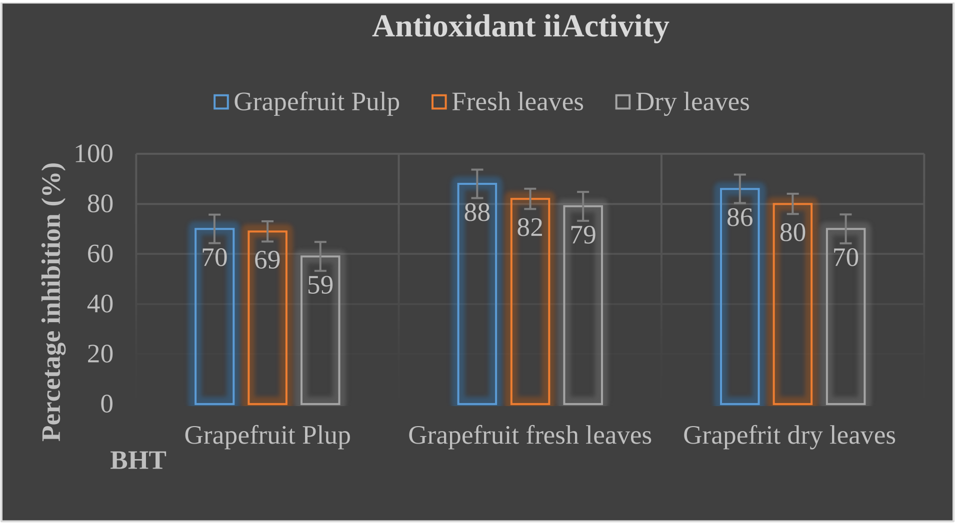

Figure 1. Antioxidant Results of Grapefruit > Grapefruit Fresh Leaves > Grapefruit Dry Leaves

Table 1. Percentage Yield of *Citrus* Essential Oils

Citrus species	Raw Material Input	Time Extract (Minutes)	Oil Volume(mL)	Productivity
1 Essential oils	(g)			(mL/2000g) %
2 Grapefruit	2000	215	9.0	0.45
3 Grapefruit fresh leaves	2000	210	6.6	0.33
4 Grapefruit dry leaves	2000	235	6.0	0.30
I5 Grapefruit pulp	2000	230	4.5	0.25

Yield of Citrus EO by Hydro Distillation

The yield of citrus oils after 3 to 4 hours of extraction ranged from 0.25% to 0.45%, which was comparable and in some cases even better than other extraction methods documented in the literature [21]. C. paradisi was found to have the largest production of any citrus essential oil that could be determined.

The output of essential oil was 0.14% and that of tangerine essential oil was 0.25%, per the earlier research [21]. There have been reports of essential oil yields ranging between 0.1-0.43% from different grapefruit varieties. Different factors such as weather conditions, soil types, and extraction techniques contribute to varying essential oil production [16].

3.4. Physicochemical Characterization

In the current study, specific gravities of citrus essential oils ranged from 0.839 to 0.857, their refractive indexes ranged from 1.456 to 1.471, and all the essential oils were discovered to be optically active (Table 2).

Table 2. Physicochemical Properties of *C. paradisi*, Grapefruit Pulp, Grapefruit Fresh Leaves, and Grapefruit Dry Leaves

Sr. no.	Parameters Mandarin	Grapefruit	Grapefruit Pulp	Grapefruit Fresh Leaves	Grapefruit Dry Leaves
1	Color	Yellow	Light yellow	Light yellow	Pale yellow
i2	Odor	Pleasant	Less Pleasant	Less Pleasant	Pleasant,
		Extreme	Extreme	Extreme	Extreme
	Refractive index	1.456	1.471	1.467	1.471
3	Optical rotation	+85	+90	+92	+88
4	Specific gravity	0.857	0.839	0.848	0.845
5	Iodine value (g 100 g^{-1})	39.99	35.34	87.14	48.69
6	Peroxide value (M/kg^{-1})	18	14	20	16
7	Saponification value (mgKOH^{-1})	155.49	161.43	208.37	198.02
8			Solubility		
	Water	Fairly Soluble	Insoluble	Insoluble	Insoluble
	Ether	Soluble	Soluble	Soluble	Soluble
	Acetone	Fairly Soluble	Fairly Soluble	Fairly Soluble	Fairly Soluble

These outcomes are consistent with earlier research on these essential oils. Table 2 shows the physiochemical parameters of grapefruit fresh leaves, grapefruit dry leaves, and pulp sample. The oil sample of grapefruit pulp and dry leaves shows low iodine values, expect fresh leaves. This indicates that it contains maximum saturated fats which are not good from a health perspective. The peroxide value of all samples was higher which showed greater chances of oxidation. This was also proved by the rancidity test that showed a red color layer due to oxidation. Grapefruit fresh leaves sample showed no rancidity, although due to a large storage time its peroxide value was also high.

3.5. Chemical Composition by GC-MS Analysis

It was determined that the essential component of citrus leaf oils is limonene. The highest quantity of limonene (86.84%) was found in grapefruit essential oil (GC-MS analysis of five citrus leaf oils), followed by other essential oils. Linolenic oxide was another chemical component found. Of all the citrus essential oils, α-terpineol (13.10%) was the second most common component, with grapefruit oil having the greatest concentration (Table 3).

Table 3. Chemical Constituents of *Citrus* Oils (GC-MS Analysis)

Sr. No.	Constituents	Retention Time	Compounds	Composition
1	Grapefruit	6.215	Limonene	86.84
		8.967	α-terpineol	13.16
		10.683	Eugenol	0.68
2	Grapefruit pulp	11.517	Spathulenol	0.56
		10.486	Caryophyllene oxide	0.89
		13.935	n-Hexadecanoic acid	0.87
		7.245	Limonene	89.94
3	Grapefruit fresh leaves	8.771	α-terpineol	10.66
		7.216	Limonene	86.45
4	Grapefruit dry leaves	8.355	Limonene oxide	6.88
		9.086	Carveol	8.97
		8.793	α-terpineol	13.55

Similarly, according to Espina et al. [22] caryophyllene oxide (0.89%), spathulenol (0.56%), n-hexadechanoic acid (0.87%), and limonene (89.94%) are present in grapefruit pulp oil. Further, α-terpineol (10.66%) was the second most important component in grapefruit fresh leaf oil, behind limonene (86.45%) (Table 3). The majority of the substances found in citrus leaf oils were discovered to be naturally occurring hydrocarbons [23]. The major constituents of *Citrus* leaf oils were investigated by (Vekiari et al., 2002). Previous research revealed that grapefruit dry leaf essential oils included α-terpineol (13.55%), limonene oxide (6.88%), and carveol (8.97%), in addition to limonene [24, 25]. It is believed that the various chemical components of various citrus species may result from various genetic traits.

4. CONCLUSION

The current results showed that fresh and dried leaves (45°C, 60°C) of grapefruit have promising antioxidant capacity; as a result, their essential oils are priceless. Many studies have reported the health benefits of grapefruit essential oils. Nonetheless, little research has been conducted on the bioactivities of grapefruit essential oil that is hydro-distilled and has an exceptional potential for usage in biomedicine. Wastes from fresh and processed grapefruit leaves are a natural source of beneficial bioactive flavonoids, primarily naringin, which could be used as medicinal agents or as food additives in pharmaceutical techniques. Lastly, *in vitro* cytoprotection of fresh and oven-dried (45°C) grapefruit leaves suggests their potential usage for these natural extracts. Nevertheless, more investigation into action mechanisms, clinical trials, and dose-effect is required.

CONFLICT OF INTEREST

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVAILABILITY STATEMENT

Data availability is not applicable as no new data was created.

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