

CHARACTERIZATION OF ESSENTIAL OIL OF GRAPEFRUIT (*CITRUS PARADISI*) BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) AND ITS ANTIOXIDANT ACTIVITY BY 2,2-DIPHENYL-1-PICRYL-HYDRAZYL FREE RADICAL

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ABSTRACT

The essential oil from the fresh peels of grapefruit (*Citrus paradisi*) was extracted through the hydro-distillation method by using Dean Stark apparatus. The extracted essential oil was dried by using anhydrous sodium sulfate, resulting in a 0.2% yield. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was conducted to identify the constituents present in the essential oil. The study showed presence of Limonene 88.15%, 5-Caranol, (1S,3R,5S,6R)-(-) 3.04%, 2-Furanmethanol 5-ethenyltetrahydro- α,α ,5-trimethyl-,cis- 1.44%, 1,6-Octadien-3-ol,3,7-dimethyl- 1.43%, Caryophyllene 1.03%, 2-Isopropenyl-5-methylhex-4-enal 0.98%, Terpinen-4-ol 0.94%, Naphthalene 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1a,4a β ,8a α)]- 0.60%, ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl 0.57%, 2-Naphthalenemethanol 1,2,3,4,4a,5,6,7-octahydro- α,α ,4a,8-tetramethyl-, (2R-cis)- 0.52%, n-Hexadecanoic acid 0.51%, alfa-Copaene 0.31%, 9,12-Octadecadienoic acid (Z,Z)- 0.27%

and D-Limonene 0.11%. The antioxidant activity of essential oil was determined by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical and it was concentration dependent. The antioxidant activity of 56.5% was observed at a concentration of 100 μ L of essential oil.

KEYWORDS: Grapefruit; Peel; Hydrodistillation; Essential oil; GC-MS; Antioxidant; DPPH.

INTRODUCTION

Grapefruit (*Citrus paradisi*) belongs to the *Rutaceae* family which is commonly recognized as the Citrus family. The *Rutaceae* family stands as the largest fruit family comprising 158 genera and 2100 species (Appelhans *et al.*, 2021). Pakistan is producing different varieties of citrus fruits. These citrus fruits have been divided into various groups. These groups include Oranges, Lemon, Grapefruit, and Mandarin (Butt *et al.*, 2004; Cheema & Jamali, 2020). In Pakistan, it is known as “Chakotrah”. Pakistan is an agricultural country and it produces 116 million tons of total crops of agriculture, involving a total fruit production of 7.01 million tons and the output of citrus fruit amounts to around 2.4 million tons constituting 35.2% of total amount of fruit produced. The grapefruit contributes only 0.1% of the total citrus produced in Punjab province (Siddique & Garnevska, 2018).

The grapefruit is an abundant reservoir of various bioactive components including limonene, ascorbic acid, pectin, flavonoids, phenolic contents, and organic acids. These components have a significant part in facilitating potential well-being benefits because of having nutritional, antiallergic, anticarcinogenic, antioxidant, and antimicrobial characteristics (Kawaii *et al.*, 1999). After the utilization of citrus fruits, the peels from the citrus fruits are produced in large amounts, these peels are discarded as agricultural and industrial waste. They provide a way to recycle discarded waste as a source of producing oils. On the industrial scale, after the utilization of the fruit the flavonoids, pulp, pectin, and other byproducts are also produced that enhance the worth of grapefruit.

The peel is recognized as a natural substance that has the potential to serve as an economic and exceptional source of antioxidants. After the processing and utilization of fruit, the peels of grapefruit play a substantial part in the formulations of health supplements or act as pharmacological substances in a variety of both in vivo and in vitro therapeutic applications (Castro-Vazquez *et al.*, 2016). Over the recent years, the need for the plant's essential oils has enhanced because of their recognized sustainability and pharmacological attributes. These

properties included anti-inflammatory characteristics, bacteriostatic effects, free radical scavenging, and inhibiting malignant tumors or cancer-causing cell proliferation (Burt, 2004; Edris, 2007; Sahay, 2015; Shaaban *et al.*, 2012).

The essential oils are broad natural systems that are comprised of primarily terpenes along with numerous other non-terpene components (Edris, 2007). The essential oils derived from peels of citrus fruits contribute about one to third of the worldwide production of essential oils (Li *et al.*, 2021) and are extensively utilized in perfume, beauty products, medicinal, and foodstuff industries (Fisher & Phillips, 2009; Hardin *et al.*, 2010; Kaur & Kaur, 2015). They are extracted by various methods including cold pressing, hydrodistillation, solvent extraction, expression, supercritical carbon dioxide extraction, and maceration (Ashurst, 2012; Njoroge *et al.*, 2006; Venkateshwarlu & Selvaraj, 2000). Factors that affect composition of essential oil included storage conditions, ripening of fruits, soil quality, climate and geography, extraction methods, varieties and species of plant used, and cultivation process (Poonkodi, 2016). The evaluation of natural sources of antioxidants has been increased because of the removal of synthetic antioxidants from the various applications of food (Iqbal, 2018). Over the past few decades, various studies have been conducted to show that the waste of citrus fruits can serve as a natural reservoir of antioxidants that provide a way to utilize these byproducts (Bocco *et al.*, 1998; Llorach *et al.*, 2003; Manthey & Grohmann, 2001; Wolfe *et al.*, 2003). The essential oil of grapefruit are extracted by hydrodistillation method which is then further characterized by GC-MS to elaborate constitutes. This study shows the presence of major contents of limonene, d-limonene, terpinene-4-ol, n-hexadecanoic acid, etc.

Studies have been conducted on the extraction of essential oils from peels of citrus fruits and determining their chemical constituents by GC-MS. The several characteristics of essential oils including antioxidant, anticancer, antifungal, and antibacterial activity were also investigated (Achimón *et al.*, 2022; Deng *et al.*, 2020; Okunowo *et al.*, 2013; Saleem *et al.*, 2008; Shahzad *et al.*). The current research was designed to extract the essential oil from the discarded peels of grapefruits and determine its chemical composition by GC-MS and antioxidant activity by DPPH free radical.

MATERIALS AND METHOD

Collection of Material

The fresh grapefruits were collected from the local fruit market of Lahore division, Punjab Pakistan.

Chemicals

The chemicals included anhydrous sodium sulphate, methanol and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) were of analytical grade and purchased from the local market of Lahore Division, Punjab Pakistan.

Extraction of Essential oil

Fresh peels of grapefruit were separated manually and weighed 3.2 kg. These peels were chopped into small pieces by using a knife and then subjected to the hydrodistillation to extract essential oil by using a Dean-Stark apparatus for a duration of 10 hours (Shahzad *et al.*, 2009). Essential oil was separated in a separating funnel and then dried by using anhydrous sodium sulphate, which yields a light yellow-colored oil after the removal of solvent. The oil was stored in an airtight bottle at 4°C in the fridge for further studies.



Cutting of Peels



Hydrodistillation Method



Storing Essential oil

GC-MS studies of essential oil

The analysis was carried out by using an Agilent 5973-6890 gas chromatograph-mass spectrometry system that was equipped with a split-splitless injector and operated in EI mode at 70 eV. The helium was used as carrier gas at a flow rate of 1 ml/min. A DP-5MS (30 m × 0.25 mm internal diameter, 0.25 µm film thickness) capillary column was utilized.

The injector temperature was set at 220°C. The initial oven temperature was at 60°C with a hold time of 4 min which was programmed to increase to 260°C at the rate of 10°C/min. A sample volume of 2 µL was injected into the column. Other MS operating parameters included quadrupole temperature 120°C, ion source temperature 230°C, solvent delay 4 minutes, and scan range 50-700 amu. Interpretation of potential components was done by comparing their retention indices, and mass spectra with those in the library of the National Institute of Standards and Technology (NIST).

Antioxidant activity of essential oil

The antioxidant activity of grapefruit's essential oil was analyzed by DPPH free radical by using the method given by (Dawidowicz *et al.*, 2012). Various concentrations of essential oil specifically 25µL, 50µL, 75µL, and 100µL, were mixed with 3 ml methanol-containing DPPH solution. Then, after 30 minutes of incubation at room temperature, the absorbance of resulting solutions and blank (comprising only DPPH) was measured at a wavelength of 517 nm utilizing a UV-Vis spectrophotometer (Shimadzu UV-1700 Pharma-Spec).

Percentage inhibition of essential oil of grapefruit was determined by using the equation below.

$$\% \text{ Inhibition (DPPH)} = \frac{\text{Absorbance of DPPH solution} - \text{Absorbance of sample}}{\text{Absorbance of DPPH solution}} \times 100$$

RESULTS AND DISCUSSIONS

Chemical Composition of Essential Oil by GC-MS

The essential oil extracted from the fresh peels of grapefruit was light yellow colored and 0.2% yield of oil was obtained which was lower than the yield reported by (Uysal *et al.*, 2011), (Ahmad *et al.*, 2006) and (Ahmad *et al.*, 2016) that might be due to the difference in the variety of fruit, soil type, environmental and harvesting conditions.

The chemical composition of the essential oil of grapefruit was determined by GC-MS which showed the presence of various volatile components. The essential oil of grapefruit was found to contain 14 different components that are represented in Table 1.

Table 1: Chemical Composition of Essential Oil.

Peak #	Components	Retention time(min)	%age
1	Limonene	4.889	88.15
2	D-Limonene	5.948	0.11
3	2-Furanmethanol, 5-ethenyltetrahydro- $\alpha,\alpha,5$ -trimethyl-,cis-	6.762	1.44
4	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate	6.822	0.57
5	1,6-Octadien-3-ol, 3,7-dimethyl-	6.904	1.43
6	Terpinen-4-ol	7.433	0.94
7	5-Caranol, (1S,3R,5S,6R)-(-)-	7.783	3.04
8	2-Isopropenyl-5-methylhex-4-enal	8.551	0.98
9	alfa-Copaene	9.453	0.31
10	Caryophyllene	10.056	1.03
11	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,[1S-(1a,4a β ,8a α)]-	11.278	0.6
12	2-Naphthalenemethanol,1,2,3,4,4a,5,6,7-octahydro- $\alpha,\alpha,4a,8$ -tetramethyl-, (2R-cis)-	12.572	0.52
13	n-Hexadecanoic acid	16.411	0.51
14	9,12-Octadecadienoic acid (Z,Z)-	17.770	0.27

These components represented presence of six different groups of hydrocarbons in essential oil including monoterpenes, sesquiterpenes, oxygenated monoterpenes, monoterpenoids, saturated fatty acids and polyunsaturated fatty acid. The monoterpenes account for 90.18% that include Limonene, D-limonene, Terpinen-4-ol, and 2-Isopropenyl-5-methylhex-4-enal. The oxygenated monoterpenes comprises 3.44% that include 2-Furanmethanol, 5-ethenyltetrahydro- $\alpha,\alpha,5$ -trimethyl-,cis- which is commonly known as cis- Linalool oxide, 1,6-Octadien-3-ol, 3,7-dimethyl- known as linalool and Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate. The monoterpenoids makeup 3.04% which include 5-Caranol, (1S,3R,5S,6R)-(-)-. Naphthalene 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,[1S-(1a,4a β ,8a α)]- which is commonly known as β -Cadinene, Caryophyllene, alfa-Copaene, and Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,[1S-(1a,4a β ,8a α)]- constitute 2.46% sesquiterpenes. While n-hexadecanoic acid known as palmitic acid constitutes 0.51% saturated fatty acid, and 9,12-Octadecadienoic acid (Z, Z)- called linoleic acid constitutes 0.27% polyunsaturated fatty acid.

Among these components, limonene 88.15%, 5-Caranol,(1S,3R,5S,6R)-(-)- 3.04%, 2-Furanmethanol, 5-ethenyltetrahydro- $\alpha,\alpha,5$ -trimethyl-,cis- 1.44%, 1,6-Octadien-3-ol, 3,7-dimethyl- 1.43%, and Caryophyllene 1.03% were the major components found in the

essential oil of grapefruit. While other components found in essential oil having percentage less than 1% were 2-Isopropenyl-5-methylhex-4-enal, Terpinen-4-ol, Naphthalene 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1a,4a β ,8a α)]-, ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate, 2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydro- $\alpha,\alpha,4a,8$ -tetramethyl-, (2R-cis)-, n-Hexadecanoic acid, α -Copaene, 9,12-Octadecadienoic acid (Z, Z)- and D-Limonene. Various studies showed the makeup of essential oils extracted from citrus peels, with constituents such as limonene, copaene, D-limonene α -Caryophyllene, etc. (Okunowo *et al.*, 2013; Uysal *et al.*, 2011) that are similar to reported results.

The GC-MS mass spectra of different components of essential oil were obtained and have been elaborated as follows.

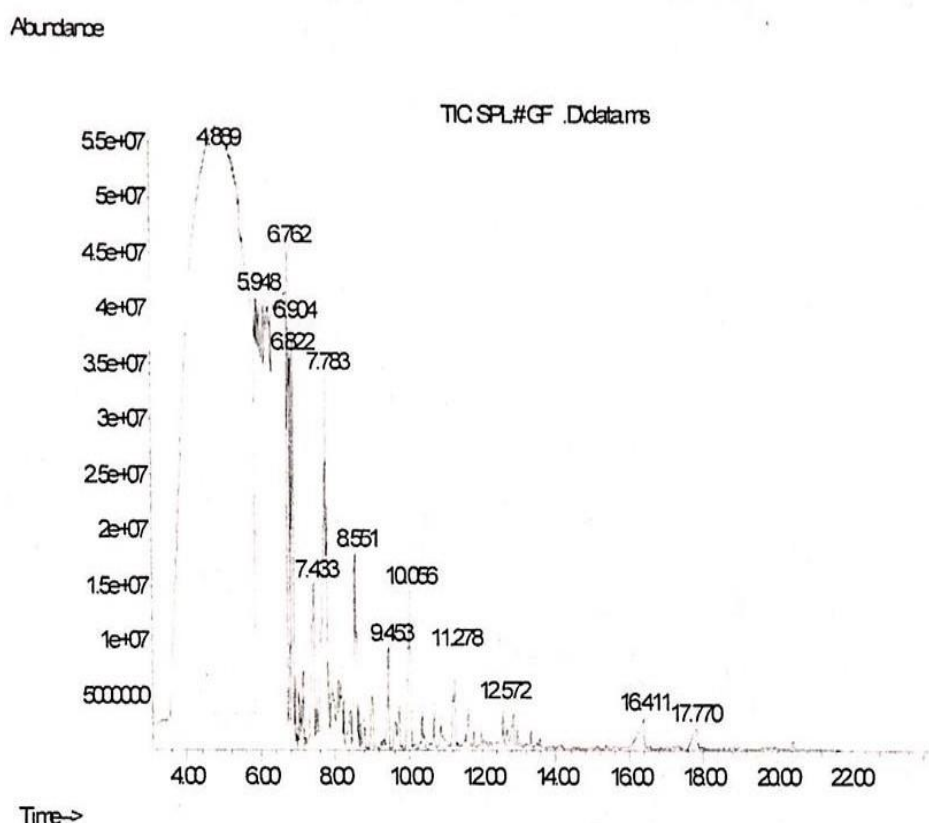


Fig 1: GC-MS Spectra of Essential oil of grapefruit (*Citrus paradisi*). Retention time corresponds to (4.889) Limonene, (5.948) D-Limonene, (6.762) 2-Furanmethanol, 5-ethenyltetrahydro- $\alpha,\alpha,5$ -trimethyl-,cis-, (6.822) Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-yl carbonate, (6.904) 1,6-Octadien-3-ol, 3,7-dimethyl-, (7.433) Terpinen-4-ol, (7.783) 5-Caranol, (1S,3R,5S,6R)-(-)-, (8.551) 2-Isopropenyl-5-methylhex-4-enal, (9.453) α -Copaene, (10.056) Caryophyllene, (11.278)

Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, [1S-(1a,4a β ,8a α)]-, (12.572) 2-Naphthalenem- ethanol, 1,2,3,4,4a,5,6,7-octahydro- $\alpha,\alpha,4a,8$ -tetramethyl-, (2R-cis)-, (16.411) n-Hexadecanoic acid and (17.770) 9,12-Octadecadienoic acid (Z,Z).

Antioxidant activity of Essential oil

The antioxidant activity of the essential oil is because of the presence of greater concentrations of phenolic contents including limonene, d-limonene, terpinen-4-ol, etc. These phenolic contents can neutralize free radicals that can harm the body, thereby lowering the risk of various diseases like cancer, Alzheimer's disease, and heart disease (Aadil *et al.*, 2013). Because they possess a high redox potential, thus they can act as radical scavengers as well as hydrogen donors (Corzo-Martínez *et al.*, 2007). The results elaborated that essential oil having a concentration of 100 μ L showed % inhibition of 56.5, a concentration of 75 μ L showed % inhibition of 46.8, concentration of 50 μ L showed % inhibition of 38.9 and concentration of 25 μ L showed % inhibition of 29.5 as shown below graph.

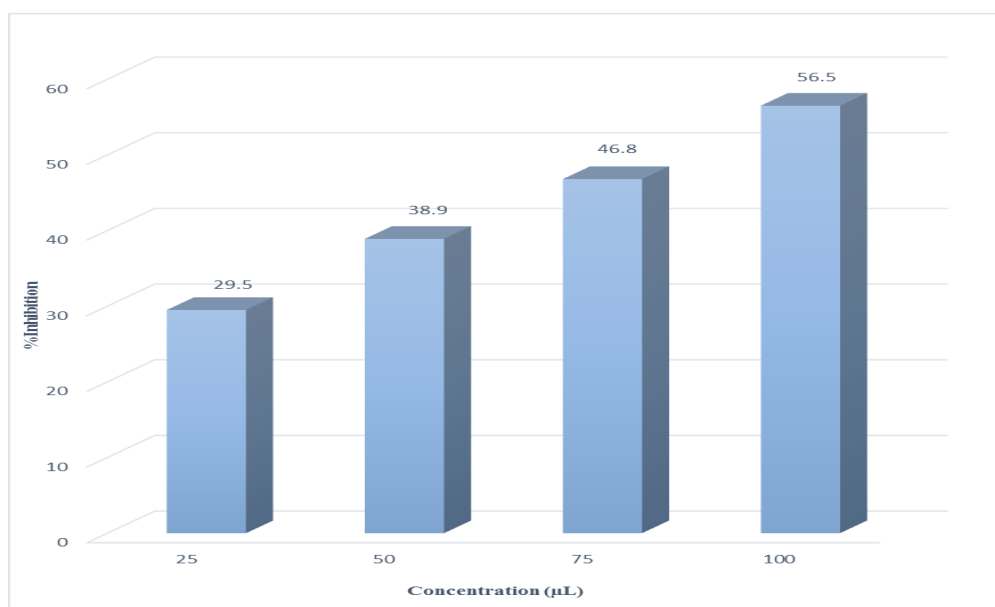


Fig 2: Antioxidant activity of Grapefruit Essential oil.

Literature showed that the antioxidant potential is directly proportional to the phenolic content, as their effect is concentration-dependent. The essential oil extracted from the peels of grapefruit possesses greater antioxidant activity because the phenolic contents are present in larger amounts as compared to the other parts of pulp and seeds (Ghasemi *et al.*, 2009; Molina-Quijada *et al.*, 2010; Sir Elkhatim *et al.*, 2018). Studies have demonstrated a strong

correlation between the phenolic content and antioxidant potential of fruits and vegetables (Li *et al.*, 2006). The results were higher than the results reported by (Vincenzo *et al.*, 2018) which showed % inhibition of 35.25% and 46.08% for the star ruby variety of grapefruit and the marsh variety of grapefruit respectively. These results were similar to those reported by (Iqbal, 2024; Jang *et al.*, 2010; Xi *et al.*, 2015; Zhang *et al.*, 2018) who investigated that the peels of various varieties of citrus fruits possess higher antioxidant activity as compared to that of pulp because of higher phenolic content.

CONCLUSION

The essential oil from peels of grapefruit (*Citrus paradisi*) was extracted through hydrodistillation. The extracted essential oil was dried by using anhydrous sodium sulfate, resulting in 0.2% yield. GC-MS was conducted to identify the constituents of essential oil. The major constituents were Limonene 88.15%, 5-Caranol,(1S,3R,5S,6R)-(-) 3.04%, 2-Furanmethanol 5-ethenyltetrahydro- α,α ,5-trimethyl-,cis- 1.44%, 1,6-Octadien-3-ol,3,7-dimethyl- 1.43%, and Caryophyllene 1.03%. Its antioxidant activity was determined by DPPH free radical. The antioxidant activity of 56.5% was observed at a concentration of 100 μ L of essential oil. So, it can be used as a natural source of antioxidants for therapeutic purposes.

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