

Research Article

Methanolic extracts of grapefruit (*Citrus paradisi*) peel, pulp and seed and their antioxidant activity

Zafar Iqbal^{a,*}, Amna Shaheen^b, Aisha Naz^b, Mian Habib ur Rehman Mahmood^b, Abeera Zafar^c, Muhammad Khalid Saeed^d

^aApplied Chemistry Research Centre, PCSIR Labs. Complex, Lahore 54600, Pakistan

^bDepartment of Chemistry, University of Education, Township, Lahore 54770, Pakistan

^cDepartment of Pharmacy, Hujvery University, Lahore 54660, Pakistan

^dFood & Biotechnology Research Centre, PCSIR Labs. Complex, Lahore 54600, Pakistan

Abstract

Fresh grapefruits (*Citrus paradisi*) were collected from the vegetable market of Lahore Division, Punjab Pakistan. Their peel, pulp, and seed were subjected to drying and grinding which was followed by extraction with methanol. These methanolic extracts were further investigated to check their antioxidant activities by using 2,2-diphenyl-1-picryl-hydrazyl (DPPH). The antioxidant activities showed that the grapefruit peel methanolic extract with concentrations of 25 μ L, 50 μ L, 75 μ L, and 100 μ L has DPPH inhibition 73.6%, 76.4%, 78.8%, and 82.7% respectively. While the grapefruit pulp methanolic extract having concentrations 25 μ L, 50 μ L, 75 μ L, and 100 μ L has DPPH inhibition 60.3%, 67.3%, 73.6%, and 78.9% respectively and the grapefruit seed methanolic extract with concentrations 25 μ L, 50 μ L, 75 μ L, and 100 μ L has DPPH inhibition 59.1%, 65.7%, 71.5%, and 75.9% respectively. The results of these extracts were concentration-dependent.

Keywords:

Grapefruit, Peel, Pulp, Seed, Methanolic Extract, Antioxidant activity, DPPH.

1. Introduction

Grapefruit (*Citrus paradisi*) belongs to the Rutaceae family and it is produced by a natural cross between sweet orange (*Citrus sinensis* L. Osb.) and pummelo (*Citrus grandis* L.) [1, 2]. Its tree grows to a height of 5.6-6 m and the fruit has a diameter of approximately 15cm. The fruit is protected by a peel that demonstrates 11-14 segments, or carpels, a sheet of membrane covers each segment or carpel and each carpel contains seeds and juice sacs [3]. In 2019, world grapefruit production was 9504.1 thousand tonnes from which Asia contributed 7115.6 thousand tonnes while Pakistan and some other countries contributed 21.8 thousand tonnes [4]. In Pakistan, 95% of the total production of citrus fruits is produced by the province of Punjab from which grapefruit cultivation over five thousand hectares constitutes only 0.3% of the overall citrus production. It is predominantly grown in the subtropical regions of central Punjab, including, Sahiwal, Layyah, Sargodha, Toba Tek Singh

and Khanewal. The main commercially cultivated grapefruit varieties are Foster Pink (seedy) and Shamber (seedless). Its harvesting commences in September-October, while the fruit is still green and the flesh color is not fully matured [1, 2].

Grapefruit is a rich source of nutrient, bioactive components and phytochemicals. The components include vitamin C, polyphenols, lycopene, fiber and pectin [5]. These components play a significant role in providing potential health benefits because of their nutritional, antiallergic, antioxidant, anticarcinogenic and antimicrobial properties [6].

Methanolic extraction is carried out to extract bioactive components from grapefruit seed, pulp and peel to determine their antioxidant activities [7]. Antioxidants play a crucial role in safeguarding lipids and oils in food from oxidative degradation. When incorporated into food, antioxidants manage the onset of rancidity, slow down the generation of harmful oxidation byproducts, preserve nutritional quality, and prolong the shelf life of products. The natural and inherent antioxidants found in spices contribute to mitigating oxidative stress. This form of stress, resulting from elevated levels of free radicals in tissues and cells, can be triggered by diverse factors, including X-ray,

*Corresponding Author:

zafarmayo2000@yahoo.com (Zafar Iqbal)

gamma and UV radiation, contaminated food, vigorous physical activity, alcoholism, psychological stress, smoking, and adverse environmental conditions [8].

The significance of natural antioxidants lies in their ability to neutralize harmful free radicals in the body. These compounds play a crucial role in protecting cells from oxidative stress, which is linked to various chronic diseases and aging processes. Consuming a diet rich in natural antioxidants, found in fruits, vegetables, and other whole foods, can contribute to overall health by reducing the risk of oxidative damage and promoting well-being [9].

Several studies have been reported to investigate the antioxidant properties in edible parts and juice of different origin and varieties oranges [10–12]. Research has been conducted on citrus peel extracts to investigate their phytochemical and antioxidant profile [13]. The antioxidant activity of extract obtained from grapefruit seed on vegetable oils was studied [14]. The phenolic profile and antioxidant activity of some citrus fruits was also determined [15]. The purpose of this research is to obtain the methanolic extract of grapefruit seed, peel, pulp and to contribute valuable insights into the characteristics and efficiency of these extracts as antioxidant and enhance our understanding of their potential benefits.

2. Materials and Methods

2.1. Collection of Material

The fresh grapefruits were collected from the vegetable market of Lahore Division, Punjab Pakistan. Their peel, pulp and seed were separated, kept under the shade and dried for 21 days.

2.2. Chemicals

Both the reagents were of analytical grade and purchased from local market of Lahore Division, Punjab Pakistan. These include methanol and 2,2-diphenyl-1-picryl-hydrazyl (DPPH).

2.3. Methanolic Extraction

2.3.1. Grapefruit Peel

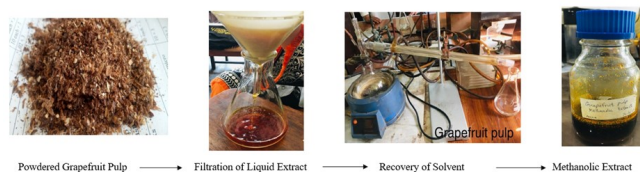
The peels of grapefruit were grounded to obtain powder by using grinder machine and passed through 500mm mesh. 100g from this powdered sample with 500ml of methanol were taken in 1000ml closed container. This mixture was allowed to macerate for 14 days with frequent shaking. After the maceration period, mixture was filtered with the help of filter paper to remove the solid residue from the liquid extract. Then, concentrated this liquid extract to evaporate the solvent by distillation method. These concentrates were then kept in air tight sample bottles and stored at room temperature.



Powdered grapefruit peel → Filtration of Liquid Extract → Recovery of Solvent → Methanolic Extract

2.3.2. Grapefruit Pulp

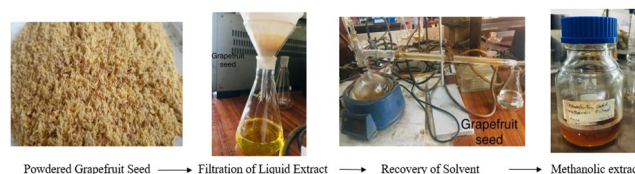
After drying, the pulp of grapefruit was pulverized using a grinder machine to obtain powder. A 100g portion of this powdered sample combined with 500ml of methanol in a 1000ml sealed container. The mixture left to macerate for 14 days with regular shaking. Following the maceration period, the mixture was filtered using filter paper to separate the solid residue from the liquid extract. Subsequently, the liquid extract was concentrated through distillation to evaporate the solvent. The resulting concentrates were then transferred to airtight sample bottles and stored at room temperature.



Powdered Grapefruit Pulp → Filtration of Liquid Extract → Recovery of Solvent → Methanolic Extract

2.3.3. Grapefruit Seed

Upon completion of the drying process, dried seeds were grounded into a powder using a grinder machine to increase their surface area. After which, 100 g portion of this powdered sample along with 500 ml of methanol was taken in a sealed 1000 ml container. The mixture underwent a 14-day maceration period with frequent shaking. After maceration, the mixture was filtered using filter paper to separate the solid residue from the liquid extract. The liquid extract was subsequently concentrated through distillation to recover the solvent. The resulting concentrates were transferred to airtight sample bottles and stored at room temperature.



Powdered Grapefruit Seed → Filtration of Liquid Extract → Recovery of Solvent → Methanolic extract

2.4. Antioxidant Activity of Methanolic Extracts

Antioxidant Activity of methanolic extracts of grapefruit were analyzed by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical by using the method described by [16]. The extracts with various concentrations of 25 μ L, 50 μ L, 75 μ L and 100 μ L were mixed with 3 ml of methanol containing DPPH solution. The absorbance of the resulting solution and the blank (containing only DPPH) was measured at a wavelength of 517nm using a UV-Vis spectrophotometer after a 30-minute incubation at room temperature. The percentage inhibition of these extract is determined by using following equation

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

3. Results

3.1. Antioxidant Activity of Grapefruit Peel Methanolic Extract

The methanolic extract of grapefruit peel showed high DPPH inhibition due to presence of high concentration of phenolic contents. These contents included a large number of volatile components e.g; limonene, ascorbic acid (vitamin C), anthocyanins, flavonoids etc. [17]. The results showed that the grapefruit peel methanolic extract with concentrations of 25 μ L, 50 μ L, 75 μ L and 100 μ L has DPPH inhibition of 73.6%, 76.4%, 78.8% and 82.7% respectively. These results have been shown in fig.(1)

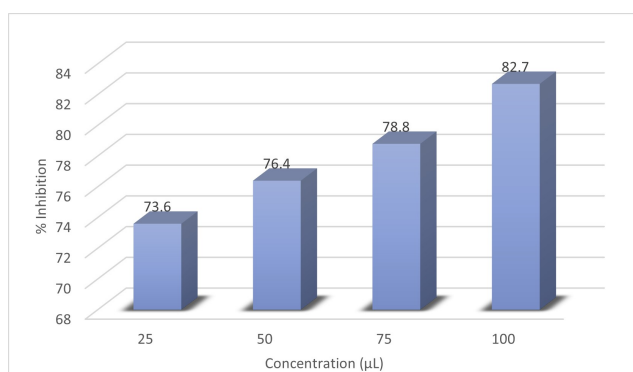


Figure 1: Antioxidant activity of Peel Methanolic Extract of Grapefruit

3.2. Antioxidant Activity of Grapefruit Pulp Methanolic Extract

The chemical composition of grapefruit pulp shows the presence of carotenoids e.g., *beta*-carotene, sugars e.g., glucose, fructose, sucrose, organic acids e.g., citric acid, quinic acid and volatile components e.g., d-limonene, caryophyllene and tert-butyl 2-methylpropanoate etc [18]. The antioxidant activity of methanolic extract of grapefruit pulp is due to the presence of the volatile components. Results showed that grapefruit pulp methanolic extract with concentrations of 25 μ L, 50 μ L, 75 μ L and 100 μ L has DPPH inhibition 60.3%, 67.3%, 73.6% and 78.9% respectively as shown in fig.(2)

3.3. Antioxidant Activity of Grapefruit Seed Methanolic Extract

The grapefruit seed contains a large number of bioactive components. These include fatty acids (linoleic acid, stearic acid, palmitic acid, and oleic acid etc), flavonoids (naringin, eriocitrin, naringenin, rutin, hesperidin, and kaempferol etc), phenolic acids (gallic acid, rosmarinic acid, syringic acid and *trans*-2-hydroxycinnamic acid etc), carotenoids (*beta*-carotene), and volatile components (d-limonene, fural, γ -Terpinene, and α -Terpinolene etc) [19]. The results of antioxidant activity showed that methanolic extract of grapefruit seed with concentrations 25 μ L, 50 μ L, 75 μ L and 100 μ L has DPPH inhibition of 59.1%, 65.7%, 71.5% and 75.9% respectively, as shown in fig.(3)

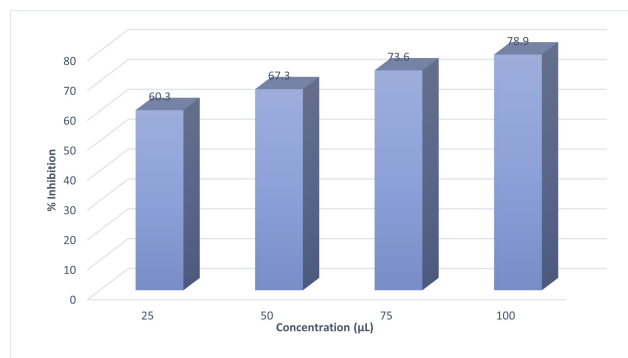


Figure 2: Antioxidant Activity of Pulp Methanolic Extract of Grapefruit

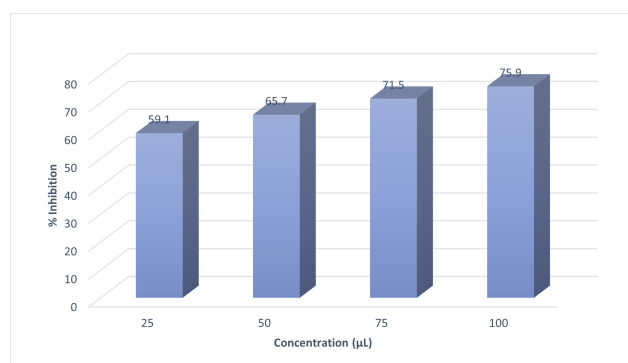


Figure 3: Antioxidant Activity of Seed Methanolic Extract of Grapefruit

4. Discussion

Literature reported that the antioxidant activity of fruits and vegetables is due to the presence of polyphenolic and phenolic compounds [20, 21]. The phenolic compounds that show antioxidant activity included ascorbic acid (vitamin C), anthocyanins, flavonoids. The antioxidant activity increases with the increase in the concentration of the phenolic and polyphenolic compounds as they were concentration-dependent. The grapefruit peel shows higher antioxidant activity than seed and pulp [17, 22, 23]. Because peels of grapefruit and other citrus fruits have larger amounts of polyphenols as compared to peeled fruits [24]. It was also reported that the dried peel extracts of lemon, orange and mandarin show higher antioxidant activity due to higher concentration of phenolic contents than the fresh ones [25–28]. Citrus fruits are abundant in phenolic acids, which exhibit varying degrees of free radical scavenging. The antioxidant activity of phenolic compounds is due to the presence of phenolic hydroxyl groups that readily donate a hydrogen atom or an electron to a free radical, and an extended conjugated aromatic system that facilitates the delocalization of an unpaired electron [29]. The relationship between overall phenolic concentration and antioxidant activity has been extensively investigated across various food items, including fruits and vegetables. Prior research has shown a direct correlation between phenolic content and antioxidant capacity in fruits and

vegetables [30]. The grapefruit methanolic extract exhibited DPPH values range from 73.6% to 82.7% in peel, 60.3 to 78.9% in pulp and 59.1 to 75.9% in seeds with concentration range from 25 μ L to 100 μ L in each sample. Their antioxidant activities were represented by order as follows: peel > pulp > seed. These results are similar to Jang, Chang et al. 2010 [31], who reported that peel of pummelo had higher antioxidant activity than its pulp when employing the DPPH method. It was because methanolic extract of grapefruit peel had higher concentration of phenolic content than pulp and seed methanolic extracts of grapefruit.

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