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Research Article

Antioxidant activity of methanolic extracts of sweet lime (*Citrus lemitta*) peel, pulp, and seed by 2, 2-diphenyl-1-picryl-hydrazyl free radical

Zafar Iqbal[a,*], Aisha Naz[b], Amna Shaheen[b], Mian Habib ur Rehman Mahmood[b], Muhammad Khalid Saeed[c]

[a]*Applied Chemistry Research Centre, PCSIR Labs. Complex, Lahore 54600, Pakistan*
[b]*Department of Chemistry, University of Education, Township, Lahore 54770, Pakistan*
[c]*Food & Biotechnology Research Centre, PCSIR Labs. Complex, Lahore 54600, Pakistan*

Abstract

Sweet lime (*Citrus lemitta*) fruits were collected from a local market in Lahore Pakistan, their peels, pulp, and seed were separated manually and dried under shade for 20 days. After drying, their extracts were prepared separately by immersing each component in methanol at a ratio of 1:5 (w/v) for 14 days at ambient conditions. The antioxidant activity of each extract was evaluated by using 2,2-diphenyl-1-picryl-hydrazyl radical. Butylated hydroxyl toluene was used as a standard, and the percentage inhibition was determined for each extract. The results indicate that peel methanolic extract with concentrations of 25μL, 50μL, 75μL and 100μL has DPPH inhibition of 70%, 74.3%, 79.1%, and 81.2% respectively. Pulp methanolic extract with concentrations of 25μL, 50μL, 75μL and 100μL have DPPH inhibition 65.3%, 69.7%, 74.1%, and 77.1% respectively. Seed methanolic extract with the same concentration has DPPH inhibition 46.2%, 49.7%, 54.6%, and 58.9% respectively. The antioxidant activity of all the extracts was concentration-dependent.

Keywords:
Sweet Lime, Peel, Pulp, Seed, Methanolic Extract, Antioxidant activity, DPPH.

1. Introduction

Citrus Lemitta belongs to the family Rutaceae, it has approximately 158 genera and 1900 species, which includes citrus [1]. In Punjab, Pakistan it is known as "Mitha". It is sweet, flavorful, full of juice and vitamin C. About 4% of the world´s citrus production comes from Pakistan, and its export contribution is merely 0.8% [2]. Citrus trees are grown almost everywhere in Pakistan, however, Punjab contributes the most to production, which is almost 70% [3]. Around the world, citrus is grown in more than 80 different countries and the most prominent producers are Pakistan, India, Egypt, and Palestine [4]. Ten species of the genus Citrus are found in Pakistan. Among them is *Citrus limetta* var. Mitha is sometimes known as sweet lime. It is a well-known native citrus fruit that is enjoyed in several regions of the subcontinent for its delicious culinary qualities in addition to its cooling and medicinal properties. Sweet lime juice has therapeutic benefit in treating fever, malaria, and jaundice according to the traditional indigenous medical system [5]. The fruit sweet lime is full in bioactive components like minerals, vitamin C, and phenolic compounds [6]. Ten species of the genus Citrus are found in Pakistan. One piece of sweet lime has total weight 159g, out of which seed, peel and pulp has 3.3g, 25g and 130.5g weight respectively. The endocarp and pericarp are the two distinct sections that make up a citrus fruit. The pericarp is made up of peel that has a lot of aromatic oil glands, which give the fruit its distinct aroma and gloss. After juice extraction, citrus fruits contribute significant amounts of non-edible residues up to 80%, which includes peel, pulp, and seeds and these are referred as "agri-wastes" [7]. After processing, the peel makes up between 30 and 40 percent of the entire citrus fruit mass that is discarded as trash. This peel can be used to make value-added goods and to fortify food items to improve the nutritional profile. If this trash is not used, it may create an unpleasant odor, contaminate the soil, serve as an insect colony, and seriously harm the ecosystem. Only the pulp of the citrus fruit is used in the juice industry, and a sizable portion of the peel and seed are thrown away as waste. Even though citrus peel has a high nutraceuti-

*Corresponding Author:
zafarmayo2000@yahoo.com (Zafar Iqbal)

cal value, it can be used as a functional ingredient in the form of powder for the food, pharmaceutical, and cosmetic industries to promote health. This is important because it promotes health and helps us make better use of the peel waste while also protecting our environment from pollution caused by waste citrus peel [8]. Methanolic extract of Sweet lime Peel, Pulp and Seed were prepared to examine their antioxidant activity. Supplying the body with antioxidants from external sources is crucial, as they help to lower the risk of heart disease and boost immunity. Oxidative stress can arise from free radicals, which include reactive oxygen species and reactive nitrogen species produced within our bodies. Maintaining a balance between free radicals and antioxidants is crucial for proper physiological function. Free radicals can negatively impact lipids, proteins, and DNA, contributing to various human diseases. Therefore, utilizing external sources of antioxidants can help mitigate oxidative stress. It's worth noting that synthetic antioxidants like butylated hydroxytoluene and butylated hydroxyanisole have recently been identified as posing potential risks to human health [9] The peel, comprising nearly half of the fruit mass, harbors the highest levels of flavonoids in Citrus fruits. Numerous studies have highlighted the presence of antioxidants in the juice and consumable sections of oranges, originating from various sources and varieties. Regarding the peel, extracts from this particular fruit component have demonstrated a notable overall radical antioxidative capacity [10]. The free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) method were employed to assess the antioxidant properties of both crude extracts and isolated compounds. In general, seeds exhibited superior antioxidant activity compared to peels. The composition analysis of all examined samples using HPLC revealed that methanol extracts were abundant in flavones and glycosylated flavanones, while hydrolyzed extracts primarily contained phenolic acids and flavonols. However, no discernible correlation was observed between antioxidant activity and the phenolic composition of the extracts [11]. *Citrus limetta* is a juicy fruit and after its juice extraction, other materials like peels, pulp and seeds are being discarded as waste. The significance of this study is to prepare methanolic extract to examine its antioxidant activity.

2. Materials and Methods

2.1. Collection of Material

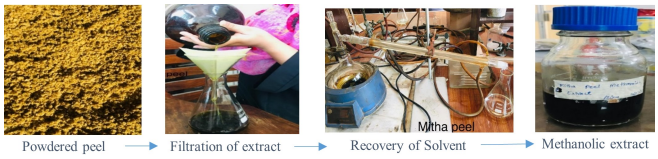
Sweet lime were collected from local vegetable market of Lahore, Punjab Pakistan. Their Peel, Pulp and Seed separated, kept under shade at ambient conditions and dried for 20 days.

2.2. Chemicals

Purchased following analytical grade chemicals from the market of Lahore, Punjab Pakistan for methanolic extraction and antioxidant activity of Peel and Pulp and Seed. Methanol, DPPH (2,2-diphenyl-1-picrylhydrazyl)

2.3. Methanolic Extraction

Methanolic extraction of Peel, Pulp and Seed were prepared.

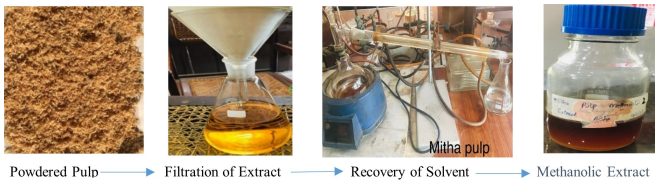


2.3.1. Methanolic extraction of Peel

Peels were dried and grounded into powder using a grinder machine to enhance the surface area and were passed through the 500mm mesh. 100g of peel powder were taken in a 1000ml sealed container and added 500 ml methanol in it. The mixture was macerate for 16 days with frequent shaking. After that filtered the mixture using filter paper to separate the solid residue from the liquid extract. The liquid extract was concentrated to eliminate the solvent through a distillation method. The resulting concentrates were transferred to airtight sample bottle and stored at room temperature.

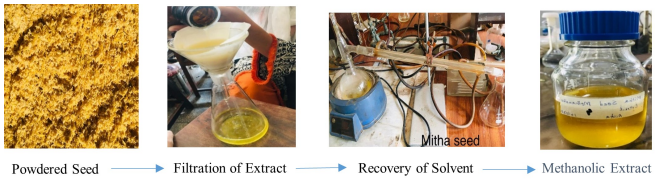
2.3.2. Methanolic extraction of Pulp

Grounded the dried pulp into powder form using a grinder machine. From this powder added 100g in to 500ml of methanol and put in a 1000ml airtight container. After maceration of 16 days filtered this mixture using filter paper to remove the solid residue from the liquid extract. Concentrated the liquid extract to eliminate the solvent through a distillation method. The resulting concentrates was transferred to airtight sample bottle and stored at room temperature.



2.3.3. Methanolic extraction of Seed

Seeds were dried, grounded into powder form to increase the surface area. 100g of this powder was taken, added into 500ml methanol and kept in a sealed jar. Macerated for 16 days, filtered the mixture using filter paper to separate the solid residue from the liquid extract. Concentrated the liquid extract through distillation to remove the solvent. Transferred the resulting concentrate to an airtight sample bottle and stored it at room temperature.



2.4. Antioxidant Activity of Methanolic Extracts

Antioxidant activity of methanolic extracts from sweet lime seed, peel and pulp was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical method, as outlined by [12]. The

methanolic extracts of sweet lime seed, peel, and pulp, at concentrations 25μL, 50μL, 75μL and 100μL, were combined with 3 ml of methanol containing DPPH solution. After 30-minutes incubation at room temperature, the absorbance of the resulting solution and the blank (containing only DPPH) was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. The percentage inhibition of these extracts is determined by the following equation

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

3. Results

3.1. Antioxidant Activity of Sweet Lime Peel

Peel methanolic extract has higher antioxidant activity due to the presence of phenolic content. Literature showed that the juices obtained from *citrus lemitta* peeled fuits had higher phenolic content such as limonene, linalool, β-myrcene and β-citronellol [6].Peel methanolic extract with concentration 25μL, 50μL, 75μL and 100μL have 70%, 74.3%, 79.1% and 81.2% DPPH inhibition respectively These results have been shown in fig.(1)

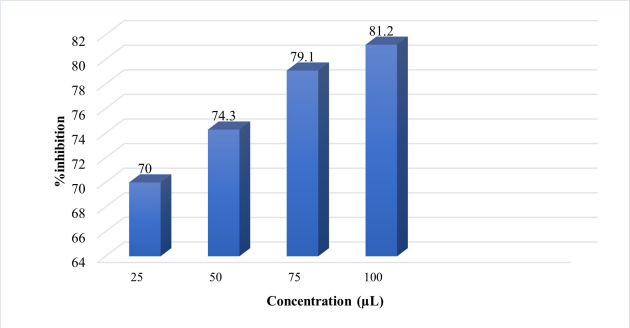


Figure 1: Antioxidant activity of Sweet Lime Peel Methanolic Extract

3.2. Antioxidant Activity of Sweet Lime Pulp

Only the pulp of the citrus fruit is used in the juice industry, and a sizable portion of the peel and seed are thrown away as waste. After juice extraction pulp residue is also thrown away. Methanolic extract of this pulp was prepared which showed DPPH inhibion of 65.3%, 69.7%, 74.1% and 77.1% at concentration of 25μL, 50μL, 75μL and 100μL respectively as shown in fig.(2)

3.3. Antioxidant Activity of Sweet Lime Seed

Citrus seed had limonoids, along with phenolic compounds like phenolic acids, flavonoids, tocopherols, and carotenoids. The positive impact of these components on human health was recognized. Citrus seed extracts had prominent phenolic acids such as caffeic, p-coumaric, and ferulic acids [13]. Al-Anbari et. al. [14] showed that seed ethanolic extract had higher quantities of phenolic content free radic al scavenging activity, total flavonoides, chelating Fe^{+2} ions, and the ability to scavenge

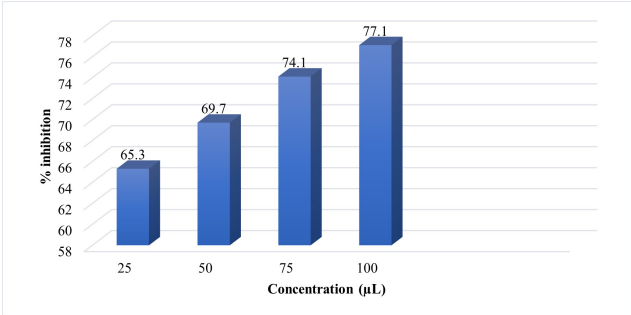


Figure 2: Antioxidant activity of Sweet lime Pulp Methanolic Extract

hydrogen peroxide. The results indicate that Seed methanolic extract with concentration 25μL, 50μL, 75μL and 100μL have DPPH inhibition 46.2%, 49.7%, 54.6% and 58.9% respectively, as shown in fig.(3)

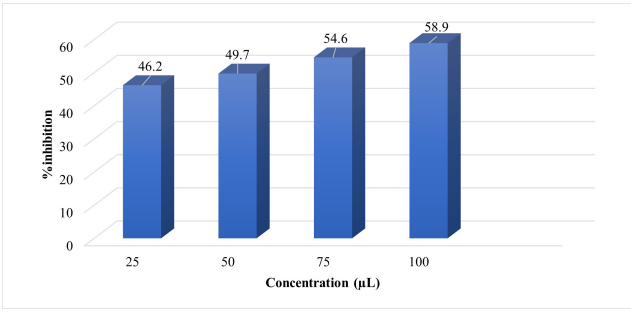


Figure 3: Antioxidant activity of Sweet lime Seed Methanolic Extract

4. Discussion

The existing literature highlights over 170 antioxidants were found in Citrus fruits, encompassing vitamins, phenolic compounds, mineral elements, terpenoids, and pectin [15]. Literature showed that the juices obtained from *citrus lemitta* peeled and unpeeled fuits had phenolic content of 109 mg/L and 88.9 mg/L respectively, while the antioxidant activities were 118 Mm Trolox/L and 93.2 Mm Trolox/L [6]. Li et. al. [16] revealed that grapefruit peels exhibit a higher total phenolic content than the peels of mandarin, yeb Ben lemon, orange, and meyer lemon. Especially the peels of lemon, orange, and grapefruit are rich in phenolic compounds and vitamin C, exhibiting a high rate of antioxidant activity. The correlation between total phenolic content and antioxidant activity, especially in the peels, was more pronounced compared to the alternative factor. A positive correlation between antioxidant activity and total phenolic content suggests that phenolics may play a significant role in the antioxidant capacities of these fruit residues. The analysis by Sir Elkhatim et. al.[17] demonstrated that peels contained elevated levels of phenolic compounds, flavonoids, vitamin C, and antioxidant activity compared to their inner discarded components including pulp and seeds. Among the peels,

grapefruit exhibited the highest total phenolic content, measuring 77.3 mg of gallic acid equivalent/g of peels, followed by lemon with 49.8 mg and orange with 35.6 mg. Vitamin C content of 113.3, 330.4, and 58.59 mg/100 g were identified in grapefruit, orange, and lemon peels respectively. However, the antioxidant activity of orange pulp and seeds was greater than that of grapefruit and lemon. According to Abeysinghe et. al.[18] and Goulas et. al.[19] the peel of citrus fruits is noted to possess a higher concentration of bioactive compounds as compared to the pulp. The ability of phenolic compounds to exhibit antioxidant activity arises from the existence of phenolic hydroxyl groups, which readily contribute a hydrogen atom or an electron to free radicals. Additionally, an extended conjugated aromatic system facilitates the delocalization of an unpaired Electron [20]. Results showed Sweet lime peel methanolic extract had higher activity of 81.2% while literature reported 755.78μM Trolex Equivalent (TE) [21]. Methanolic extract of sweet lime pulp showed antioxidant activity of 77.1% having concentration of 100μL while results reported that the orange pulp had 99.14mmol Trolox/g [22]. He also reported that sweet lime peel had higher antioxidant activity than pulp due to the presence of higher phenolic content [22]. While the sweet lime seed methanolic extract had antioxidant activity of 58.9% with concentration of 100μL at 517nm wavelength while literature showed that ethanolic seed extract of sour oranges had 60.4% which is nearly equivalent to our results [14]. As compared to other citrus fruits the antioxidant activity of *Citrus lemitta* can vary depending on fruit culture, area and growth conditions. Extraction method also effect both phenolic content and antioxidant activity of Sweet lime Peel, Seed, and Pulp.

References

[1] D. J. Mabberley, et al., Mabberley's Plant-book: a portable dictionary of plants, their classifications and uses., no. Ed. 3, Cambridge university press, 2008.

[2] A. Tahir, Forecasting citrus exports in pakistan, Pakistan Journal of Agricultural Research 27 (1).

[3] A. Nawaz, W. Ahmed, M. Jiskani, High density planting-an approach to increase citrus yields (2007).

[4] K. Chang, The evaluation of citrus demand and supply, Proceeding of International Society Citric. Italy 3 (1992) 1153–1155.

[5] M. M. Cowan, Plant products as antimicrobial agents, Clinical microbiology reviews 12 (4) (1999) 564–582.

[6] D. Topi, Volatile and chemical compositions of freshly squeezed sweet lime (citrus limetta) juices, The Journal of Raw Materials to Processed Foods 1 (1) (2020) 22–27.

[7] A. Zayed, M. T. Badawy, M. A. Farag, Valorization and extraction optimization of citrus seeds for food and functional food applications, Food Chemistry 355 (2021) 129609.

[8] M. Iftikhar, S. Wahab, N. ul Haq, S. N. Malik, S. Amber, N. U. Taran, S. U. Rehman, Utilization of citrus plant waste (peel) for the development of food product, Pure and Applied Biology (PAB) 8 (3) (2019) 1991–1998.

[9] Z. Iqbal, H. K. Mehmood, M. Hussain, M. Mehmood, M. N. Choudhry, Antioxidant activity of essential oil from the leaves and stems of murraya koenigii, World Journal of Pharmaceutical Research 6 (7) (2017) 267–273.

[10] N. J. Miller, C. A. Rice-Evans, The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink, Food Chemistry 60 (3) (1997) 331–337.

[11] A. Bocco, M.-E. Cuvelier, H. Richard, C. Berset, Antioxidant activity and phenolic composition of citrus peel and seed extracts, Journal of agricultural and food chemistry 46 (6) (1998) 2123–2129.

[12] A. L. Dawidowicz, D. Wianowska, M. Olszowy, On practical problems in estimation of antioxidant activity of compounds by dpph method (problems in estimation of antioxidant activity), Food chemistry 131 (3) (2012) 1037–1043.

[13] B. Falcinelli, F. Famiani, A. Paoletti, S. DÂ´Egidio, F. Stagnari, A. Galieni, P. Benincasa, Phenolic compounds and antioxidant activity of sprouts from seeds of citrus species, Agriculture 10 (2) (2020) 33.

[14] A. K. H. Al-Anbari, M. A. Hasan, Antioxidant activity in some citrus leaves and seeds ethanolic extracts, AABES London UK (2015) 93–7.

[15] Z. Zou, W. Xi, Y. Hu, C. Nie, Z. Zhou, Antioxidant activity of citrus fruits, Food chemistry 196 (2016) 885–896.

[16] B. Li, B. Smith, M. M. Hossain, Extraction of phenolics from citrus peels: I. solvent extraction method, Separation and Purification Technology 48 (2) (2006) 182–188.

[17] K. A. Sir Elkhatim, R. A. Elagib, A. B. Hassan, Content of phenolic compounds and vitamin c and antioxidant activity in wasted parts of sudanese citrus fruits, Food science & nutrition 6 (5) (2018) 1214–1219.

[18] D. Abeysinghe, X. Li, C. Sun, W. Zhang, C. Zhou, K. Chen, Bioactive compounds and antioxidant capacities in different edible tissues of citrus fruit of four species, Food chemistry 104 (4) (2007) 1338–1344.

[19] V. Goulas, G. A. Manganaris, Exploring the phytochemical content and the antioxidant potential of citrus fruits grown in cyprus, Food chemistry 131 (1) (2012) 39–47.

[20] J. Dai, R. J. Mumper, Plant phenolics: extraction, analysis and their antioxidant and anticancer properties, Molecules 15 (10) (2010) 7313–7352.

[21] O. K. Buyukkurt, G. Guclu, H. Kelebek, S. Selli, Characterization of phenolic compounds in sweet lime (citrus limetta) peel and freshly squeezed juices by lc-dad-esi-ms/ms and their antioxidant activity, Journal of Food Measurement and Characterization 13 (2019) 3242–3249.

[22] H. R. de Moraes Barros, T. A. P. de Castro Ferreira, M. I. Genovese, Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from brazil, Food chemistry 134 (4) (2012) 1892–1898.