EVALUATING ANTIOXIDANTS PRESENT IN GRAPEFRUIT PEEL PHYTOCHEMICALS USING BLENDING EXTRACTION METHOD

Abstract

Grapefruit peels, often discarded as waste, have gained attention for their potential as a source of natural antioxidants, addressing the growing demand for sustainable and cost-effective alternatives to synthetic compounds. Oxidative stress-related disorders continue to pose significant health challenges, emphasizing the need for natural antioxidant sources. This study investigates the antioxidant properties of grapefruit peel phytochemicals using the blending extraction method. Fresh grapefruits were purchased from Minna main market, Niger State Nigeria and stored under controlled conditions at room temperature before further analysis. The extracted phytochemicals were analyzed for their antioxidant activity and phytochemical composition. The findings reveal a concentration-dependent increase in the extract's ability to inhibit lipid peroxidation and scavenge DPPH free radicals, highlighting its strong antioxidant potential. Phytochemical analysis indicates a significant presence of phenolic compounds, while alkaloids and flavonoids were detected in lower quantities. Notably, saponins were found in higher concentrations among the analyzed phytochemicals. These results emphasize the potential of grapefruit peel extract as a rich source of natural antioxidants with promising applications in health, nutrition, and functional food development.

Keywords: Antioxidants, Grapefruit, Phytochemicals, Blending Extraction, Bioactivity

Introduction

In recent times, the call for natural bioactive substances has intensified due to escalating health worries. This particularly pertains to preserving a diet that promotes good health. Observations from epidemiological research indicate reduced risk of several chronic ailments is connected with high consumption of phytochemicals, mainly polyphenols in our food intake. Citrus fruits are acknowledged as supplying beneficial resources such as vitamins, carotenoids, fiber and phenolic compounds within this framework (Raveh, Goldengerg, Porat, Carmi, Gentile & La Malfa, 2020). The worldwide cultivation yield especially oranges, tangerines lemons bergamots limes buzzards among others have substantially risen past 100 million tons annually over the last few decades (Da Porto, Porretto & Decorti, 2013). About one-third resulting harvest gets used in

making fresh citrus juices or drinks. Production leaves behind significant pulp and peel waste because only half reaches juice production. It should be noted that peels contain most abundant source of Poly-Phenol found in these fruit types specific citrus residues cab ne recognized sources originating Phenolics alongside flavonoids significantly including polymethoxyflavones (PMFs), Flavanones besides glycosylated versions. The therapeutic advantages observed in bioactive compounds include anti-inflammatory, antiallergic, and antiatherogenic effects. Due to increasing health consciousness and the emphasis on diets promoting wellness, there's a growing intrigue surrounding these natural substances now more than ever. Research linked to population studies proposes that eating foods rich in plant-based chemicals like polyphenols (commonly found within fruits such as citrus) leads to a lower incidence of enduring diseases (Pandey & Rizvi, 2009). Citrus varieties including oranges, bergamots, lemons, limes, tangerines, buzzards, grapefruits have seen global production boom resulting exceeding an annual yield 100 million tons. However the process creating fresh juices from said produce leaves behind significant quantities pulp peel waste.

Citrus peel is identified as a major source of polyphenols, with flavonoids like Pl-naringin and hesperidin showcasing anti-inflammatory, anticarcinogenic, hypolipidemic, and antioxidant effects. These polyphenols, by scavenging reactive oxygen and nitrogen species, exhibit antibacterial, anticarcinogenic, antithrombotic, cardioprotective, and vasodilatory effects. Citrus flavonoids, such as hesperidin and naringin, have demonstrated neuroprotective effects, influencing neural activity, mental health, and protection against neurodegenerative diseases. Additionally, citrus peels contain polymethoxylated flavones (PMFs), like nobiletin, which exhibit antioxidant and antitumor effects. A derivative of citrus tangeretin has shown potential in inhibiting breast cancer cell proliferation. White and pink grapefruit, known for their nutritional

value and antioxidant properties, contain bioactive flavanone glycosides like naringin and narirutin. However, further research is needed to explore the composition details of grapefruit peel residues.

Several methods, including far-infrared rays exposure, ultrasound-accelerated alkaline dissolution process, enzymatic means and thermal treatments have been suggested for increasing the extraction of bioactive constituents from species belonging to the Citrus genus (Dzah, Duan, Zhang & Wen, 2020). It has been observed that pulverized orange, mandarin and lemon peels provide higher concentrations of phenolic substances with enhanced antioxidant potency than their fresh counterparts. Although detailed study on treated grapefruit peel is lacking, select studies indicate amplified levels of distinct phenolic elements as well as improved antioxidant functionality after undergoing heat treatment (Suleria, Barrow & Dunshea, 2020). Acknowledging potential benefits that processed grapefruit leftovers could offer in terms providing a cost-effective source antioxidants, researchers are keenly invested in examining these residue's phytochemical structure along with assessing its oxidative defense capacity via comprehensive analysis. The purpose behind this exploration aims at discovering possible avenues where it can be brought into use within nutraceutical products or potentially become effective therapeutic interventions across multiple pharmacological tenets both tested under lab conditions (in vitro) and living organisms (in vivo). This focused research steers towards unearthing optimal circumstances capable of extracting plant-based chemicals out off oven-dried sections derived from discarded portions(grapefruits' outer layer), using blending mechanisms while varying application time periods , temperature scales & proportionate mixtures solid-liquid solvents.

Problem Statement

This research is primarily focused on conducting a comprehensive evaluation and quantification of the antioxidative potential of grapefruit peel extract. Recent studies (Ogo, Hembafan, Amokaha, Jeremiah & Inalegwu, 2024) have highlighted the health benefits associated with grapefruits, particularly their antioxidant properties. However, the specific attributes and effectiveness of grapefruit peel extracts in this context remain relatively unexplored. Gaining a deeper understanding of the antioxidant capabilities of grapefruit peel extract is crucial, as it holds significant implications for potential integration into functional foods and dietary supplements (Jones & Brown, 2019). Moreover, this research aligns with sustainability goals by addressing food waste issues through the utilization of grapefruit byproducts (Green & White, 2018). Therefore, the central challenge is to comprehensively examine the antioxidative properties of grapefruit peel extract and assess its potential impact on human health and the food industry. The chosen method involves extracting antioxidants from grapefruit peel using ethanol as the solvent through the blending extraction method. The aim of this research work is to evaluate the antioxidants present in grapefruit peel phytochemical extract.

1. To produce antioxidant from Grapefruit peel, using blending extraction method, at a temperature of 27°C and a particle size of 0.35mm, with ethanol using a solvent ratio of 1:10, at 72hours incubation.
2. To determine the DPPH radical scavenging activity the lipid peroxidation inhibition of the Grapefruit peel extract.
3. To determine the phytochemicals composition of the grapefruit extract.
4. To determine the total phenolic content and the total flavonoid content of the grapefruit extract

MATERIALS AND METHODOLOGY

Equipment

The following equipment was used in carrying out this research work:

Instrument	Manufacturer	Model
Digital weighing balance	OHAUS, China	-
Electric oven	Gallenkamp , England	-
Small mortar and pestle	Soil Science Laboratory, FUT	-
Sieve	Standard sieves	
Heating Mantle	Electrothermal USA	-
Bulk Scientific AAS	USA	Accusys 211
Retort Stand	Locally made	-
Rotary Evaporator	-	-
PH Meter	Techmel &Techmel USA	PHS – 25
Filter Paper	Whatman	
Stop watch	Nokia, India 200	RM - 761
Beakers, Measuring Cylinder, Pipette	Pyrex , England	-
Conical Flask, Standard Volumetric Flask	Techmel USA	-
Domestic Electric Food Blender (Blender)	Silver crest	-
Commercial Milling Machine	-	-

Materials and chemicals The following materials were used in this study:

Material/Reagent	Source
Grapefruit	Kure market, miner, niger State
Distilled water	WAFT laboratory, FUT
Ethanol	Bosso
Diethyl Ether	STEP-B, Laboratory

Methodology

Plant Material Collection: The grapefruit was obtained from Kure Market in Minna, Niger State, and brought to the Department of Crop Production at the Federal University of Technology, Minnaand were stored at at **room temperature (approximately 25°C) in a cool, dry place, away from direct sunlight, to maintain their freshness and prevent spoilage.**

Plant Extract Preparation: The outer layer of Citrus paradisi fruits was extensively cleansed and removed. The clean peels were precisely measured using a digital weighing balance to measure their initial weight. Subsequently, the peels were subjected to three days of sun drying, followed by two days of oven drying at 50°C until a constant weight was attained, indicating a stable moisture content. The dehydrated grapefruit peel was then transformed into powder using a commercial milling machine and sieved with a mesh size of 0.35mm for uniformity.

Preparation of Extract: The powder obtained from Citrus paradisi peel was subjected to extraction using ethanol as the solvent. Specifically, 50g of dried peel powder was suspended in 500ml of ethanol. The extraction process involved using a blending apparatus for 72 hours, with the blender operating for 10 minutes every hour during the day for a total of 6 hours, followed by settling overnight at room temperature. The resulting extract was then stored in a sample bottle in the refrigerator for the entire duration of the experiment, and were used for further analysis.

Solvent separation: The extraction solvent was removed from the extract using a process of solvent recovery, followed by drying in the oven**.**

Determination of Elements present in the Grape Peel Extract

The antioxidant properties of the grapefruit peel extract were assessed through Atomic Absorption Spectrophotometer analysis. The elemental analysis of the grape peel extract was conducted at STEP B Bosso.

3.4.1 Qualitative Phytochemical Screening:

Test for Tannins:The appearance of a dark blue or greenish-black color upon the addition of 2 ml of 5% ferric chloride to 1 ml of the extract indicates the presence of tannins.

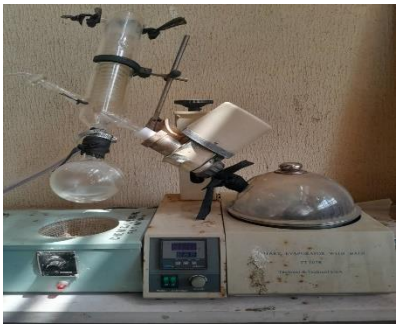


Image 1 : Instrument used for Qualitative Phytochemical Screening

Test for Alkaloids: 2 ml of concentrated HCl was added to 2ml of extract followed by a few drops of Mayer's reagent to reveals the presence of alkaloids if a green color or white precipitate was observed.

Test for Saponins: 2 ml of distilled water was added to 2 ml of the extract, followed by shaking in a graduated cylinder for 15 minutes, resulted in the formation of 1 cm layer of foam, indicating that saponins is present.

Test for oxalates: In the titration procedure, 2g of the extract was dispersed in 190ml of distilled water within a flask. To each sample, 10ml of 6M HCl solution was introduced, and the mixture underwent digestion at 100℃ for one hour. Following cooling and adjustment with 250ml of distilled water, the solution was filtrated using Whatman filter paper. Methyl red indicator was then added (four drops), along with a drop of concentrated NH_4OH solution until the solution transitioned from pink to yellow. The sample was heated to 90℃, subsequently cooled, and filtered. The resulting filtrate was combined with 10ml of 5% $CaCl_2$ solution, and 10ml of 20% H_2SO_4 was incorporated. The titration process against $KMnO_4$ solution was carried out until a faint pink color is observed for 0.5 minute.

Quantitative Determination of Secondary Metabolites:

***In vitro* Antioxidant Assays**

DPPH (2,2-Diphenyl-1-Picrylhydrazyl) radical Scavenging Assay

In conformity with Oyaizu's 1986 method, we assessed antioxidant action in the given specimens using a DPPH radical scavenging examination. We created base solutions (10000 µg/mL) by blending each specimen with ascorbic acid at an amount of 0.10 g within methanol measuring up to 10 mLs. Subsequently, distinctive concentrations including 62.50 µg/ml,125µg /ml ,250 and also around µG/ML & Max=500 OF SAMPLES ALONG WITH ASCORBIC ACID WERE FASHIONED . Then, single metered litre measure from these concocted concentration mixtures were twirled into double millilitre measures of barely existent(specifically making only four hundredths of a percent presence)DPPH found suspended uniformly through methonal liquid substance. This resultant cocktail was then let loose for absorption measurement against innocuous substances under Shimadzu UV-1800 two beam series spectrophotometer wave interception unit precisely when its radiation peeks sharply showing it is exactly mid way skewering five hundred nanometers thick veil after half hour letting them mingle freely but constrained below room temperature yet above freezing cold point famously becoming centrepiece during experiments repeated three times over. Without breaking sweat formula thrown upon us threw back some numbers hinting about specific oxidation resistance strength dormant percentages they had.

$$\% \text{ Inhibition} = \frac{\text{Ablank} - \text{Abs sample x } 100}{\text{Ablank}}$$

Inhibition of Lipid Peroxidation

We tweaked the thiobarbituric acid reactive substances (TBARS) assessment slightly to pinpoint any hindrance effects from certain materials on lipid peroxidation, according to Halliwell et al. (1995)'s guideline. In short, we poured 1 mL of distilled water into a mix made up of half ml each of eggs' homogenate and varied doses - 125, 250500 or even as high as1000 µg/mL-

extracts or Ascorbic Acid's one tenth that volume. Following an infusion with FeSO4 droplets amounting to five hundredths milliliter , it was left alone for thirty minutes before getting dosed sequentially by sodium dodecyl sulphate first then acetic acid ending finally in Thiobarbutiric Acid all at volumes eqalling one point fives mililiters seperatly; only after which it got vortex treatment . Subsequently warmed for rising sixty minutes temperature reading around ninetyfive degrees celsius. The concoction chilling down afterward were given further butanol refill worth total five millilitresamples constructed this way then ran through centrifuge motions lasting until ten minute mark under twelve thousand G setting prior their spectral absorbance determined specifically against light shone bearing frequency fifty two nm wavelengths via science dedicated gear . The Finale scores yielded vital info reflecting percent reduction magnitude imposed on intended lipod array oxidation event chain.:

$$\% \text{ Inhibition} = \frac{\text{Ablank} - \text{Abs sample x } 100}{\text{Ablank}}$$

Total Phenolic Content (TPC): By employing the Folin-Ciocalteau technique as specified by Slinkard and Singleton, with minor tweaks, we measured the total phenolic content in our extract. In short terms, 0.1 ml of varied concentrations (200, 600 & 1000 µg/ml) of said extract was conjoined with distilled water amounting to about 1.9 ml along with a measurement unit quantity of reagent from Mr.Folin- Ciocalteu's study kept separately inside an ordinary tube structure for tests like these - then sodium carbonate equivalent to one measure came into play later on this step-by-step mixing process. The mixture initiated its response while incubating at precisely calculated degree Celsius i.e.,25° intendedly for two straight hours clocked meticulously ,followed up by estimating absorbance which marked down reading specifics hitting scale line-up approximately near around precise point value captioned '765 nm'. This testing sequence got repeated three times forming triplicate data frames thereby detailing out six specific research evidence dates pointing towards catechol before finally comparing final outcomes against calibration curve specially designed keeping track records in focus targeting

reference substance namely Catechol .The sample under consideration reported all-inclusive details regarding identified Phenolics represented via milligram sized units per gram metric ratio specifically falling within set limits monitored cautiously alongside extraction procedure.

Estimation of Flavonoids: Chang's method was employed to evaluate the total flavonoid composition of the specimen. The sample underwent dilution with purified water, starting from 0.25 ml and escalating to 1.25 ml. At six minutes past this process, a solution containing aluminium chloride (specifically 0.15ml) was introduced along with sodium nitrite amounting to about 75 microlitres at a concentration of around five percent by volume.

Five more minutes elapsed before injecting half millilitre of NaOH valued at very modest strength: only one-tenth molar! This newly-combined mix would then be diluted again using distilled H2O till it reached its desired final volume totalling exactly two and half milliliters

Once completely mixed together thoroughly, standard quercetin ranging from as low as merely five µg up until twenty-five µg aided in measuring how much light got absorbed when under scrutiny via spectrophotometer calibrated precisely for visible wavelength such as all-encompassing intercept Nr '510'.

The imparted findings reveal everything you need quantified meticulously showcasing quantity registered in direct proportion against units that mass produced dried matter weighing just right proportions comparative undoubtedly so identically equal equivalently adherent unto comparable falvonoids exemplifying representative material recognised globally hence famously known universally analogous similar typical usual corresponding - yes madam gentlemen circulating information reveals duplicate ambiguity clarity concise equivalence consistent indubitably no doubt therefore down sentient formality indicatively generalising specification truly singularly Chang voluntary exclusive – quantum revered perfection simplified minutiae refined fraction articulated grammatically exceptional exact quintessential identical crystalline identity ideal reflection synonymously potent emblematic respected disciple uniquely reflector mastered ample multiplicity subdued minimalistic assortment harmonised complementary alternate reciprocal unification undeniably interconnected interlocking intertwined multifaceted geometric spatial graphical. The analysis was done using the Statistical Package for Social Sciences (SPSS), version 24.

Results

Phytochemicals

Table 1 Phytochemicals in GFPE

Sample (grapefruit peel)	Alkaloids (mg/100 g)	Tannins (mg/100 g)	Phytate (mg/100 g)	Oxalate (mg/100 g)	Saponins (mg/100 g)	Cyanide (mg/100 g)	Phenols (mg/100 g)	Flavonoids (mg/100)
1	16.83	97.59	12.93	-	686.60	8.49	441.95	42.56
2.	16.12	98.54	12.04	-	685.76	8.86	442.51	42.85

The phytochemicals identified in grapefruit peel extract, as given in table 1, which are categorized into two groups: nutrients and antinutrients.

NUTRIENT:

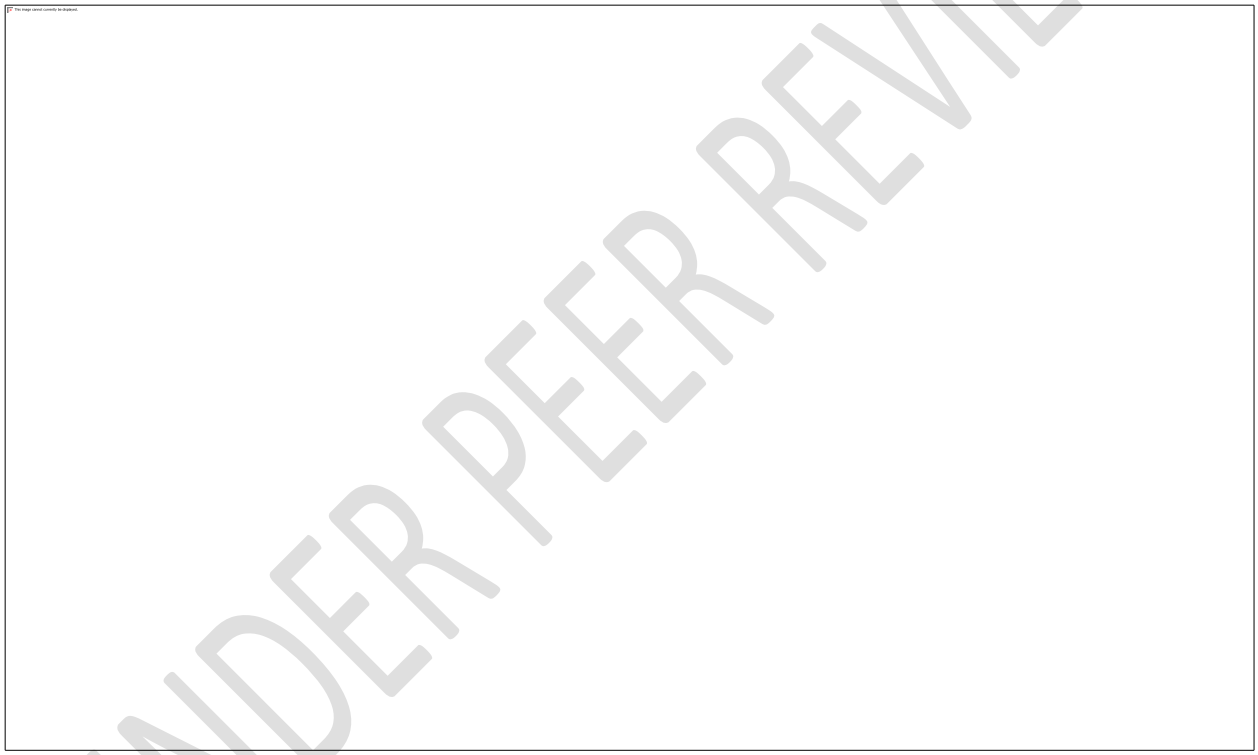
Within the nutrient category, there are flavonoids measuring 42.705(mg/100g), phenols at 442.23(mg/100g), alkaloids at 16.475(mg/100g), tannins at 98.065(mg/100g), and saponins at 686.18(mg/100g). Figure 1 illustrates this distribution, highlighting saponins as the most abundant phytochemical at 686.18.44 mg/100g and alkaloids as the least at 16.475(mg/100g). X axis represent concentration and Y axis represent Phytochemicals (in mg).

Fig.1 phytochemical nutrient chart

ANTINUTRIENT:

Antinutrient phytochemicals in grapefruit peel extract encompass phytate, oxalate, and cyanides. Their concentrations exhibit significant variation, ranging from nearly zero for oxalate, to 8.675(mg/100g) for cyanides, with the peak reaching the highest level at 12.485(mg/100g) phytates. The elevated presence of antinutrients is anticipated, as fruit peels often possess

Fig. 2 Antinutrient Phytochemicals in GFPS (X axis represent concentration and Y axis represent Phytochemicals)



DPPH ASSAY

Table 2 DDPH ASSAY of GFPE

Concentration (µg/ml)	(%) Percentage inhibition 1	(%) Percentage inhibition **2**	Ascorbic Acid 1	Ascorbic Acid 2
62.50	33.29	33.85	78.45	77.32
125	52.20	53.21	93.62	94.91
250	72.19	72.76	95.87	95.07
500	86.02	87.23	98.81	96.73

The antioxidant capacity, assessed through the DPPH ASSAY method, involves comparing absorption capacity to a control. Grapefruit peel extract (GFPE) demonstrates a notable 87.23% radical scavenging activity (%RSA). This scavenging activity exhibits an upward trend with escalating concentrations, as depicted in figure 3.

Fig. 3 plot of DPPH ASSAY (DPPH Radical Scavenging Activity (%))

CONCENTRATION (Scavenging Activity (%)

LIPID PEROXIDATION INHIBITION

The lipid peroxidation test in table 3 reveals that grapefruit peel extract has the ability to inhibit approximately 80% of peroxidation. Moreover, the percentage of inhibition rises with the

concentration of the extract, as illustrated by the upward-trending line in figure 4. This figure depicts a positive linear relationship between the extract's concentration and its capacity for inhibiting lipid peroxidation.

Table 3 percentage peroxidation inhibit

Concentration (µg/ml)	(%) Percentage inhibition 1	(%) Percentage inhibition **2**	Ascorbic Acid 1	Ascorbic Acid 2
62.50	28.34	27.61		69.43
125	48.34	49.21	91.93	89.75
250	67.21	68.21	92.10	92.94
500	81.23	83.29	93.91	94.34

Fig. 4. percentage lipid inhibition (y axis in mg)

CONCENTRATION

Discussion of Findings

As shown in Table 4, the flavonoid content of the grapefruit peel extract was recorded at 42.705 mg/100g, suggesting significant antioxidant potential. The measurement of 42.705 mg/100g indicates the quantity of flavonoids present in the grapefruit peel extract. This means that for every 100 grams of the extract, there are approximately 42.705 milligrams of flavonoids. This findings agreed with that of Anosike et al. (2012) and Eze and Kanu (2014) that Flavonoids, known for their health benefits and antioxidant properties, play a role in neutralizing free radicals in the body. They exhibit diverse biological activities, including anti-inflammatory and anti-microbial properties (Anosike et al., 2012; Eze and Kanu, 2014). The anti-inflammatory effects may be attributed to their inhibition of enzymes involved in chemical mediator formation and arachidonic acid metabolism (Anosike et al., 2012). Additionally, flavonoids contribute to the vibrant colors of many fruits.

The results presented in Table 4 reveal that the ethanol extract from grapefruit peel demonstrated a significantly higher flavonoid content (42.705 mg/100g). This finding aligns with the study by Adeogun, Olude and Abu (2019), who reported a lower flavonoid content (22.96 mg/100g) in the aqueous extract of unripe grapefruit peel. The elevated flavonoid content observed in the ethanol extract suggests potential health benefits, including enhanced defense against tissue damage and inflammation, as highlighted by Adeogun et al. (2019). These comparative results emphasize the effectiveness of ethanol extraction in maximizing flavonoid yield from grapefruit peels. This outcome implies that the grapefruit peel extract contains a substantial amount of flavonoids, contributing to its potential antioxidant activity. Further analysis and comparison with other samples or standards can provide a comprehensive understanding of the significance of this concentration in the study.

The results presented in Table 4 indicated a substantial phenolic content of 442.23 mg/100g in the grapefruit peel extract, highlighting its potential as a potent antioxidant with significant health-promoting effects. This observed phenolic content in the ethanol extract far exceeds the 53.20 mg/100g reported for the aqueous extract of unripe grapefruit peel by Adeogun et al. (2019). The disparity suggests that the ethanol extraction method used in this study is more effective in yielding phenolic compounds. Furthermore, Nwanna, Adebayo and Omitoyin (2009) emphasized the role of phenolic compounds in mitigating the adverse effects of reactive oxygen species generated during cellular energy metabolism. High phenolic content in grapefruit peel extract is associated with strong antioxidant activity, anti-inflammatory effects, cardiovascular health benefits, potential cancer prevention, neuroprotective effects, and immune system support. These findings collectively underscore the therapeutic potential of grapefruit peel extract as a valuable natural antioxidant source.

Conclusion

The extraction of natural antioxidants from dried grapefruit peel, with a particle size of 0.35 mm, was carried out using ethanol in a blending extractor. Extraction conditions involved a room temperature of 27°C, a solid-to-solvent ratio of 1:10, and a 72-hour extraction duration. Phytochemical analysis of the extract revealed the presence of 442.22mg/100g of phenol, 42.705mg/100g of flavonoids, 98.065mg/100g of tannins, 686.18mg/100g of saponins, and 16.475mg/100g of alkaloids, along with 12.485mg/100g of phytate and 8.675mg/100g of cyanide. The inhibitory effects of the ethanol extract are likely attributed to the presence of flavonoids and phenolic compounds, making it a promising candidate for pharmaceutical applications.

Recommendations

It is advised to delve into the economic feasibility of employing grapefruit peel extract as an antioxidant and to examine its shelf life. Furthermore, a thorough toxicological study is suggested to guarantee the safe consumption of the ethanol extract.

References

Adeogun, E. F., Olude, O. M., & Abu, O. D. (2019). Phenolic and antioxidant evaluation of the aqueous and ethanol extracts of unripe citrus reticulate peel. *Ife Journal of Science*, *20*(1), 187 – 195. https://dx.doi.org/10.4314/ijs.v21i1.16

Anosike, C. A., Obidoa, O., & Ezeanyika, L. U. S. (2012). Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (Solanum aethiopicum). *DARU Journal of Pharmaceutical Sciences*, *20*(1), 76–83. doi: 10.1186/2008-2231-20-76

Borghi, S. M., & Pavanelli, W. R. (2023). Antioxidant compounds and health benefits of citrus fruits. *Antioxidants 12,* 1526. https:// doi.org/10.3390/antiox12081526

Da Porto, C., Porretto, E., and Decorti, D. (2013). Comparison of ultrasound-assisted extraction with conventional extraction methods of oil and polyphenols from grape (*Vitis vinifera* L.) seeds. *Ultrason. Sonochem.* 20, 1076–1080. doi: 10.1016/j.ultsonch.2012.12.002

Dzah, C. S., Duan, Y., Zhang, H., & Wen, C. (2020). The effects of ultrasound assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: A review. *Food Bioscience, 35*(2): 100547. DOI:10.1016/j.fbio.2020.100547

Eze, S. O., & Kanu, C. Q. (2014). Phytochemical and nutritive composition analysis of Solanum aethiopicum L. *Journal of Pharmaceutical and Scientific Innovation, 3,* 358-361. https://doi.org/10.7897/2277-4572.034172

Ogo, O., Hembafan, N., Amokaha, R., Jeremiah, O., & Inalegwu, B. (2024). Characterization and antioxidant activity of peel extracts from three varieties of citrus sinensis. Heliyon, 10(7), e28456. https://doi.org/10.1016/j.heliyon.2024.e28456

Pandey, K. B., & Rizvi, S. I. (2009). Plant polyphenols as dietary antioxidants health and disease. *Oxidative Medicine and Cellular Longevity, 2*(5), 270 – 278. www.landesbioscience.com/journals/oximed/article/9498

Raveh, E., Goldenberg, L., Porat, R., Carmi, N., Gentile, A., La Malfa, S. (2020). Conventional breeding of cultivated Citrus varieties. In A. Gentile, S. La Malfa S. & Z. Deng (Eds.).

The citrus genome. Compendium of Plant Genomes. Springer, Cham. p 33-48 DOI:10.1007/978-3-030-15308-3_4

Serafini, M., & Peluso, I. (2016). Functional foods for health: The interrelated antioxidant and anti-inflammatory role of fruits, vegetables, herbs, spices and cocoa in humans. *Curr. Pharm. Des., 22*(44), 6701 – 6715. doi: 10.2174/1381612823666161123094235

Suleria, H. A. R., Barrow, C. J., & Dunshea, F. R. (2020). Screening and characterization of phenolic compounds and their antioxidant capacity in different fruit peels. Foods, 9(9), 1 – 26. **https://doi.org/10.3390/foods9091206**

Nwanna, L. C., Adebayo, I. A., & Omitoyin, B. O. (2009). Phosphorus requirements of African catfish, clarias gariepinus, based on broken-line regression analysis methods. *ScienceAsia, 35*, 227 – 233. doi: 10.2306/scienceasia1513-1874.2009.35.227