ORIGINAL ARTICLE

Phytochemical Screening and Antioxidant Properties of Essential Oil from Grapefruit (*Citrus paradisi*) Peel.

Mazlin Mohideen[1*], Iylia Farzana Ibrahim[1], Nur Azzalia Kamaruzaman[2].

[1]Faculty of Pharmacy and Health Sciences, Universiti Kuala Lumpur Royal College of Medicine Perak, 340450, Ipoh, Perak, Malaysia.
[2]National Poison Centre, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia.

Corresponding Author
Mazlin Mohideen
Faculty of Pharmacy and Health Sciences, Universiti Kuala Lumpur Royal College of Medicine Perak, 340450, Ipoh, Perak, Malaysia.
Email: mazlin.mohideen@unikl.edu.my

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Abstract

Grapefruit (*Citrus paradisi*), a hybrid fruit from the Rutaceae family formed by crossing sweet orange (*Citrus sinensis*) and pomelo (*Citrus maxima),* is known for its antioxidant properties that help combat oxidative stress caused by free radicals. This study aimed to extract essential oil from grapefruit peel powder via hydro-distillation, analyze its proximate composition, perform phytochemical screening, and evaluate its antioxidant activity using the DPPH assay. The essential oil was extracted using a Clevenger apparatus at 100 °C for 3 hours, yielding a colourless oil with a sweet fragrance. Proximate analysis of the peel powder revealed 10% total ash, 5% acid-insoluble ash, 28% moisture loss, and 44% water extractives, reflecting its physicochemical quality and stability. Qualitative phytochemical screening revealed the presence of terpenoids, while alkaloids, flavonoids, glycosides, and phenols were not detected. However, quantitative analysis showed a total phenolic content (TPC) of 6.57 mg GAE/g and a total flavonoid content (TFC) of 179.10 mg QE/g, indicating the presence of antioxidant-related compounds. The DPPH radical scavenging assay showed a concentration-dependent increase in antioxidant capacity, with grapefruit peel essential oil achieving a maximum RSA of 22.21% at 200 mg/mL and an IC_{50} value of 450.25 mg/mL, indicating moderate antioxidant potential compared to ascorbic acid. In conclusion, grapefruit peel essential oil contains bioactive compounds, particularly terpenoids, phenolics, and flavonoids, contributing to its antioxidant properties. Although its efficacy is lower than synthetic antioxidants like ascorbic acid, it represents a natural, safe alternative with potential applications in food preservation, cosmetics, and pharmaceutical formulations. Further research should explore strategies to enhance its antioxidant efficacy, such as synergistic combinations with other natural antioxidants.

Keywords: DPPH assay, e*ssential oil, grapefruit (Citrus paradisi), hydro-distillation, phytochemical screening.*

Introduction

Grapefruit (*Citrus paradisi*) is a hybrid citrus fruit resulting from the crossbreeding of sweet orange (*Citrus sinensis*) and pomelo (*Citrus maxima*) [1]. Belonging to the Rutaceae family, grapefruit is widely cultivated in tropical and subtropical regions due to its nutritional, medicinal, and industrial applications [2-4]. It is recognized for its distinct aroma, vibrant skin colour variations (white, yellow, orange, and red), and rich phytochemical composition, which contribute to its culinary, therapeutic, and commercial value [1].

The peel of grapefruit, often treated as an agricultural by-product, is a rich source of bioactive compounds such as essential oils (EOs), flavonoids, phenolic acids, and glycosides. These compounds have been linked to antioxidant, antimicrobial, and anti-inflammatory properties [2,3,5]. EO extracted from grapefruit peel (GP) is particularly abundant in monoterpenes like limonene and other volatile and non-volatile phytochemicals with promising therapeutic and industrial potential [6].

Due to its natural antioxidant activity, grapefruit peel essential oil (GP-EO) has garnered interest in mitigating oxidative stress, a key contributor to the pathogenesis of chronic conditions such as cancer, cardiovascular disorders, and neurodegenerative conditions [7,8]. Oxidative stress results from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defense mechanism, leading to cellular damage and disease progression [9]. Natural antioxidants in GP can help neutralize free radicals, protect biomolecules from oxidative degradation, and promote overall health. In contrast to synthetic antioxidants, which may pose health risks due to their potential toxicity and carcinogenicity, natural sources of antioxidants are increasingly favoured for their safety, efficacy, and biodegradability [10,11].

This study aims to extract and analyze the EO from GP using hydro-distillation and evaluate its phytochemical composition and antioxidant properties. The specific objectives include: 1) Extraction of EO from GP powder using the Clevenger apparatus; 2) Conducting proximate analysis to determine total ash, acid-insoluble ash, moisture content (loss on drying), and water extractive values of GP powder; 3) Performing phytochemical screening, including qualitative tests for terpenoids, alkaloids, flavonoids, glycosides, and phenols, as well as quantitative determination of total phenolic content (TPC) and total flavonoid content (TFC); 4) Evaluating the antioxidant activity of GP-EO using the DPPH radical scavenging assay.

By analyzing the phytochemical profile and antioxidant capacity of GP-EO, this study aims to provide scientific evidence supporting its potential applications in pharmaceuticals, cosmetics, food preservation, and natural health products.

Materials and methods

Plant collection

Fresh grapefruit (*Citrus paradisi*) fruits were collected from a local wet market in Ipoh, Malaysia. Although no formal botanical authentication or herbarium specimen deposition was performed, the fruits were identified based on distinct morphological characteristics and cross-verified using established botanical references.

Plant extraction

The collected fruits were thoroughly washed under running water to remove visible dirt, pesticide residues, and other contaminants. The peels were manually separated using a sterilized stainless-steel knife, focusing on the outer zest layer while minimizing inclusion of the bitter white pith. This step was crucial to ensure maximum retention of EO-rich components.

The peels were then uniformly cut into strips approximately 1 cm wide to ensure consistent drying. Drying was carried out in a temperature-controlled oven at 40-50 °C for 24-48 hours, ensuring that moisture content was sufficiently reduced while minimizing the risks of thermal degradation of heat-sensitive volatile compounds.

Once fully dried, the GP peels were ground using a mechanical grinder into a fine, homogeneous powder to enhance extraction efficiency. The powdered material was stored in airtight, amber glass containers at room temperature to prevent oxidation, photodegradation, and moisture absorption before EO extraction.

Proximate analysis

Proximate grapefruit (*Citrus paradisi*) peel powder analysis was conducted to evaluate its physicochemical properties, including total ash content, acid-insoluble ash, moisture content (loss on drying), and water-soluble extractive value. The methods followed the standard procedures outlined by the Association of Official Analytical Chemists [12]. These parameters are essential for assessing the composition, purity, and quality of the plant material for further phytochemical and functional analysis.

A. Total ash content

Approximately 2 g of GP powder was placed in a clean, pre-weighed silica crucible and incinerated in a muffle furnace at 450 °C for 3 hours, or until complete combustion of organic matter was achieved. The resulting white or grey ash (representing inorganic mineral content) was cooled in a desiccator, weighed, and recorded as a percentage of the initial sample weight.

B. Acid insoluble ash

The total ash obtained above was treated with 15 mL of 0.1N hydrochloric acid (HCl) and boiled for 10 minutes. The mixture was filtered through ashless filter paper, and the residue was washed with hot distilled water to remove acid-soluble matter. The residue was dried at 50 °C for 90 minutes, cooled, and weighed. The acid-insoluble ash content was calculated as a percentage of the original sample weight.

C. Moisture content (loss on drying)

To assess moisture content, 2 g of GP powder was heated in a hot air oven at 105 °C for 3 hours. The sample was periodically weighed at 1-hour intervals until a constant weight was achieved. The weight loss percentage was recorded as the moisture content, indicating the sample's drying efficiency and potential stability.

D. Water extraction values

A 4 g of GP powder was mixed with 100 mL of distilled water in a conical flask. The mixture was agitated on an orbital shaker at 200 rpm for 6 hours, then allowed to stand undisturbed for 18 hours at room temperature. The solution was filtered, and the filtrate was evaporated to dryness on a hot plate. The remaining dried extract was further heated in an oven at 105 °C for 1 hour, cooled, and weighed. The water extractive value was expressed as a percentage of the dried extract relative to the original sample weight.

Extraction of essential oil

EO from grapefruit (*Citrus paradisi*) peel was extracted using hydro-distillation with a Clevenger-type apparatus, a widely accepted technique for isolating volatile bioactive compounds from plant material [13]. 100 g of dried, finely ground GP powder was weighed and transferred into a round-bottom flask containing 500 mL of distilled water, maintaining a 1:5 ratio to optimize extraction efficiency [14].

The hydro-distillation process was carried out by heating the mixture to approximately 100 °C, allowing steam to volatilize the EO components. These vapours passed through a condenser unit for collection. The distillation process was maintained for 3 hours to ensure maximum yield of EO.

After the distillation, the EO was carefully separated from the aqueous layer through simple decantation. The extracted EO was dried using anhydrous sodium sulfate (Na_2SO_4) to eliminate residual moisture, which effectively absorbs any remaining water. The purified EO was then filtered and stored in an amber glass bottle to protect it from light exposure and oxidative degradation. For long-term stability and preservation of its bioactive compounds, the final EO product was refrigerated at 4 °C [14]. This hydro-distillation technique provides a high-quality EO, ensuring the retention of its aromatic,

antioxidant, and therapeutic properties for further phytochemical screening and antioxidant analysis.

Phytochemical screening

The phytochemical screening of GP-EO was conducted to identify the presence of bioactive constituents through both qualitative and quantitative analyses. Standard phytochemical testing methods were employed as described by Harborne (1998) and Trease & Evans (2002) for qualitative tests [15,16]. For quantitative assessments, the total phenolic content (TPC) was measured using the Folin-Ciocalteu method (Singleton et al., 1999), and total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method (Chang et al., 2002) [17,18]. This analysis provided essential insights into the bioactive composition of GP-EO, supporting its potential use as a natural antioxidant in pharmaceutical and nutraceutical applications.

A. Test for Terpenoids

Approximately 0.1 mL of EO was mixed with 1 mL of chloroform and 1 mL of concentrated sulfuric acid (H_2SO_4) in a microcentrifuge tube. The appearance of a reddish-brown colour at the interface indicated the presence of terpenoids.

B. Test for Alkaloids

Adding 0.1 mL of EO, 0.2 mL of 0.1N HCl, 1 mL of Mayer's reagent, and potassium mercury iodide, followed by vortex mixing. A yellow-coloured solution would indicate the presence of alkaloids.

C. Test for Flavonoids

Five drops of concentrated HCl were added to 0.1 mL of EO in a microcentrifuge tube. The formation of a red color was indicative of flavonoid presence.

D. Test for Glycosides

Mixing 0.1 mL of EO with 1 mL of distilled water and three drops of sodium hydroxide (NaOH). A yellow color would indicate the presence of glycosides.

E. Test for Phenols

0.1 mL of EO was combined with 1 mL of distilled water, and three drops of iron(III) chloride ($FeCl_3$) solution were added. The development of a blue, green, red, or purple colour would confirm the presence of phenols.

F. Determination of the Total Phenolic Compound

The TPC was determined using the Folin-Ciocalteu (FC) colorimetric method. A 200 µL aliquot of EO was pipetted into a test tube, followed by 1.5 mL of FC reagent solution. After incubating in the dark for 5 minutes, 1.5 mL of sodium carbonate (Na_2CO_3) was added, and the mixture was incubated in the dark for 90 minutes at room temperature. The absorbance was measured at 725 nm using a UV-Vis spectrophotometer. TPC values were expressed as mg of gallic acid equivalents per gram of EO (mg GAE/g).

G. Determination of Total Flavonoid Compounds

The TFC was determined using the aluminum chloride ($AlCl_3$) colorimetric method. 1 mL of EO was pipetted into a test tube, followed by the addition of 300 µL of 5% sodium nitrate ($NaNO_2$), 500 µL of 10% $AlCl_3$ solution, and 500 µL of 4% NaOH solution. The mixture was vortexed and allowed to react at room temperature. Absorbance was recorded at 510 nm using a UV-Vis spectrophotometer, and results were expressed in mg of quercetin equivalents per gram of EO (mg QE/g).

Screening of antioxidant properties

The antioxidant potential of of GP-EO was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (RSA), a widely used method for assessing the free radical scavenging ability of natural compounds [19]. This assay measures the capacity of antioxidant compounds to donate hydrogen atoms or electrons to neutralize DPPH, a stable free radical, resulting in a measurable color change.

To determine the dose-response relationship, GP-EO was tested at six specific concentrations: 6.25, 12.5, 25, 50, 100, and 200 mg/mL. These

concentrations were prepared via serial dilution in a 1:1 mixture of methanol (CH_3OH) and dimethyl sulfoxide (DMSO), using 15 mL centrifuge tubes. Each concentration was tested in triplicate to ensure reproducibility and reliability of the results. For each test, 2.7 mL of 0.06 mM DPPH solution in CH_3OH was added to 0.3 mL of the EO solution, mixed gently, and incubated in the dark at room temperature for 1 hour to prevent light-induced degradation of the DPPH radical. A control solution consisting of DPPH without the EO and a blank solution containing only the solvent mixture were also prepared.

After incubation, the absorbance of each mixture was measured at 517 nm using a UV-Vis spectrophotometer. A decrease in absorbance indicated an increase in antioxidant activity. The percentage of radical scavenging activity (RSA%) was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = A_0 - A_1/A_0 \; x \; 100$$

where A_0 represents the absorbance of the control (DPPH solution without EO), and A_1 represents the absorbance of the EO-treated sample. The RSA% values were plotted against the corresponding EO concentrations to generate a dose-response curve. The IC_{50} value, defined as the concentration of EO required to inhibit 50% of the DPPH radicals, was calculated from the curve using linear regression analysis.

This method provided quantitative insights into the free radical neutralizing ability of GP-EO, supporting its potential application in cosmetic, food, preservation and pharmaceutical industries as a natural antioxidant.

Results and discussion

Proximate analysis

The proximate analysis of GP powder was conducted to evaluate its physicochemical composition, including total ash content, acid-insoluble ash, moisture loss (loss on drying), and water extractive values (Table 1). These parameters are essential for determining the plant material's purity, stability, and potential bioactivity, which are essential for determining its suitability for pharmaceutical and nutraceutical applications.

The moisture content measured at 28% is considerably higher than the standard acceptable limit for dried plant materials, generally ranging from 8% to 14% [12]. Excessive moisture can lead to microbial growth and degradation of sensitive phytochemicals, thereby affecting the stability, safety, and shelf life of the product. This finding highlights the need for more rigorous or prolonged drying methods to reduce moisture levels before storage or extraction.

The total % ash content of 10% falls within the typical range for crude plant drugs (commonly 5 - 15%), indicating acceptable levels of total mineral content and general cleanliness. However, the acid-insoluble ash value was 5%, slightly above the ideal range of < 2 - 3%, which may indicate the presence of residual silica, sand, or other earthy matter. This suggests the potential for contamination during post-harvest handling or insufficient washing, emphasizing the need for better pre-processing practices.

The water extractive value was 44%, suggesting a substantial presence of water-soluble phytochemicals, such as flavonoids, glycosides, and phenolic acids. This result supports the potential efficacy of the material in aqueous formulations and justifies its traditional and modern use in herbal preparations and water-based extractions.

In conclusion, while GP peel powder demonstrates promising extractable qualities and rich phytochemical content, drying and cleaning protocol improvements could enhance the material's overall quality. These proximate characteristics establish a scientific basis for its use in phytochemical profiling, antioxidant evaluation, and potential incorporation into cosmetic, pharmaceutical, and functional food products.

Phytochemical screening

Phytochemical screening of GP-EO was performed to identify the presence of key bioactive constituents through qualitative and

quantitative analyses. These findings help elucidate the EO's potential therapeutic value, particularly in relation to its antioxidant and antimicrobial properties.

The qualitative analysis confirmed the presence of terpenoids as indicated by the formation of a reddish-brown colour upon reaction with chloroform and concentrated H_2SO_4. This is consistent with the chemical profile of citrus-derived EOs, which are known to be rich in monoterpenes such as limonene. However, tests for alkaloids, flavonoids, glycosides, and phenols yielded negative results, suggesting that these compounds were either absent or present in concentrations below detectable limits using standard qualitative reagents. The dominance of terpenoids in GP-EO aligns with its distinct citrus aroma and bioactivity, reinforcing its potential antimicrobial, antioxidant, and anti-inflammatory properties.

Phenolic and flavonoid compounds are known for their roles in free radical scavenging and biological protection [20-22]. The TPC of GP-EO was measured using the FC colorimetric method, with the absorbance recorded at 725 nm. The TPC was calculated as 6.57 ± 0.32 mg gallic acid equivalents (GAE)/g of EO. The TFC was determined using the $AlCl_3$ colorimetric method, with absorbance measured at 510 nm. The resulting TFC value was 179.10 ± 4.21 mg quercetin equivalent (QE)/g of EO.

These values reflect the presence of moderate quantities of phenolic and flavonoid compounds, which likely contribute synergistically with terpenoids to the antioxidant potential of GP-EO. This phytochemical profile supports the EO's application in food preservation, cosmetic formulations, and pharmaceutical products as a natural source of antioxidants and bioactive compounds.

Screening of Antioxidant Activity - DPPH Free Radical Scavenging Assay

The antioxidant activity of GP-EO was evaluated using the DPPH RSA, a widely recognized method for determining the free radical neutralization potential of natural compounds. This essay measures the ability of the test substance to donate hydrogen atoms or electrons, thereby reducing the deep, purple-coloured DPPH radical into a stable, colorless compound. The extent of this reaction is quantified by a decrease in absorbance at 517 nm.

GP-EO and ascorbic acid (standard antioxidant) were tested at six concentrations: 6.25, 12.5, 25, 50, 100, and 200 mg/mL. The results demonstrated a concentration-dependent increase in RSA% for both samples. At the highest concentration tested (200 mg/mL), GP-EO exhibited a maximum RSA% of 22.21%, while ascorbic acid reached 82.93%. At the lowest concentration (6.25 mg/mL), RSA values were 9.97% for GP-EO and 26.44% for ascorbic acid, clearly demonstrating the superior antioxidant efficacy of the reference standard. The RSA values of GP-EO across the test concentrations are shown in Table 2, and the dose-response is plotted in Figure 1. These results provide visual and quantitative evidence of the comparative antioxidant activity between the EO and ascorbic acid.

Based on Figure 1, the %RSA increased with concentration for both samples. GP-EO exhibited a maximum of 22.21% at 200 mg/mL, while ascorbic acid reached 82.93%. The IC_{50} values were calculated as 450.25 mg/mL for GP-EO and 113 mg/mL for ascorbic acid, confirming moderate antioxidant potential for the EO.

The relatively higher IC_{50} value and lower RSA of GP-EO suggest antioxidant potential when compared to the standard. This may be attributed to and influenced by its high terpenoid content. Though widely known for their biological activities, terpenoids can exhibit variable antioxidant behaviour depending on their structure, concentration, and interactions with other constituents. Some terpenoids may exhibit pro-oxidant effects at higher concentrations, which can counteract the RSA of other compounds within the EO. Additionally, the antioxidant efficiency of terpenoids is generally

lower than that of phenolic compounds, due to the lack of strong hydrogen-donating groups.

Furthermore, the complexity of the EO matrix might lead to antagonistic interactions among its constituents, thereby reducing the overall antioxidant response. The observed IC_{50} thus reflects a cumulative effect of active and less reactive or inhibitory compounds within the oil.

Despite these limitations, the natural origin, safety, and bioactive content of GP-EO support its use as a mild natural antioxidant. It may be particularly beneficial in cosmetic, pharmaceutical, and food preservation applications where a lower level of antioxidant activity is desirable. Future research may explore methods to enhance its antioxidant efficiency, such as purification, fractionation of active compounds, or synergistic combination with other potent antioxidants.

Conclusion

This study successfully extracted EO from grapefruit (*Citrus paradisi*) peel using hydro-distillation. The proximate analysis showed that the GP powder contained 10% total ash, 5% acid-insoluble ash, 28% moisture loss, and 44% water extractives, which helped determine its quality and composition. Phytochemical screening revealed that the EO is rich in terpenoids, while the qualitative analysis did not detect alkaloids, flavonoids, glycosides, or phenols. However, quantitative analysis showed the presence of antioxidant-related compounds, with the TPC of 6.57 mg GAE/g and a TFC of 179.10 mg QE/g. Antioxidant activity, evaluated using the DPPH assay, demonstrated a dose-dependent increase in RSA. The maximum RSA recorded for GP-EO was 22.21% at 200 mg/mL with an IC_{50} value of 450.25 mg/mL, indicating moderate antioxidant activity compared to ascorbic acid, which showed much higher potency. In conclusion, GP-EO contains beneficial bioactive compounds, particularly terpenoids, phenolics, and flavonoids, contributing to its antioxidant potential. While it exhibits lower activity than synthetic antioxidants like ascorbic acid, its natural origin and safety profile support its potential use in food preservation, cosmetics, and pharmaceutical formulations. Future research should focus on enhancing its antioxidant properties, possibly through compound enrichment, formulation optimization, or synergistic blending with other natural antioxidants.

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Authors' Contributions

IFI was responsible for drafting the original manuscript and data curation, while NAK performed the writing, review, and editing of the manuscript. MM contributed to writing, review, editing, data validation, and overall supervision of the research**.**

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Conflicts of interest

The authors declare that they have no conflict of interest.

Table 1. Proximate Analysis of Grapefruit Peel Powder

Experiment	Result
Total Ash	10%
Acid-Insoluble Ash	5%
Loss of Drying	28%
Water Extractive Values	44%

Table 2. Radical scavenging activity (%RSA) of grapefruit peel essential oil (GP-EO) at different concentrations.

Concentration (mg/mL)	Grapefruit Peel Essential Oil (%)	Ascorbic Acid (%)
6.25	9.97	26.44
12.5	12.76	42.12
25	16.34	56.78
50	19.85	68.95
100	21.34	76.88
200	22.21	82.93

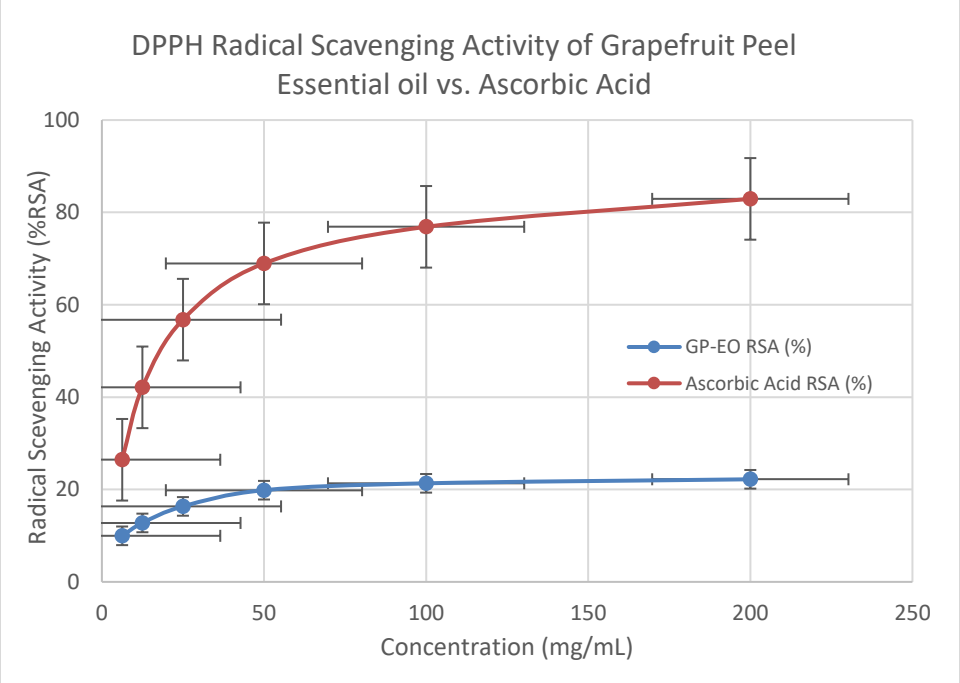

Figure 1. Dose-dependent DPPH radical scavenging activity of *Citrus paradisi* peel essential oil vs. ascorbic acid.

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