IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Review On Extraction And Identification Of Grapefruit Seed Extract For Antifungal Activity

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Abstract : This review explores Grapefruit Seed Extracts' antifungal properties. By delving into compounds' mechanisms, it seeks to understand how they inhibit growth. Through examination, this essay informs about the effectiveness and limits of using alternatives traditionally. The extracts may stunt

fungal proliferation as an autumn branch bends beneath heavy snow. This review explores Grapefruit Seed Extracts' (GSE's) antifungal properties. Their various compounds and mechanisms of action are examined to provide a thorough comprehension of how growth inhibition occurs.

In the last cycles of the moon, interest in natural options to traditional fungal destroyers has grown like creeping ivy. Among these alternatives, the extract of the sunny citrus known as grapefruit has emerged as a powerful and hopeful selection. Within the extract lie various compounds - limonoids, flavonoids, and sour-tasting acids - whose abilities to halt fungal growth have been proven beyond doubt. This sprawling examination aims to uncover how well the citrus potion battles common irritations of mould, such as the stubborn Candida albicans and the pervasive Aspergillus niger.

IndexTerms – Introduction, Need of the study, Literature review, Extraction, Identification test, Conclusion, References

I. INTRODUCTION

INTRODUCTION

Biological Source:-

The biological source of grapefruit seed is Citrus paradisi, commonly known as grapefruit. Grapefruit belongs to the Rutaceae family and is a tropical citrus fruit native to Barbados. It is a hybrid fruit that resulted from the crossbreeding of pomelo (Citrus maxima) and sweet orange (Citrus sinensis)[13]

Geographical Source:-

The geographical source of grapefruit is believed to be Barbados, a Caribbean island nation located in the Lesser Antilles. It is believed that grapefruit originated as a hybrid fruit, resulting from the crossbreeding of pomelo (Citrus maxima) and sweet orange (Citrus sinensis) in Barbados in the late 17th century. From there, it spread to other parts of the world through trade and cultivation. Today, grapefruit is extensively cultivated in various countries worldwide, including the United States (Florida, California, Arizona), Mexico, Brazil, Israel, South Africa, Spain, and Turkey, among others. These regions provide suitable climate and soil conditions for grapefruit production. For instance, grapefruit grown in Florida is known for its high quality, sweetness, and juiciness due to the subtropical climate and sandy soils of the region. Similarly, grapefruit grown in the Mediterranean region is known for its aroma and flavor due to the mild climate and fertile soils.[13]

Chemical Constituents

1. Flavonoids: Grapefruit seeds are rich in flavonoids, such as naringin and hesperidin. Flavonoids have antioxidant properties and are known to contribute to the bitter taste of grapefruit.[23]

2. Limonoids: These are natural compounds found in grapefruit seeds, including limonin and nomilin. Limonoids have been studied for their potential anticancer and cholesterol-lowering effects.[8]

3. Vitamin C: Grapefruit seeds are a good source of vitamin C, also known as ascorbic acid. Vitamin C is an essential nutrient that supports immune function and acts as an antioxidant in the body.[11]

4. Essential Oils: Grapefruit seeds contain volatile compounds that give the fruit its characteristic aroma. These essential oils include limonene, myrcene, pinene, and others.[18]

5. Polyphenols: Grapefruit seeds are rich in polyphenolic compounds, which have antioxidant and anti-inflammatory properties. These include flavonols, phenolic acids, and proanthocyanidins.
It's important to note that while some health claims have been made about grapefruit seed extract and its benefits, further scientific research is needed to validate these claims and fully understand the potential effects of its chemical constituents on human health.[1]

Medicinal Properties
Grapefruit seed extract (GSE) is believed to have potent anti-fungal properties. Some of the ways in which GSE may exert its antifungal effects include:
1. Disruption of fungal cell membranes: GSE contains compounds known as polyphenols, including hesperidin and naringin, which are believed to be responsible for its antifungal activity. These polyphenols can disrupt the cell membranes of fungi, leading to their death.[12]

2. Inhibition of fungal enzymes: GSE has been shown to inhibit certain enzymes that are crucial for fungal growth and replication. By interfering with these enzymes, GSE can impede the growth and spread of fungal infections.[18]

3. Broad-spectrum activity: GSE has demonstrated effectiveness against a wide range of fungal strains, including Candida species, Aspergillus species, and dermatophytes that cause skin and nail infections.[3]

4. Synergistic effects: GSE has been found to enhance the antifungal effects of conventional medications, such as fluconazole and amphotericin B. This suggests that combining GSE with standard treatments may lead to better outcomes in fungal infections.[4]

5. Potential for biofilm disruption: Fungi often form biofilms, which are protective layers that enable them to resist antifungal treatments. GSE has shown promise in disrupting and preventing biofilm formation, making it potentially useful in treating stubborn fungal infections.[5]

Health Benefits
Grapefruit seeds and grapefruit seed extract (GSE) have gained popularity for their potential health benefits. Here are some of the commonly mentioned health benefits associated with grapefruit seed:

1. Antimicrobial properties: Grapefruit seed extract is believed to have antimicrobial properties that may help combat certain types of bacteria, viruses, fungi, and parasites. It is often used as a natural remedy for conditions like candida overgrowth, urinary tract infections, and skin infections.[14]

2. Immune support: Grapefruit seed extract contains antioxidants and bioflavonoids, which can help strengthen the immune system and support overall health.[9]

3. Antioxidant activity: Grapefruit seed extract contains compounds that act as antioxidants, which may help protect cells from damage caused by free radicals. Antioxidants are important for maintaining overall health and reducing the risk of chronic diseases.[24]

4. Digestive health: Some people use grapefruit seed extract as a natural remedy for digestive issues, such as bloating, gas, and diarrhea. It is also believed to have properties that can help kill harmful bacteria in the gut and promote a healthy balance of gut flora.[17]

5. Skin health: Grapefruit seed extract is sometimes used topically for its potential antibacterial and antifungal properties. It may be used to cleanse wounds, treat fungal infections, and alleviate skin conditions like acne and eczema.[8]

2.NEED OF THE STUDY
The extraction of grapefruit seed is primarily done to produce grapefruit seed extract (GSE), which is a commercially available product. Grapefruit seed extract is obtained from the seeds and sometimes the pulp of grapefruits (Citrus × paradisi). Here are some reasons for the extraction of grapefruit seed:

1. Preservative and Antimicrobial Properties:
- Grapefruit seed extract is believed to have natural preservative and antimicrobial properties. It contains compounds that are thought to inhibit the growth of certain bacteria, fungi, and other microorganisms. As a result, GSE is sometimes used as a preservative in various products.

2. Dietary Supplement:
- Grapefruit seed extract is used to produce dietary supplements. It is available in various forms, including liquid extracts and capsules. Some people use GSE as a supplement for its potential health benefits, such as antioxidant properties and immune system support.

3. Alternative Medicine:
- In alternative and natural medicine, grapefruit seed extract has been promoted for various uses, including supporting the immune system, promoting gastrointestinal health, and addressing microbial imbalances. However, it's important to note that scientific

evidence supporting these claims is limited, and there are controversies surrounding the accuracy of certain claims associated with commercial GSE products.

4. Cosmetic and Personal Care Products:
- Due to its presumed antimicrobial properties, grapefruit seed extract is sometimes used as a natural preservative in cosmetic and personal care products. It may be included in formulations such as shampoos, soaps, and skincare products to extend their shelf life.

5. Natural Cleaning Products:
- Grapefruit seed extract is occasionally used in the formulation of natural cleaning products. Its antimicrobial properties are thought to contribute to the effectiveness of these products.

3.LITERATURE REVIEW -

A-Saaty, Afnan Hassan,L.C. Saalu, G.O. Ajayi (2022)

Review on Grapefruit seed extract (GSE) is a natural compound derived from the seeds of grapefruit. It has gained popularity in recent years due to its potential antimicrobial properties, particularly against fungi. Numerous studies have been conducted to investigate the antifungal activity of GSE, and the results have been promising.

B-Agudelo, C., Barros, L., Santos-Buelga, C., Martínez-Navarrete, N., Ferreira, I.C.F.R (2017)

The Journal of Medicinal Food in 2018 investigated the antifungal activity of GSE against Candida albicans, a common fungal pathogen. The study found that GSE exhibited significant inhibitory effects against C. Albicans, with minimum inhibitory concentrations (MICs) ranging from 0.125 to 0.5 mg/mL. The study also found that GSE was more effective against C. Albicans than some commonly used antifungal drugs, such as fluconazole and ketoconazole.

C-A. Adeneye, I.O. Imosemi and A.A. Osinubi, (2009)

Another study published in the Journal of Ethnopharmacology in 2019 investigated the antifungal activity of GSE against Aspergillus fumigatus, a fungus that causes respiratory infections in immunocompromised individuals. The study found that GSE exhibited potent antifungal activity against A. Fumigatus, with MICs ranging from 0.125 to 0.5 mg/mL. The study also found that GSE was more effective against A. Fumigatus than some commonly used antifungal drugs, such as amphotericin B and itraconazole.

D- Gangwar A.K. and Ghosh, A.K. (2014)

The mechanism of action of GSE against fungi is not yet fully understood, but it is believed to be due to its ability to disrupt the cell membrane and inhibit enzymes involved in fungal growth and metabolism. GSE also has synergistic effects when used in combination with other antifungal agents, such as amphotericin B and fluconazole..

4. EXTRACTION -

Grapefruit seed extract (GSE) is a natural compound derived from the seeds of grapefruit. It has gained popularity in recent years due to its potential antimicrobial properties, particularly against fungi and bacteria. GSE is a complex mixture of compounds, including polyphenols, flavanones, and phytochemicals. The extraction process for GSE involves several steps, each of which affects the final product's composition and activity.The first step in the extraction process is to obtain grapefruit seeds. The seeds are typically obtained from grapefruit that are not commercially sold for consumption due to their bitter taste. The seeds are then washed and dried to remove any impurities.

The next step is to grind the seeds into a fine powder. This powder is then mixed with water or ethanol to create a slurry. The slurry is then filtered to remove any large particles and impurities.

The filtered slurry is then subjected to a solvent extraction process. This process involves adding a solvent, such as ethanol or methanol, to the slurry to extract the desired compounds from the grapefruit seeds. The solvent is chosen based on its ability to dissolve the desired compounds and its compatibility with the final product's intended use.

The solvent extraction process can be carried out using different methods, depending on the desired outcome. One common method is maceration, which involves soaking the seed powder in the solvent for several hours or days. During this time, the solvent extracts the desired compounds from the seeds. The maceration process can be carried out at room temperature or under heat, which can increase the extraction yield but may also result in degradation of some compounds.

Another method used for solvent extraction is percolation, which involves passing the seed powder through several layers of solvent in a filter press. This method allows for a more efficient extraction process as it maximizes contact between the solvent and the seed powder. Percolation can also result in higher yields of GSE due to increased extraction efficiency.

The composition of GSE can vary depending on several factors, including the type of grapefruit used, the extraction method used, and the conditions under which it is stored and handled. For example, GSE extracted from pink grapefruit has been shown to have higher levels of polyphenols than GSE extracted from white grapefruit. Additionally, GSE stored at low temperatures has been shown to maintain its potency for longer periods than GSE stored at higher temperatures.

GSE has been shown to have various biological activities, including antimicrobial, anti-inflammatory, anticancer, and antioxidant properties.[21]

Its antimicrobial properties make it an attractive candidate for use as a natural preservative in food products and as an alternative to traditional antibiotics in medical applications. Its anti-inflammatory properties make it a potential candidate for use in treating inflammatory diseases such as arthritis and asthma. Its anticancer properties make it a potential candidate for use in cancer prevention and treatment strategies. Its antioxidant properties make it a potential candidate for use in preventing oxidative stress-related diseases such as cardiovascular disease and neurodegenerative diseases.[21]

Objectives Of Extraction-

1. To obtain grapefruit seeds from discarded grapefruit fruits, which would otherwise go to waste.

2. To extract bioactive compounds, such as polyphenols, flavanones, and phytochemicals, from the seeds.

3. To develop an efficient and cost-effective extraction process that ensures the highest possible yield of these bioactive compounds.

4. To standardize the extraction process to ensure consistency in product quality and potency.

5. To investigate the biological activities of the extracted compounds, such as antimicrobial, anti-inflammatory, anticancer, and antioxidant properties, and to evaluate their safety and efficacy under clinical conditions.

6. To explore potential applications of grapefruit seed extract in various medical fields, such as food preservation, alternative antibiotics for medical applications, anti-inflammatory agents for treating inflammatory diseases, anticancer agents for cancer prevention or treatment strategies, and antioxidant agents for preventing oxidative stress-related diseases.

7. To address concerns regarding the safety and efficacy of grapefruit seed extract due to variability in production methods and lack of standardization by conducting further research into standardization of production methods and evaluation of safety and efficacy under clinical conditions.

Various Extraction Process

There are several extraction processes for grapefruit seed that have been reported in the literature. Here are some of the most commonly used methods:

1. Solvent extraction: This is the most commonly used method for grapefruit seed extraction. The seeds are ground into a fine powder and mixed with a solvent, such as ethanol or methanol, to extract the desired compounds. The solvent is chosen based on its ability to dissolve the desired compounds and its compatibility with the final product's intended use. The solvent extraction process can be carried out using different methods, such as maceration or percolation, depending on the desired outcome.[18]

2. Ultrasound-assisted extraction: This method involves using ultrasound waves to enhance the extraction process. The seeds are ground into a fine powder and mixed with a solvent, such as ethanol or water, and then subjected to ultrasound waves. The ultrasound waves help to break down the cell walls of the seeds, releasing more of the desired compounds into the solvent.[6]

3. Microwave-assisted extraction: This method involves using microwaves to enhance the extraction process. The seeds are ground into a fine powder and mixed with a solvent, such as ethanol or water, and then subjected to microwaves. The microwaves help to heat up the mixture, which can increase the extraction yield by breaking down the cell walls of the seeds and releasing more of the desired compounds into the solvent.[1]

4. Supercritical fluid extraction: This method involves using supercritical fluids, such as carbon dioxide, to extract the desired compounds from grapefruit seeds. The seeds are ground into a fine powder and mixed with supercritical carbon dioxide, which helps to dissolve the desired compounds and separate them from the seeds. This method has been shown to be more efficient than traditional solvent extraction methods because it uses less solvent and produces fewer impurities in the final product.[18]

5. Enzyme-assisted extraction: This method involves using enzymes to break down the cell walls of grapefruit seeds and release more of the desired compounds into the solvent. The seeds are ground
into a fine powder and mixed with an enzyme solution, such as cellulase or pectinase, which helps to break down the cell walls and release more of the desired compounds into the solvent. This method has been shown to be more efficient than traditional solvent extraction methods because it uses less solvent and produces fewer impurities in the final product.[10]

The choice of extraction method depends on several factors, such as the desired compounds, the intended use of the final product, and the availability of resources. For example, solvent extraction is often used for large-scale production because it is relatively simple and cost-effective, while supercritical fluid extraction is often used for small-scale production because it is more efficient and produces fewer impurities in the final product. Enzyme-assisted extraction is often used for specialized applications where specific compounds need to be extracted from grapefruit seeds.

5.IDENTIFICATION TEST

Pharmacognosy is the scientific study of medicinal plants and their derivatives. In pharmacognosy, identification tests are performed to ensure the authenticity, purity, and safety of plant-derived products. Here is a brief overview of some commonly used identification tests for grapefruit seed extract:

1. Microscopic examination: This test involves examining the morphological features of grapefruit seed extract under a microscope to identify characteristic structures, such as starch grains, oil bodies, and cellulose fibers. This test can help to confirm the identity of grapefruit seed extract and differentiate it from other plant-derived products.[2]

2. Thin-layer chromatography (TLC): This test involves separating the constituents of grapefruit seed extract using a TLC plate coated with a specific stationary phase and developing it with a specific mobile phase. The constituents are then visualized using a UV lamp or a specific reagent, such as iodine or sulfuric acid, to identify characteristic spots that correspond to specific compounds.

This test can help to confirm the presence of specific compounds in grapefruit seed extract and differentiate it from other plant-derived products.[5]

3. High-performance liquid chromatography (HPLC): This test involves separating and quantifying the constituents of grapefruit seed extract using an HPLC system equipped with a specific column and mobile phase. The constituents are then detected using a UV detector or a specific detector, such as an electrochemical detector or a mass spectrometer, to identify characteristic peaks that correspond to specific compounds. This test can help to confirm the purity and potency of grapefruit seed extract and differentiate it from other plant-derived products.[13]

4. Fourier transform infrared (FTIR) spectroscopy: This test involves analyzing the infrared spectrum of grapefruit seed extract using an FTIR spectrometer equipped with an attenuated total reflectance (ATR) accessory. The spectrum is then compared with reference spectra to identify characteristic peaks that correspond to specific functional groups, such as hydroxyl (-OH), carbonyl (-C=O), and methyl (-CH3) groups. This test can help to confirm the chemical structure of grapefruit seed extract and differentiate it from other plant-derived products.[6]

6. Nuclear magnetic resonance (NMR) spectroscopy: This test involves analyzing the nuclear magnetic resonance spectrum of grapefruit seed extract using an NMR spectrometer equipped with a specific probe and solvent. The spectrum is then compared with reference spectra to identify characteristic peaks that correspond to specific nuclei, such as protons (-H) and carbons (-C). This test can help to confirm the chemical structure of grapefruit seed extract and differentiate it from other plant-derived products.[6]

Chemical tests

1. Saponification test: This test involves treating grapefruit seed extract with a strong base, such as sodium hydroxide, to convert any ester groups into carboxylate ions. The resulting solution is then acidified with a strong acid, such as hydrochloric acid, to precipitate any saponifiable material, such as triterpenoid saponins. The remaining solution is then analyzed for the presence of non-saponifiable material, such as sterols and fatty acids, using TLC or GC-MS. This test can help to confirm the presence of characteristic saponins in grapefruit seed extract and differentiate it from other plant-derived products that lack saponins.[8]

2. Starch iodine test: This test involves treating grapefruit seed extract with iodine and potassium iodide to form a blue-black color in the presence of starch. This test can help to confirm the presence of starch granules in grapefruit seed extract and differentiate it from other plant-derived products that lack starch.[11]

3. Fehling's test: This test involves treating grapefruit seed extract with a solution of copper sulfate and potassium tartrate in the presence of concentrated sulfuric acid to form a blue color in the presence of reducing sugars, such as glucose and fructose. This test can help to confirm the presence of reducing sugars in grapefruit seed extract and differentiate it from other plant-derived products that lack reducing sugars.[13]

4. Molisch's test: This test involves treating grapefruit seed extract with a solution of phenol and concentrated sulfuric acid to form a red color in the presence of non-reducing sugars, such as sucrose and raffinose.This test can help to confirm the presence of non-reducing sugars in grapefruit seed extract and differentiate it from other plant-derived products that lack non-reducing sugars.[1]

6.CONCLUSION

In conclusion, the review on the extraction and identification of grapefruit seed extract (GSE) for antifungal activity reveals a dynamic and promising field of research. The diverse methods of extraction, ranging from solvent extraction to steam distillation and supercritical fluid extraction, highlight the versatility in obtaining GSE with potential antifungal properties. These methods have been instrumental in isolating a complex mixture of bioactive compounds from grapefruit seeds.

Identification techniques, including Thin-Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), and Nuclear Magnetic Resonance (NMR), have played a crucial role in characterizing the chemical composition of GSE. These techniques allow researchers to identify and quantify specific compounds, providing insights into the diverse phytochemical profile responsible for antifungal activity.

The antifungal activity of GSE, observed in numerous in vitro studies against Candida species, dermatophytes, and filamentous fungi, underscores its potential as a natural antifungal agent. The mechanisms of action, including disruption of cell membranes, enzyme inhibition, and the generation of reactive oxygen species, contribute to its broad-spectrum efficacy.

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