JAZ INDIA

Journal of Advanced Zoology

ISSN: 0253-7214
Volume ***44*** *Issue* ***04 Year 2023*** *Page* ***921:927***

Exploring the Antibacterial Potency of Citrus Fruit Peel Extracts Using Various Solvents

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Article History

Received: 06 June 2023
Revised: 05 Sept 2023
Accepted: 01 Dec 2023

Abstract

The study evaluates the inhibitory effect of different solvent-extracted citrus peel extracts (Citrus lemon, Citrus sinensis, Citrus maxima, Citrus reticulata and Citrus aurantiifolia) on the growth of various bacterial strains (Klebsiella. pneumonia, Staphylococcus. aureus, Pseudomonas aeruginosa, and Escherichia coli). Citrus lemon extract demonstrated the most significant inhibition with methanol and acetone showing the highest zones of inhibition. Citrus sinensis extract showed antibacterial activity, with the choice of solvent influencing the effectiveness. Methanol and acetone generally performed well. Citrus maxima extract demonstrated antibacterial activity, with methanol and butanol being effective solvents. Citrus reticulata extract's effectiveness varied depending on the solvent and bacterial strain tested. Ethanol and butanol were more effective solvents for inhibiting the growth of some bacteria, while acetone and methanol showed limited effectiveness. The Citrus aurentiifolia peel extract exhibited varying degrees of antibacterial activity against the tested bacteria. The effectiveness of the extract depended on the solvent used and the type of bacteria. For some bacteria, such as E. coli and S. aureus, the extract showed significant inhibitory effects, particularly when using Ethanol as the solvent. Further research and optimization may be necessary to enhance the antibacterial properties of Citrus aurentiifolia peel extract.

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Keywords: *Citrus lemon, Citrus sinensis, Citrus maxima, Citrus reticulata, Citrus aurantiifolia, Citrus fruit, Antibacterial, Citrus peel.*

1. Introduction

Numerous fruits are rich sources of diverse antioxidants, including ascorbic acid, flavonoids, and tannins, which are believed to play a crucial role in disease prevention. In the realms of biochemistry and medicine, antioxidants refer to enzymes or other organic substances like Vitamin E or β-carotene that possess the capacity to counteract the harmful effects of oxidation in both animal tissues and food. It has been suggested that, in addition to inherent defense mechanisms within the body, the intake of dietary antioxidants, particularly phenolic compounds, plays a pivotal role in safeguarding against the detrimental impact of reactive oxygen species (Han et al., 2008). The presence of plant phytochemicals, including phenolics, has been linked to health benefits, stemming from the consumption of higher quantities of fruits and vegetables. The most important commercial fruit in the world is citrus fruit, cultivated in almost all regions. Citrus fruits are primarily utilized by juice processing industries, with the peels typically going to waste. Given that the juice yield of citrus accounts for less than half of the fruit's weight, substantial quantities of byproduct waste, including peels, accumulate annually (Manthey and Grohmann,2001).

The perishable and seasonal nature of peel waste poses challenges for both processing industries and pollution monitoring agencies. There is a growing interest in extracting value from waste materials, and citrus waste is no exception. Appropriate methods must be employed to harness their potential for transforming them into value-added products (Nand, 1998).

Recovering by-products from fruit waste can enhance the overall economics of processing units and significantly reduce environmental pollution. Citrus peels, abundant in nutrients and a variety of phytochemicals, have the potential to serve as efficient sources for pharmaceuticals and dietary supplements. With the rising prevalence of antibiotic-resistant pathogens, there is a continuous quest for alternative, safe drugs. If it is established that citrus peels possess antibacterial properties, they could also find application within the food industry, especially in sectors generating substantial amounts of peel waste, as a natural food preservative. Food processors, food safety researchers, and regulatory bodies have shown mounting concern regarding the escalating instances of foodborne illness outbreaks attributed to certain pathogens (Friedman *et al*. (2002).

Citrus fruit derivatives exhibit antimicrobial properties against both bacteria and fungi. The significance of citrus products extends beyond their role in physiology, as they hold substantial commercial value in the global food and pharmaceutical industries (Palombo, E. A., 2011). Citrus fruits and their juices are particularly noteworthy for their rich content of bioactive compounds. These compounds are of vital importance to human nutrition and encompass antioxidants such as ascorbic acid, phenolic compounds, flavonoids, and pectins (Vaishali and Geetha, 2018).

India is the largest producer of medicinal herbs and is appropriately called the botanical garden of world. The extract of orange peel exhibits numerous medicinal properties, including its efficacy against conditions such as colic, upset stomach, cancer, diuretic, carminative, immune enhancement, stomachic, and tonic for the digestive, immune, and skin systems. Furthermore, it is employed in the treatment and prevention of vitamin deficiencies, colds, flu, scurvy, and as a defense against viral and bacterial infections. Notably, research has demonstrated the antibacterial effects of orange peel extract. For instance, Mehmood et al. (2015) reported potent antibacterial activity against enteric pathogens, and Akdemir (2015) found it to be effective against *Klebsiella pneumonia*.

Citrus species are renowned for their abundance of bioactive components, nutraceuticals, and functional compounds found in the peels' flavedo and albedo. These fruits contain flavonoids such as flavanones (neohesperidosides, rutinosides), flavanol glycosides, and flavones (polymethoxyflavones, hydroxylated polymethoxyflavones), with prominent bioactive compounds like naringin and hesperidin (Escobedo-Avellaneda et al., 2014; Ramful et al., 2011). Phenolic compounds, including flavonoids, are recognized for their various health benefits, encompassing antioxidant, antiatherogenic, anti-inflammatory, anticarcinogenic, antiviral, antimicrobial, and antiallergenic activities (Escobedo-Avellaneda et al., 2014). Citrus fruit peels are abundant in flavanones and numerous polymethoxylated flavones, making them a unique and scarce source compared to other plants (Ahmad *et al*. (2006). The antimicrobial properties of essential oils, including citrus oils, are an exceptionally intriguing area for potential applications in the food and cosmetic sectors. (Caccioni *et al*., (1998). It has also been utilized as an anti-diabetic agent (Hamendra and Anand, 2007), an antimicrobial substance (Caccioni et al., 1998), an antifungal remedy (Stange Jr et al., 1993), a hypotensive agent (Kumamoto et al., 1986), an antioxidant (Proteggente et al., 2003;), a carminative, an insect repellent, an antibacterial agent, a larvicidal agent, an antiviral agent, a uricosuric agent, an anti-yeast agent, an antihepatotoxic agent, and an antimutagenic agent (Han, 1998).

This study primarily aimed to address waste minimization in the fruit juice processing industry. By implementing strategies to minimize waste during production and recovering valuable products, the study effectively reduces waste and enhances the environmental sustainability of the fruit juice processing sector. This study explores the antibacterial properties of citrus fruits. It assesses the inhibitory effects of solvent-extracted citrus peel extracts (*Citrus lemon, Citrus sinensis, Citrus maxima, Citrus reticulata, and Citrus aurantiifolia*) on the growth of various bacterial strains, including *Staphylococcus aureus*, *Klebsiella pneumonia*, *Bacillus subtilis*, and *Pseudomonas aeruginosa.*

2. Materials And Methods

Collection of samples

Fresh citrus fruits (*Citrus limon, Citrus sinensis, Citrus maxima, Citrus reticulate, Citrus aurantiifolia)* were purchased from the from the Irrulappapuram Market, Nagercoil, Kanyakumari District, Tamil Nadu. Following the collection process. These fruits were subsequently forwarded to the Department of Botany at Holy Cross College (Autonomous), Nagercoil, for the purpose of authentication.

Microorganisms used

The antibacterial activity was assessed using bacterial strains, including *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa.*

Extracts preparation

The peel of *C. limon*, *C. sinensis*, *C. maxima*, *C. reticulate* and *C. aurantiifolia* were Initially, citrus fruits were sourced from the Irrulappapuram Market. These fruits underwent a thorough cleaning process, first with tap water and then with distilled water. Subsequently, the fruit peels were meticulously separated from the fruits. Each gram of these samples was then immersed in 2 ml of various solvents (ethanol, acetone, butanol, and methanol), and the mixtures were thoroughly ground. Afterward, these ground mixtures underwent centrifugation and filtration through Whatman filter paper, at room temperature. The resulting extracts were collected, and were preserved in a refrigerator for future antibacterial studies.

Antibacterial activity Assay

The antibacterial activity of citrus extracts from the peels of Fresh citrus fruits (*C. limon, C. sinensis, C. maxima, C. reticulate* and *C. aurantiifolia*), dissolved in various solvents, was assessed using the standard disc diffusion method as described by Bauer et al. in 1966. In this experiment, sterilized Muller Hinton Agar (20 ml) was poured into sterile glass petriplates and allowed to solidify. Once solidified, 100 µl of a fresh culture of pathogenic bacteria were evenly spread on the surface of the Muller Hinton Agar plates. Sterile 6 mm discs, loaded with 25 µl of the citrus peel extracts, as well as positive and negative control discs for comparison, were carefully placed on the plates. The positive control disc contained streptomycin (25 µg disc), while the negative control disc was sterile. The plates were then incubated for 24 hours at 37°C. Following incubation, the diameter of the inhibitory zones formed around each disc was measured in millimeters (mm) and recorded for analysis.

3. Results and Discussion

Plants have long been proven to be a source of natural products and medicine for mankind. Although once the source of all medicine, synthetic chemistry has long been used as an active agent in plants, animals, and human disease mitigation. They have adverse effects on the environment and can cause pathogen resistance. Therefore, biological control, or the use of microorganisms or their release to prevent disease, is often an active alternative or adjunct to disease management without the adverse effects of chemical control. The use of medicinal plants plays an important role in meeting basic health needs in developing countries. There are four different types of bacteria tested in this experiment. *E. coli, K. pneumonia, P. aeruginosa,* and *S. aureus* are all common bacteria that can cause various types of infections in humans. The antibacterial activity of Citrus fruit peel extract was tested using four different solvents: Ethanol, Methanol, Acetone, and Butanol.

Antibacterial activity of *C. lemon* peel extract against bacteria

The table 1. shows the results of an antibacterial activity assay using *C. lemon* extract against four different bacteria (*E. coli, K. pneumonia, P. aeruginosa,* and *S. aureus).* The antibacterial activity was tested using different solvents (Ethanol, Methanol, Acetone, and Butanol) and compared to a positive control and a negative control.

Table : 1 Antibacterial activity of *C. lemon* peel extract against bacteria

Bacteria	Zone of inhibition (mm)					
	Ethanol	Methanol	Acetone	Butanol	Positive control	Negative control
E. coli	7±0.7	9.5±1.5	9±0.8	8.3±0.4	18.7±0.8	Na
K. pneumonia	8±0.7	8.5±0.5	9±0.7	8.2±0.8	23.7±0.8	Na
P. aeruginosa	7±0.7	7.5±0.5	10.7±0.8	8±1.2	19.5±1.1	Na
S. aureus	6.5±0.5	8±1.2	10.3±1.2	9±0.7	12±0.8	Na
	Na: not active					

Acetone extract of *C. lemon* showed a maximum zone of inhibition against *P. aeruginosa* (10.7mm), *S. aureus* (10.3mm) followed by *E. coli.* (9mm), and *K. pneumonia* (9mm). Methanol extract of *C. lemon* showed a maximum zone of inhibition against *E. coli.* (9.5mm), whereas the butanol and ethanol extract of *C. lemon* did not show such high antibacterial activity. Acetone had the largest zone of inhibition at 10.7 mm, followed by Ethanol at 7 mm. Acetone produced the largest zone of inhibition at 10.3 mm, followed by Methanol at 8 mm. Acetone seems to be the most effective solvent for inhibiting the growth of these bacteria, particularly against *S. aureus*. *Citrus lemon* extract also exhibits antibacterial activity against *Staphylococcus aureus*, with Acetone having the highest zone of inhibition is comparable to the positive control.

Antibacterial activity of *C. sinensis* peel extract against bacteria

The table. 2 presents the results of an antibacterial activity assay using *C. sinensis* (orange) extract against the bacteria (*E. coli*, *K. Pneumonia*, *P. aeruginosa, and S. aureus*) with different solvents (Ethanol, Methanol, Acetone, and Butanol). *C. sinensis* extract in Methanol resulted in the largest zone of inhibition towards *E. coli*, followed by Acetone at 9.3 mm. Ethanol extract of *C. sinensis* showed a maximum zone of inhibition against *S. aureus* (10mm) followed by *P. aeruginosa* (9mm) *E. coli.* (8mm), and *K. pneumonia* (7mm).

Methanol extract of *C. sinensis* showed a maximum zone of inhibition against *E. coli.* (10mm), Acetone extract showed a maximum zone of inhibition against *K. pneumonia* and *S. aureus* (10mm). whereas the butanol extract of *C. sinensis* did not show such high antibacterial activity.

Table: 2 Antibacterial activity of *C. sinensis* peel extract against bacteria

Bacteria	Zone of inhibition (mm)					
	Ethanol	Methanol	Acetone	Butanol	Positive control	Negative control
E. coli	8.2±1	10.6±0.4	9.3±0.4	7.3±0.4	21.7±2	Na
K. pneumonia	7.6±0.4	7.6±0.4	10±0.8	8.3±0.4	20±3.5	Na
P. aeruginosa	9±0.4	7.7±0.4	8.6±0.4	8±0.8	20.5±1.8	Na
S. aureus	10±0.4	8.5±1.1	10±1.6	8±0.8	16.2±2.5	Na
Na: not active						

Antibacterial activity of *C. maxima* peel extract against bacteria

The table 3 presents the results of an antibacterial activity assay using *C. maxima* (pomelo) extract against the same set of bacteria (*E. coli, K. pneumonia, P. aeruginosa, and S. aureus*) with different solvents (Ethanol, Methanol, Acetone, and Butanol). Methanol extract of *C. sinensis* showed a maximum zone of inhibition against *P. aeruginosa.* (11mm). Ethanol extract of *C. maxima* showed a maximum zone of inhibition against *P. aeruginosa* (10mm) followed by *E. coli.* (9mm), *K. pnemonia* (8mm) and *S. aureus* (7mm). Acetone extract showed a maximum zone of inhibition against *P. aeruginosa.* (10mm). and *E. coli.* (9mm). whereas the butanol extract of *C. maxima* showed such high antibacterial activity with *P. aeruginosa* (10mm).

Table : 3 Antibacterial activity of *Citrus maxima* peel extract against bacteria

Bacteria	Zone of inhibition (mm)					
	Ethanol	Methanol	Acetone	Butanol	Positive control	Negative control
E. coli	9.2±0.8	10.5±1.1	9±0.7	9±0.8	23.7±2.1	Na
K. pneumonia	8±0.7	10.7±0.8	7.6±0.4	9.2±0.8	18.7±0.8	Na
P. aeruginosa	10±1.2	11.6±1.2	10±0.8	10.6±1.2	19.5±0.8	Na
S. aureus	7.3±0.4	10±0.8	7.7±0.8	9.3±0.4	19.5±0.5	Na
Na: not active						

Antibacterial activity of *C. reticulata* peel extract against bacteria

The table 4 provides the results of an antibacterial activity assay using *C. reticulata* (mandarin orange) extract against the same set of bacteria (*E.coli*, *K. pneumonia*, *P. aeruginosa*, and *S. aureus*) with different solvents (Ethanol, Methanol, Acetone, and Butanol). The *C. reticulata* peel extract exhibited varying degrees of antibacterial activity against the tested bacteria. Ethanol extract of *C. reticulata* showed a maximum zone of inhibition against *S. aureus* (14mm) followed by *P. aeruginosa* (10mm), *E. coli.* (9mm), *K. pneumonia* (7mm) whereas methanol, acetone, butanol extract of *C. reticulata* did not show such high antibacterial activity. The effectiveness of the extract depended on the solvent used and the type of bacteria. For some bacteria, such as *E. coli* and *S. aureus*, the extract showed significant inhibitory effects, particularly when using ethanol as the solvent.

Table: 4 Antibacterial activity of *Citrus reticulata* peel extract against bacteria

Bacteria	Zone of inhibition (mm)					
	Ethanol	Methanol	Acetone	Butanol	Positive control	Negative control
E. coli	9±0.8	7.6±0.4	Na	8±0.7	19.7±1	Na
K. pneumonia	7.2±1.6	7.5±0.5	Na	8±0.7	20±0.7	Na
P. aeruginosa	10±0.8	7.3±0.4	Na	7.3±0.4	17.6±2	Na
S. aureus	14±0.8	8±0.8	7.3±0.4	8.5±1.1	7.5±2	Na
Na: not active						

Antibacterial activity of *C. aurentiifolia* peel extract against bacteria

The table 5 results suggest that the choice of solvent significantly impacts the antibacterial activity of the citrus peel extracts. In most cases, the positive control outperformed the citrus extracts, indicating the need for further research and optimization to enhance their antibacterial properties. The table 5 presents the antibacterial activity of *C. aurentiifolia* peel extract against different bacteria, as indicated by the zones of inhibition (mm) in response to various solvents (Ethanol, Methanol, Acetone, and Butanol).

Table : 5 Antibacterial activity of *C. aurentiifolia* peel extract against bacteria

Bacteria	Zone of inhibition (mm)					
	Ethanol	Methanol	Acetone	Butanol	Positive control	Negative control
E. coli	9±0.8	7.2±0.8	7.6±0.4	11±0.8	19.5±0.8	Na
K. pneumonia	7.6±0.4	7±1.6	8.5±1.1	7.6±0.4	18.7±0.8	Na
P. aeruginosa	9±0.7	7.2±1	7.3±0.4	10±0.8	14.2±0.8	Na
S. aureus	7±0.8	8.3±0.4	8.6±0.9	9.5±0.5	14±0.8	Na
Na: not active						

The *C. aurentiifolia* peel extract exhibited varying degrees of antibacterial activity against the tested bacteria. Butanol extract of *C. aurentiifolia* showed a maximum zone of inhibition against *E. coli* (11mm) followed by *P. aeruginosa* (10mm), *S. aureus* (9.5mm) whereas ethanol, extract of *C. aurentiifolia* showed a maximum zone of inhibition against *E. coli* (9mm) followed by *P. aeruginosa* (9mm), acetone, butanol extract of *C. aurentiifolia* did not show such high antibacterial activity. The effectiveness of the extract depended on the solvent used and the type of bacteria. For some bacteria, such as *E. coli* and *S. aureus*, the extract showed significant inhibitory effects, particularly when using ethanol as the solvent.

Discussion

The World Health Organization promotes and facilitates health programs in developing countries. The potential of higher plants as a source of new drugs is still unexplored. Therefore, the last decade has witnessed an increase in research on new biomolecular plant sources for the management of human diseases.

The in vitro evaluation of plants for antimicrobial properties is the first step towards achieving the goal of developing environmentally friendly management of human infectious diseases through the discovery of new plant biomolecules. The use of plant extracts with certain antimicrobial properties can be of great value for therapeutic treatment. The present study was conducted to determine the antimicrobial activity of *citrus* plant using five bacterial strains. Certain Citrus species possess antibacterial potential against clinically significant bacterial strains. Notably, acid-hydrolyzed Citrus fruit peel extract has been shown to inhibit *Bacillus cereus*, *Staphylococcus aureus*, and *Listeria monocytogenes* (Min et al., 2014). As antimicrobial agents, these polyphenols can permeate the semi-permeable cell membrane and interact with the cytoplasm or cellular proteins. The citrus fruit is usually eaten whole or processed into juice after the peeling of the external rind (flavedo). This peeling process leads to the generation of substantial wastes. Citrus peels, which are often discarded as waste products in the food industry, are rich sources of bioactive compounds including flavonoids, limonoids, essential oils, and phenolic compounds, which have been demonstrated to possess antimicrobial properties (Bocco et al., 2018), with diverse pharmacological activities, including antimicrobial effects. Citrus fruits, belonging to the Rutaceae family, include widely consumed varieties such as oranges, lemons, limes, and grapefruits. The peels of these fruits are particularly rich in bioactive compounds. These compounds are known to exhibit inhibitory effects against various skin pathogens (Mahmoud and Nassar, 2018).

In the case of *C. lemon*, the acetone extract exhibited the highest antibacterial activity, particularly against *P. aeruginosa* and *S. aureus*. This extract demonstrated a maximum zone of inhibition against *P. aeruginosa* (10.7 mm) and *S. aureus* (10.3 mm), suggesting its potential as a potent antibacterial agent. Methanol extract of *C. lemon* also showed significant activity against *E. coli*. On the other hand, butanol and ethanol extracts displayed comparatively lower antibacterial activity.

For *C. sinensis*, the ethanol extract displayed notable antibacterial activity against *S. aureus*, while the methanol extract showed efficacy against *E. coli*. Acetone extract exhibited a significant zone of inhibition against *K. pneumonia* and *S. aureus*. The butanol extract, however, did not show high antibacterial activity. *C. maxima* demonstrated antibacterial activity, with the ethanol extract being particularly effective against *P. aeruginosa*. Acetone extract also showed notable inhibition against *P. aeruginosa* and *E. coli*. In contrast, butanol extract displayed high antibacterial activity against *P. aeruginosa*. The antibacterial activity of *C. reticulata* peel extract varied depending on the solvent used. Ethanol extract showed the highest activity against *S. aureus*, while other solvents did not exhibit as high antibacterial effects. In the case of *C. aurentiifolia*, butanol extract displayed significant antibacterial activity against *E. coli*, *P. aeruginosa*, and *S. aureus*. Ethanol extract also showed notable inhibition against *E. coli* and *P. aeruginosa*.

These results highlight the importance of the choice of solvent in extracting bioactive compounds from citrus fruit peels. Additionally, the findings suggest that certain extracts, particularly those obtained with acetone and ethanol, have potential applications as antibacterial agents against specific bacteria. Further investigations, including the isolation and identification of specific bioactive compounds, are warranted to understand the mechanisms behind the observed antibacterial activity and to optimize the extraction process for potential practical applications. This study could be considerable interest to the development of new drugs, further studies on the chemical characteristics of the extract is in progress in order to identify the leads with antimicrobial activity. Citrus peel has significant bioactive chemicals that can be exploited to create a value-added product. The concentration of bioactive chemicals varies depending on the approach utilized. Modern technology and process parameter optimization can considerably improve extraction efficiencies. As a result, there is still much to learn about extraction methods and technology in order to improve flavonoid extraction (Hannah Sakile et al., 2023). In the search for new pharmaceuticals, screening of such different natural organic compounds and the proper identification of bio active agents must be considered as a fruitful approach.

The study indicates that the effectiveness of various Citrus peel extracts against tested bacteria varies depending on the solvent used for extraction and the specific type of bacteria under examination. In general, the study found that the choice of solvent influences the inhibitory effect of the Citrus extracts, with Acetone and Methanol performing well in some cases. However, it's important to note that, in most instances, the positive control (presumably an antibiotic) exhibited a more robust inhibitory effect compared to the Citrus extracts. .*Citrus lemon* extract demonstrated antibacterial activity against the tested bacteria, with Acetone being the most effective solvent in inhibiting the growth of these bacteria, particularly against *Staphylococcus aureus*. *Citrus sinensis* extract also displayed antibacterial activity, with Acetone and Methanol generally performing well as solvents for *Citrus sinensis* extract. However, the positive control (likely an antibiotic) was more potent in most cases. *Citrus maxima* extract exhibited antibacterial activity, and Methanol and Butanol were effective solvents in some cases. The effectiveness of *Citrus reticulata* extract varied depending on the solvent and the type of bacteria. Ethanol and Butanol were more effective solvents in some cases, but, as with the other Citrus extracts, the positive control usually exhibited stronger inhibitory effects. The study suggests that Citrus extracts have antibacterial properties, but their effectiveness varies based on the choice of solvent for extraction and the specific bacteria being targeted. Further research and optimization may be necessary to enhance the antibacterial properties of these Citrus extracts, as the positive control often outperformed them.

4. Conclusion

Repurposing fruit waste through recycling stands out as a crucial method for leveraging it in various innovative ways, leading to the creation of novel products that fulfil the essential needs in human, animal, and plant nutrition, as well as within the pharmaceutical industry. In this research, the antibacterial potential against test organisms *K. pneumonia, S. aureus, P. aeruginosa*, and *E. coli* in the extracts from the peels of *Citrus lemon, Citrus sinensis, Citrus maxima, Citrus reticulata, and Citrus aurantiifolia* obtained through different solvents have been successfully carried out. Nevertheless, to draw a conclusive link between bioactive compounds and antimicrobial activity, additional assessment using pure compounds is imperative. While the specific nature and quantity of active components in each extract remain unclear, they exhibit promising attributes. This finding serves as a foundational platform for subsequent investigations aimed at optimizing the preparation of herbal extracts.

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