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Original Article

Structural Characterization, Antioxidant, Antidiabetic and Antimicrobial Activities of Citrus Limettarisso, Citrus Nobilis X Citrus Deliciosa and Citrus Maxima

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ABSTRACT

Background: The pharmacological properties of citrus fruits have long been recognized in traditional medicine, with recent scientific studies corroborating their potential as sources of natural bioactive compounds. Citrus limettarisso, Citrus nobilis x Citrus deliciosa, and Citrus maxima, in particular, have garnered attention for their antioxidant, antidiabetic, and antimicrobial properties. Prior research has highlighted these species' capabilities, with various studies reporting on their significant health benefits.

Objective: This study aims to evaluate and compare the antioxidant, antidiabetic, and antimicrobial activities of essential oils extracted from the peels of Citrus limettarisso, Citrus nobilis x Citrus deliciosa, and Citrus maxima, thereby contributing to the understanding of their potential therapeutic applications.

Methods: Essential oils were extracted from the peels of the three citrus species using hydro distillation. The antioxidant activity was assessed through Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and DPPH Radical Scavenging Assay. Antiglycation potential and alpha-amylase inhibition were evaluated for antidiabetic properties, while antimicrobial activity was determined using the Agar Well Diffusion Method.

Results: Citrus limettarisso showed the highest TPC (301.4474 ± 2.930402 mg GAE/100g) and significant antioxidant activity (68.26347% DPPH scavenging). Citrus maxima exhibited a high TFC (143.8727 ± 5.454545 mg CE/100g) but lower TPC (115.8333 ± 3.553444 mg GAE/100g). Citrus nobilis x Citrus deliciosa demonstrated considerable TPC (244.1667 ± 5.862774 mg GAE/100g) and TFC (50.17576 ± 0.457566 mg CE/100g). Antiglycation and alpha-amylase inhibition assays revealed Citrus limettarisso as the most potent antidiabetic agent with the highest inhibition percentages. All three species showed significant antimicrobial activity.

Conclusion: The study confirms the substantial pharmacological potential of Citrus limettarisso, Citrus nobilis x Citrus deliciosa, and Citrus maxima, particularly in terms of their antioxidant and antidiabetic properties. Citrus limettarisso emerged as the most potent in most assays, underscoring its potential for therapeutic use. These findings support the use of these citrus species as natural sources of bioactive compounds for health and medical applications. It carries special importance for public health advancements.

Keywords: Citrus limettarisso, Citrus nobilis x Citrus deliciosa, Citrus maxima, Antioxidant, Antidiabetic, Antimicrobial, Phytochemicals, Essential oils.

INTRODUCTION

The extensive use of plant products in traditional medicinal systems has garnered significant attention from researchers, leading to a renewed focus on the pharmacological potential of plant-derived secondary metabolites (1). These metabolites impact disease-causing organisms by interfering with vital metabolic processes, signaling pathways, and gene expression, as demonstrated by Velu et al. (2018)(2). Among these, aromatic plants stand out for their diverse applications in pharmaceuticals, agrochemicals, and as food additives and insecticides (3, 4). Their medicinal properties are attributed to secondary metabolites, with polyphenols playing a critical role in inhibiting mutagenesis and carcinogenesis. Phytoconstituents, responsible for antioxidant activity, and flavonoids, which act as reducing and antimicrobial agents, are particularly notable in this regard, as highlighted by Hussein et al. (2019) (5).

Citrus maxima, commonly known as pomelo and scientifically referred to as Chakotra, is a prime example of this. As the largest citrus fruit in the Rutaceae family and a progenitor of the grapefruit, Citrus maxima possesses a plethora of nutrients, dietary fibers,

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vitamins, and pectins (6, 7). Notably, it contains active phytochemicals such as phenolic acids, flavanones, flavones, and flavonols, which contribute to its antiatherogenic, anti-inflammatory, anticancer, anticlotting, antibacterial, and antioxidant properties (8). Its high antioxidant activity and total phenolic content position Citrus maxima as a superior natural antioxidant additive for foods, offering a healthier alternative to synthetic additives known for carcinogenic side effects, as indicated by Chappalwar et al. (2018) (9).

Another citrus species, Citrus limettarisso, known as mousami, sweet lime, or sweet limetta, is a cultivar of Citrus limon and C. limon 'Limetta'. This species boasts a wide spectrum of biological effects, including antibacterial, antifungal, antidiabetic, anticancer, and antiviral properties (10). The flavonoids in Citrus limettarisso not only act as direct antioxidants and free radical scavengers but also regulate enzyme activity, restrict cellular proliferation, and protect against DNA damage (11). These properties also contribute to the fruit's protective role against pathogens such as bacteria, viruses, and fungi, enhancing its utility in skincare for its antioxidant, antibacterial, and disinfecting capabilities, as Khanal et al. (2022) observed (12).

Lastly, the kinnow, a hybrid of Citrus nobilis (King) and Citrus deliciosa (Willow Leaf), is remarkable for its high vitamin C content and anti-aging properties. This has led to its frequent use in cosmetics. Beyond its skin benefits, the kinnow's minerals contribute to overall metabolism and diabetes management (13). The fruit's antioxidant activity is crucial in the prevention and treatment of various chronic and degenerative diseases, making it a recommended source of dietary antioxidants (14). Rich in essential metabolites like amino acids, nutrients (ascorbic acid, provitamin-A, and folate), and secondary metabolites such as bioactive chemicals (limonoids, flavones, phenolics, and flavonoids), and carotenoids, kinnow is a powerhouse of nutrition and therapeutic potential, as per the findings of Singh (2021) (15).

The investigation of Citrus maxima, Citrus limettarisso, and the kinnow hybrid reveals a wealth of therapeutic and nutritional benefits, underscoring the importance of these fruits in both traditional and modern medicinal practices. Their diverse phytochemical profiles offer promising avenues for the development of natural remedies and health supplements, reinforcing the relevance of plant-derived secondary metabolites in contemporary medical research.

MATERIAL AND METHODS

In the study of the pharmacological properties of citrus peels, an intricate methodological approach was employed to assess their antioxidant, antimicrobial, and antidiabetic activities. The plant materials, specifically the peels of Citrus limettarisso, Citrus nobilis x Citrus deliciosa, and Citrus maxima, were sourced from local markets. These were then carefully washed, sliced into small pieces, and subjected to hydro distillation to extract essential oils.

To evaluate the antioxidant profile of these citrus peels, several assays were conducted. The Total Phenolic Content (TPC) was determined using the Folin-Ciocalteu reagent method. This involves the oxidation of phenols in a solution, followed by the reduction of the resulting blue-colored molybdenum and tungsten oxides. The absorbance of the resulting blue color, which correlates with the total phenolic content, was measured at a wavelength of 750 nm. The assay combined $Na2CO3$ solution, test samples, and diluted reagent, with results expressed in mg GAE/100g, as per the method described by Hussain (16).

Additionally, the Total Flavonoid Content (TFC) was assessed using the AlCl3 colorimetric method. This method is based on the formation of acid-stable AlCl3 complexes with specific hydroxyl and keto groups in flavonoids. Test samples were mixed with NaNO2 and AlCl3, and the absorbance was measured at 510 nm. The findings, expressed as mgCE/100g, were compared against a standard curve.

The DPPH Radical Scavenging Assay further complemented the antioxidant evaluation. This assay measures the ability of the plant extracts to scavenge the 2,2-diphenyl-l-picrylhydrazyl radical. Plant extract was mixed with DPPH solution, and the mixture was covered and allowed to react. The percentage of DPPH scavenging capability was calculated using a specified formula, and the IC50 values were derived from the concentration curve.

For the determination of antibacterial activity, the Agar Well Diffusion Method was utilized. Samples were prepared by mixing ethyl acetate extract with DMSO solution. Agar solution was then prepared, autoclaved, and allowed to cool before being mixed with bacteria and poured onto petri plates. Wells were created in the solidified agar, and samples, along with a positive control (ciprofloxacin), were added. After incubation, the diameter of the inhibition zones was measured.

The antidiabetic potential of the citrus peels was assessed through two assays: the antiglycation assay and the alpha-amylase inhibition assay. The antiglycation assay involved maintaining a reaction solution containing D-glucose, plant extract, and bovine serum albumin at a controlled temperature. Absorbance was measured at specific wavelengths, with metformin used as a reference compound. The alpha-amylase inhibition assay, as conducted by Hussain et al. (2021), involved incubating samples with an amylase solution and a starch solution, followed by the addition of HCl and iodine solution. Absorbance was then measured, and the percentage inhibition was calculated.

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Overall, these methodologies, characterized by their precision and standardization, provided a comprehensive analysis of the pharmacological properties of Citrus limettarisso, Citrus nobilis x Citrus deliciosa, and Citrus maxima, contributing valuable insights into their potential applications in medicine and health.

RESULTS

In the presented study, the results highlight the significant pharmacological potential of essential oils extracted from the peels of Citrus limettarisso, Citrus nobilis x Citrus deliciosa, and Citrus maxima. A comprehensive analysis revealed notable differences in their Total Phenolic Content (TPC), Total Flavonoid Content (TFC), DPPH Radical Scavenging activity, Antiglycation potential, and Alpha-Amylase inhibition.

The Total Phenolic Content (TPC) varied significantly among the three citrus varieties. Citrus limettarisso exhibited the highest phenolic content with 301.4474 ± 2.930402 mg GAE/100g, followed by Citrus nobilis x Citrus deliciosa with 244.1667 ± 5.862774 mg GAE/100g, and Citrus maxima with 115.8333 ± 3.553444 mg GAE/100g. This variation in TPC was statistically significant ($P<0.05$), indicating distinct phytochemical profiles for each peel oil extract.

In terms of Total Flavonoid Content (TFC), Citrus maxima led with 143.8727 ± 5.454545 mg CE/100g, surpassing Citrus limettarisso's 129.3273 ± 3.636364 mg CE/100g, and Citrus nobilis x Citrus deliciosa's 50.17576 ± 0.457566 mg CE/100g. These results also demonstrated a significant difference ($P<0.05$) among the citrus peel oils, suggesting varied potential in their flavonoid-related bioactivities.

The DPPH Radical Scavenging Assay showed that all three citrus peel oils had considerable antioxidant activity, with Citrus limettarisso displaying the highest percentage of DPPH scavenging at 68.26347%. This was closely followed by Citrus nobilis x Citrus deliciosa (68.06387%) and Citrus maxima (67.66467%). The percentage of DPPH activity ranged from 0 to 70%, positioning Citrus limettarisso as the most potent scavenger. The observed differences in antioxidant activity among the extracts were statistically significant ($P<0.05$).

The Antiglycation potential of the essential oils was also examined, revealing that Citrus limettarisso exhibited the highest percentage inhibition, followed by Citrus nobilis x Citrus deliciosa and Citrus maxima. These findings were statistically significant ($P<0.05$) and suggest a descending order of efficacy in inhibiting glycation.

Lastly, the Alpha-Amylase inhibition assay showed that Citrus limettarisso had the highest inhibitory effect with 0.99% inhibition, while Citrus nobilis x Citrus deliciosa and Citrus maxima showed lower inhibitory activities at 0.13% and 0.11%, respectively. This descending order of alpha-amylase inhibition among the citrus peel oils was significant ($P<0.05$), highlighting Citrus limettarisso's potential in managing postprandial blood sugar levels.

Table 1 Phenolic Content

Sample	Total Phenolic Content (TPC) [mg GAE/100g]	Total Flavonoid Content (TFC) [mg CE/100g]	% DPPH Scavenging	Mean Antiglycation	Mean Alpha-Amylase Inhibition (%)
Citrus limettarisso	301.4474 ± 2.930402	129.3273 ± 3.636364	68.26347	0.992845	0.93628
Citrus nobilis x Citrus deliciosa	244.1667 ± 5.862774	50.17576 ± 0.457566	68.06387	0.13923	0.11378
Citrus maxima	115.8333 ± 3.553444	143.8727 ± 5.454545	67.66467	0.110462	0.11649

In summary, these results demonstrate a profound difference in the pharmacological properties of the essential oils derived from Citrus limettarisso, Citrus nobilis x Citrus deliciosa, and Citrus maxima, with Citrus limettarisso generally showing the most potent bioactivities across the assays conducted. The statistical significance of these differences ($P<0.05$) underscores the potential of these citrus peel oils in various therapeutic applications.

DISCUSSION

In this study, the essential oils extracted from the peels of Citrus limettarisso, Citrus nobilis x Citrus deliciosa, and Citrus maxima were evaluated for their pharmacological properties, revealing significant insights into their potential health benefits and therapeutic applications. The findings demonstrated notable variances in Total Phenolic Content (TPC), Total Flavonoid Content (TFC), antioxidant

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activity, antiglycation potential, and alpha-amylase inhibition, each offering a unique perspective on the medicinal value of these citrus fruits.

Monteiro 2022 study on the fresh juice of C. maxima aligns well with the current findings, particularly in highlighting the significant antioxidant and antidiabetic activities of this species (17). This supports the notion that different parts of the citrus fruit, be it peel or juice, contain valuable bioactive compounds (18, 19). The high Total Phenolic Content and Total Flavonoid Content observed in Citrus maxima in the present study corroborate Abirami's findings, suggesting a broad spectrum of health benefits across different extracts of the same fruit (20, 21).

Similarly, Malik's 2021 research on the peel extract of C. nobilis emphasizes its strong antioxidant, antimicrobial, and anti-inflammatory properties (22). This complements the findings of the current study, where Citrus nobilis x Citrus deliciosa exhibited significant antioxidant activity. The consistency between these studies underscores the reliability of the antioxidant potential of Citrus nobilis, reinforcing its utility in natural health products (23).

Maurya 2018 investigation into the essential oil of C. limetta var. Mitha as an effective antimicrobial and antioxidant agent provides further context to the current study’s findings on Citrus limettarisso (24). The potent bioactivities observed in C. limetta var. Mitha resonate with the high antioxidant and antimicrobial properties noted in Citrus limettarisso, suggesting a common thread of beneficial phytochemicals in different varieties within the Citrus genus.

Damián-Reyna's 2018 study, which highlights the antioxidant and antibacterial properties of C. limetta and C. reticulata, offers a broader perspective. While the current study did not specifically investigate C. reticulata, the mention of C. limetta’s potent effects parallels the findings on Citrus limettarisso (25). This comparison further cements the role of citrus peels as reservoirs of natural compounds with significant therapeutic potential.

CONCLUSION

In conclusion, the amalgamation of the current research with preceding studies offers a detailed and multi-faceted view of the medicinal capabilities of Citrus limettarisso, Citrus nobilis x Citrus deliciosa, and Citrus maxima. The consistency in findings across various scientific inquiries underscores the significant potential of these citrus species as reservoirs of natural bioactive compounds, with implications for health enhancement and disease prevention. Such insights not only affirm the outcomes of this study but also illuminate the broad spectrum of applications these fruits hold in the realms of healthcare and wellness. Looking ahead, further exploration into the specific active compounds within these citrus species and their precise mechanisms of action is warranted. This could pave the way for their incorporation into functional foods, nutraceuticals, and bespoke pharmacological treatments. Additionally, these findings bear relevance for public health policy, as understanding the health benefits of readily available natural resources like citrus fruits could inform nutritional guidelines and health promotion strategies. The potential for these fruits to contribute to non-pharmacological interventions and preventive healthcare measures aligns with a growing global emphasis on natural and holistic approaches to health, thereby influencing public health policy and practices.

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