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Abstract

Profiling of Phenolic Compounds in *Citrus* Flowers and their Biological Activities

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Citrus, a genus of flowering plants in the Rutaceae family, holds substantial market importance as a crop worldwide. After prolonged periods of breeding and extensive hybridization, numerous species have emerged, each possessing a unique metabolism that produces a diverse array of secondary metabolites. It is important to note that phenolic compounds, in particular phenols and flavonoids, are among the most important secondary metabolites in *Citrus* flowers. These chemical compositions of *Citrus* flowers differ depending on a variety of factors, such as variety, fruit maturity, environmental conditions, storage conditions, and extraction methods. Nevertheless, phenolic compounds extracted from *Citrus* flowers are well-recognized for their bioavailability characteristics and exhibit numerous health-promoting effects, including antioxidative, anti-inflammatory, anticancer and antibacterial activities in humans. The information contained in this document provides a comprehensive summary of the latest investigations conducted on the subject matter. The aim is to thoroughly comprehend the biological functions of the bioactive compounds in the *Citrus* flowers and their potential impacts on various biological systems, shedding light on their potential therapeutic applications.

Keywords: *Citrus* flowers, phenolic compounds, biological functions, biological activities, therapeutic applications.

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1. Introduction

Citrus encompassing both fresh fruit and their derived products hold considerable significance within the global market and the agricultural section. Their pivotal role is underscored by their widespread cultivation across over 140 countries worldwide, contributing to annual consumption and trade [1]. In the year 2021, the total output of fresh *citrus* fruit reached a staggering 143 thousand tonnes (Figure 1) [2]. The attraction of *citrus* fruits resides not only in their delectable flavor but also in their captivating colourings and aromas. Moreover, these fruits have a combination of health-enhancing constituents, comprising vitamins, minerals, essential macronutrients like carbohydrates, dietary fibers, crude proteins, lipids, and critical phenolic compounds [3]. Remarkably, the utilization of *citrus* fruits extends beyond their consumption in their natural form. Approximately one-third of the global *citrus* output is allocated to various industries, further attesting to their extensive utility [4].

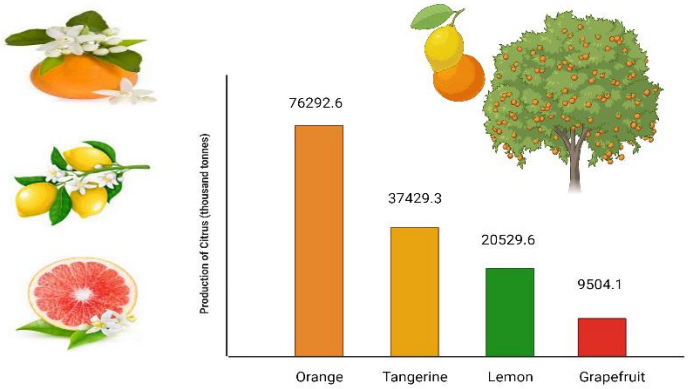

Figure 1. Global total production of four different Citrus species in the year 2021.

The *citrus* genus demonstrates classification into four principal groupings: oranges, encompassing both sweet orange (*Citrus sinensis*) and sour orange (*Citrus aurantium*); tangerines (*Citrus reticulata*); grapefruits (*Citrus paradisi*); and lemons (*Citrus limon*) or limes (*Citrus aurantiifolia*). Amongst the extensively cultivated *citrus* varieties, oranges emerge as the foremost contributors, comprising over fifty percent of the global *citrus* yield. Notably, they also dominate the international *citrus* trade, followed in succession by tangerines, lemons, and grapefruits. Also, in industry, approximately 80% of processed *citrus* fruits belong to the orange category, constituting the predominant share within the total processed citrus volume [1,2,5].

In recent times, there has been a surge in research focusing on the constituents of *citrus* fruits. This encompasses various parts such as fruit juice, flowers, seeds, leaves, and peels, with a primary emphasis on the composition of bioactive compounds [4]. It's worth noting, however, that despite the extensive exploration of these components, there remains a scarcity of comprehensive information particularly concerning *citrus* flowers. Consequently, in order to attain a complete comprehension of the biological functionalities inherent to the bioactive compounds within citrus flowers and their potential ramifications across diverse biological systems, a thorough investigation is imperative. This study aims to unravel the latent therapeutic utilities that these compounds might offer.

2. Phenolic compounds and their biological activities in *Citrus* flowers

The bioactive phytochemicals present in *citrus* flowers exhibit a remarkable diversity, encompassing various subclasses, most notably polyphenols which further include phenolic acids and flavonoids. In relation to phenolic compounds, recent results have highlighted the feasibility of distinguishing citrus species through analysis of their distinctive phenolic compound profiles.

The flowers of *Citrus aurantium* L. (Bitter orange) are considered as a promising natural source of ingredients in the development and formulation of functional foods and pharmaceutical products. A study investigated the potential in vitro antioxidant activity of *C. aurantium* L. flowers and concerning polyphenols content, Total phenolic content (TPC), and total flavonoid content (TFC) were 18.22 ± 1.44 mg GAE /g dw and 111.44 ± 20.60 µg QE /g dw, respectively, thus confirming the efficiency of the extraction procedure in extracting bioactive compounds [6]. One research represented the investigation of the potential anti-ageing and anti-wrinkle effects attributed to the flowers of *Citrus aurantium*; Conducting this investigation involved an examination of the physiological activity of a 50% ethanolic extract derived from citrus flowers, both prior to and subsequent to the fermentation process. These mentioned compounds had the highest amount; Phenolic (pyrogallol, syringic acid, ferulic acid and caffeic acid) and Flavonoids (neohesperidin,

rutin and naringin) [7]. In another study, Zhao et el. aimed to isolate constituents from the flowers of *Citrus aurantium* L. var. amara Engl (a specific type of *Citrus aurantium* L.) in order to assess their potential activities in terms of antitumor, antioxidant, antivirus, and anti-inflammatory effects. The main compound identified was 5-hydroxy-6,7,3,4-tetramethoxyflavone (HTF) [8]. Nabavi et al. compared four flowers of *Citrus* in terms of antioxidant activities. *Citrus aurantium, Citrus sinensis, Citrus nobilis, and Citrus limon* have TPC contents of 78.76, 78.47, 62.10 and 60.01 mg gallic acid equivalent/g of extract, respectively. Furthermore, *Citrus limon* and *Citrus aurantium* exhibited the highest value in TFC [9]. Anthocyanins are a subgroup within the extensive category of phenolic compounds, which are collectively classified under the group of flavonoids. The presence of numerous types of anthocyanins in flowers of lemon (*Citrus limon* (L.) Burm. f.), citron (*Citrus medica* L.), Piretto lemon (*Citrus limoni medica* Lush.), Rangpur lime (*Citrus limonia* Osbeck), and Meyer lemon (*Citrus meyeri* Y. Tan.) has been confirmed [10]. Moreover, coumarins, a subset of polyphenolic compounds, have demonstrated noteworthy anti-cancer attributes through the engagement of multiple mechanisms of action [11]. Pellizzeri et al., found coumarins in flowers of two citrus species, namely lemon (*Citrus limon (L.)* Osbeck) and grapefruit (*Citrus paradise* Macfad.) and they were determined as valuable resources for the perfumery and cosmetic industries [12].

3. Concluding Remarks

Citrus flowers emerge as valuable reservoirs of bioactive compounds, particularly phenolics and flavonoids, renowned for their pivotal role as natural antioxidants. In regards to abundant production and notable scientific interest, it is evident that the benefits offered by *Citrus aurantium* flowers surpass those of other citrus flower variants. Numerous comparative investigations have consistently demonstrated that C. aurantium flowers boast the highest levels of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). These secondary metabolites yield a host of advantageous biological and medicinal effects. These encompass a wide array of benefits, ranging from antioxidant and antimicrobial properties to potential anti-cancer, anti-diabetic, anti-obesity, and anti-anxiety effects. Moreover, their application extends to the cosmetic and food industries. In light of these findings, it is evident that C. aurantium flowers, among other citrus species, hold significant promise for future research endeavors.

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References

1. Chen Y, Barzee TJ, Zhang R, Pan Z. Citrus. Integr Process Technol Food Agric By-Products [Internet]. Elsevier; 2019. p. 217–42. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780128141380000095

2. FAO. 2021. Citrus Fruit Statistical Compendium 2020. Rome.

3. Zhao C, Wang F, Lian Y, Xiao H, Zheng J. Biosynthesis of citrus flavonoids and their health effects. Crit Rev Food Sci Nutr [Internet]. Taylor & Francis; 2020;60:566–83. Available from: https://doi.org/10.1080/10408398.2018.1544885

4. Tayengwa T, Katiyatiya chenaimoyo L., Mahachi LN, Chikwanha OC, Mapiye C. Mediterranean Fruits Bio-wastes. In: Ramadan MF, Farag MA, editors. Mediterr Fruits Bio-wastes. Springer; 2022. p. 140–77.

5. Rahman MM, Jahan FI, Mim SA. A brief phytochemical investigation and pharmacological uses of citrus seed – a review. Pharmacologyonline. 2019. p. 94–103.

6. Seyyedi-Mansour S, Chamorro F, Donn P, Carpena M, Pereira A., Otero P. Exploring the potential of Citrus aurantium L . flowers as a source of antioxidant compounds. Santiago de Compostela (Spain); 2022. Report No.: The XXVIth Galician-Portuguese International Conference On Chemistry. Poster.

7. Chen CY, Hu CY, Chen YH, Li YT, Chung YC. Submerged fermentation with Lactobacillus brevis significantly improved the physiological activities of Citrus aurantium flower extract. Heliyon. 2022;8:0–9.

8. Zhao H-Y, Yang L, Wei J, Huang M, Jiang JG. Bioactivity evaluations of ingredients extracted from the flowers of Citrus aurantium L. var. amara Engl. Food Chem. 2012;135:2175–81.

9. Nabavi SF, Nabavi SM, Ebrahimzadeh MA. Antioxidant activity of hydro-alcoholic extracts of 4 citrus species flower. Prog Nutr. 2016;18:74–80.

10. Fabroni S, Ballistreri G, Amenta M, Rapisarda P. Anthocyanins in different citrus species: An UHPLC-PDA-ESI/MSn-assisted qualitative and quantitative investigation. J Sci Food Agric. 2016;96:4797–808.

11. Davatgaran-Taghipour Y, Masoomzadeh S, Farzaei MH, Bahramsoltani R, Karimi-Soureh Z, Rahimi R, et al. Polyphenol nanoformulations for cancer therapy: experimental evidence and clinical perspective. Int J Nanomedicine [Internet]. 2017;Volume 12:2689–702. Available from: https://www.dovepress.com/polyphenol-nanoformulations-for-cancer-therapy-experimental-evidence-a-peer-reviewed-article-IJN

12. Pellizzeri V, Costa R, Grasso E, Dugo G. Valuable products from the flowers of lemon (Citrus limon (L.) Osbeck) and grapefruit (Citrus paradisi Macfad.) Italian trees. Food Bioprod Process [Internet]. 2020;123:123–33. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0960308520304442