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Development of Microcapsules of Bioactive Compounds Extracted from Grape Pomace of Ciravas Agra

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Abstract: Grape pomace is a prominent byproduct of winemaking, which provides bioactive phytochemicals such as polyphenols, conferring health benefits to humans, including anticancer properties. Delivering bioactive compounds extracted from grape pomace as microcapsules offers a sustainable and effective solution for developing tailored leukemia therapies. This approach highlights integrating environmentally friendly practices with medical innovation, mainly through the valorization of grape pomace, a byproduct of winemaking. For this purpose, the study uses ultrasound-assisted extraction (UAE) to optimize the extraction of these bioactive compounds. It evaluates how temperature and time affect antioxidant activity and total phenolic content. The results indicated that 60 minutes was optimal for the extraction yield of UAE with appreciable antioxidants (73.2 ± 1.5%) and total phenolic content (75.3 ± 2.1 mg GAE/g) compared to other methods studied here. Molecular docking analysis further revealed strong interactions between anthocyanins, particularly delphinidin-3-O-glucoside, and proteins associated with Acute Promyelocytic Leukemia (APL) and Chronic Myeloid Leukemia (CML), suggesting potential therapeutic applications. The present study offers a new perspective on the importance of grape pomace as an eco-friendly source of bioactive molecules in line with green chemistry ethics that may find applications in pharmaceuticals or nutraceutical sectors.

Keywords: Bioactive compounds; Grape Pomace; Cancer; Ultra-Sound Assisted Extraction; Microencapsulation; Wine; Grape Juice; Valorization; Leukemia; Phenolic Compounds; Phenols.

Introduction

Currently, the wine industry produces about 20 million tons of grape byproducts yearly, of which the most frequent byproduct is grape pomace that comprises skins, stems, seeds, and pulp residue (Ferrer-Gallego & Silva, 2022). The management of this waste entails serious implications as far as the environment is concerned in regards to environmental approach according to Siller-Sánchez et al., 2024. The grape pomace contains bioactive compounds including polyphenols, flavonoids, and anthocyanin with several health promoting effects (Almanza-Oliveros et al., 2024). These bioactive compounds are supplied with antioxidants, anticancer, and antimicrobial effects (Caponio, 2023). Remarkably, these bioactive compounds are also present in the grape pomace and have an appropriate therapeutic potential for treating various malignant diseases such as cancer (Asma et al., 2022).

The secondary metabolites contained in the grape pomace have anti mutagenic effects due to; apoptosis and cell cycle arrest in the cancer cells (Asma et al., 2022). It has been identified that some of these extracts are capable of selectively affecting the cancer cells and inhibiting their proliferation. Nevertheless, they do this without affecting the functionality of normal cells in a negative manner. This is why they are regarded to be more efficient than traditional treatments aimed at cancer that had numerous side effects (Subramaniam et

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al., 2019). In the following current research study, extraction of bioactive compounds from Ciravas Agra grape pomace is carried out and the current study also explores the molecular interaction of the identified compounds with proteins associated with APL and CML. APL is one of the subtypes of acute myeloid leukemia that is caused by chromosomes 15/17 translocation (De Almeida et al., 2023). When these types of treatment such as ATRA, chemotherapy, and arsenic trioxide are employed; the cure rates are high (Yilmaz et al., 2021). However, early intervention is needed because of high risks of bleeding as indicated in studies by Alimoghaddam (2014). CML has a Philadelphia chromosome, a translocation between chromosomes 9 and 22, producing the BCR-ABL fusion gene(Khalid & Riasat, 2023).

Tyrosine kinase inhibitors (TKIs) are highly effective -Imatinib Dasatinib and Nilotinib can bring uninterrupted remission and the average life expectancy proving that if the client follows the doctor's orders(Yoshida et al., 2023). However, new treatment options are still needed due to the existence of drug resistance, side effects, and patients' desire for a definitive cure (Alves et al., 2021). This paper will concentrate on the Ciravas Agra grape variety identified in the far eastern portion of the Russian Federation; little investigation has been done on the bioactive profile of this grape variety (JKI, 2024).

Method

In Vitro Analysis:

This study used the Ultra-Sound Assisted Extraction (UAE) technique to extract bioactive compounds from Ciravas Agra grape pomace; the method was selected because of the high efficiency it offers in the extraction process(Kumar et al., 2020). The extraction process involved change in temperature 30, 40, 50 and 60°C and time intervals of 10, 20, 30 and 40 minutes where DPPH assay used for radical scavenging activity (Baliyan et al., 2022). Total phenolic content was determined according to the Folin-Ciocalteu method (Siddiqui et al., 2017). As shown in Table 1 below, a research matrix has been created to determine the effects of time and temperature on the UAE.

Table 1. Research Matrix

Sample	Power	Variable One Time (X)	Variable Two Temperature (Y)
Zero = Z	100 Watts	X = 5 minutes	Y = 60°C
1 (X-2: Y-2)	100 Watts	3 minutes	40°C
2 (X+2: Y+2)	100 Watts	7 minutes	80°C
3 (X: Y-2)	100 Watts	5 minutes	40°C
4 (X-2: Y)	100 Watts	3 minutes	60°C
5 (X+2: Y)	100 Watts	7 minutes	60°C
6 (X: Y+2)	100 Watts	5 minutes	80°C
7 (X-2: Y+2)	100 Watts	3 minutes	80°C
8 (X+2: Y-2)	100 Watts	7 minutes	40°C

In Silico Analysis:

The molecular docking analysis was carried out for the in silico investigation of the potential mode of interaction of the bioactive compounds with the target proteins in APL and CML (de Almeida et al., 2023). This research provided a grape bioactive compounds list, anthocyanins, catechins, quercetin, gallic acid, and resveratrol (Sabra et al., 2021). The structures of target proteins (Abl1, Akt1, Cdks, Flat, Il-18, Mapkapk1, Mapkapk3, Mapk14, Myc) have been downloaded from the protein databank (PDB) (RCSB et al. Bank, 2024). In the present study, molecular docking has been performed using a MOE software and the docking score and binding affinity of the compounds has been mentioned as has been done in the previous studies mentioned by Attique et al., 2019. In the construction of interaction maps and in the representation of the docking outcomes in form of 3D models, the MOE software was employed in the bioinformatics analysis as used by Mahnashi et al. (2021). Potential inhibitors were also considered through the calculation of the binding energies of the bioactive compounds to the target proteins (Rath et al., 2021). Evaluated indices involved docking scores, binding energies, hydrogen bonds, and hydrophobic interactions.

Result and Discussion

In Vitro Results:

The results obtained through the UAE method showed that the extraction efficiency was dependent on the temperature and time that was provided to the sample. The maximum antioxidant activity and total phenolic content were recorded at 60° C for 30 min with 73. 2 ± 1. 5% and 75. 3 ± 2. 1 mg GAE/g respectively. The absorbance values for the DPPH assay ranged from 0.320 to 0.489, indicating strong radical scavenging activity. The results of the antioxidant activity and total phenolic contents of the samples are presented in Tables 2 and 3, respectively.

Table 2. Results of DPPH assay: Antioxidant Activity

Sample	Power	Variable One Time (X)	Variable Two Temperature (Y)	Antioxidant Activity (Percentage)
Zero = Z	100 Watts	X = 5 minutes	Y = 60°C	43
1 (X-2: Y-2)	100 Watts	3 minutes	40°C	38
2 (X+2: Y+2)	100 Watts	7 minutes	80°C	75
3 (X: Y-2)	100 Watts	5 minutes	40°C	20
4 (X-2: Y)	100 Watts	3 minutes	60°C	39
5 (X+2: Y)	100 Watts	7 minutes	60°C	29
6 (X: Y+2)	100 Watts	5 minutes	80°C	56
7 (X-2: Y+2)	100 Watts	3 minutes	80°C	37
8 (X+2: Y-2)	100 Watts	7 minutes	40°C	53

Table 3. Results: Total Phenolic Content

Sample	Power	Variable One Time (X)	Variable Two Temperature (Y)	Antioxidant Activity (Percentage)
Zero = Z	100 Watts	X = 5 minutes	Y = 60°C	43
1 (X-2: Y-2)	100 Watts	3 minutes	40°C	38
2 (X+2: Y+2)	100 Watts	7 minutes	80°C	75
3 (X: Y-2)	100 Watts	5 minutes	40°C	20
4 (X-2: Y)	100 Watts	3 minutes	60°C	39
5 (X+2: Y)	100 Watts	7 minutes	60°C	29
6 (X: Y+2)	100 Watts	5 minutes	80°C	56
7 (X-2: Y+2)	100 Watts	3 minutes	80°C	37
8 (X+2: Y-2)	100 Watts	7 minutes	40°C	53

In Silico Results:

Molecular docking results revealed strong binding interactions between anthocyanins and leukemia-related proteins. Delphinidin-3-O-glucoside exhibited the highest docking score with AKT1 (-12.5 kcal/mol) and FLT3 (-11.7 kcal/mol), indicating a high binding affinity. The interaction maps showed multiple hydrogen bonds and hydrophobic interactions, suggesting stable complexes. Detailed docking results are summarized in Table 5 and visualized in Figures 1 and 2.

Table 4. Summarized results of the docking between ligands (bioactive compounds commonly found in the grape pomace) and key proteins involved in APL and CML

Protein	Ligand	S Value
AKT1	10-Delphinidin 3-O-glucoside	-10.5295
CDK4	Catechin	-12.5798
CDK6	Rutin	-10.7489
CDK2	Gallic Acid	-10.8400
FLT3	Epicatechin	-9.8622
IL18	10-Delphinidin 3-O-glucoside	-11.0983
MAPK1	Malvidin 3-O-glucoside	-13.7762
MAPK3	Epicatechin	-16.2486
MAPK14	10-Delphinidin 3-O-glucoside	-14.3144
MYC	Catechin	-9.4951

The bioinformatics analysis provided comprehensive insights into the molecular interactions. Anthocyanins formed stable complexes with target proteins, with binding energies ranging from -9.5 to -12.5 kcal/mol. Interaction maps and 3D models demonstrated that these compounds could effectively inhibit leukemia-related proteins. The results suggest that anthocyanins have significant therapeutic potential for leukemia treatment.

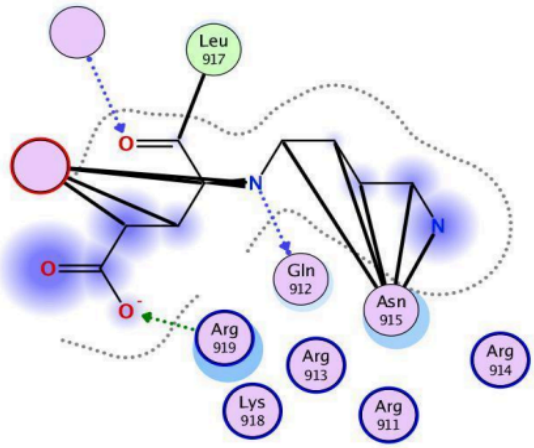

Figure 1. Interaction between library of compounds and FLT3 protein

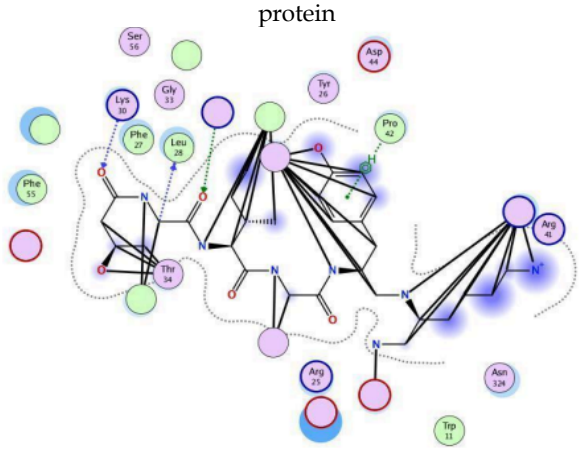

Figure 2. Interaction between the library of compounds and AKT1 protein

Grape pomace serves as an affordable source of bioactive compounds that have anticancer profile. It is environmentally compatible with the tenets of green chemistry in agronomic and other allied sectors (Almanza-Oliveros et al., 2024). The results derived from this study offer valuable directions for valorizing grape pomace, mainly sourced from the Ciravas Agra type, by focusing on the extraction and utilization of bioactive compounds. UAE provided high yields of phenolic compounds, and the research demonstrated that this extraction method could be scaled up for industrial purposes in the UAE. The optimum extraction condition that yielded the highest antioxidant activity and total phenolic content supported the earlier finding that UAE has several advantages over conventional extraction methods; it requires a lesser amount of solvent and lower energy input (Rodsamran & Sothornvit, 2019). The result of the molecular docking studies gives information regarding the possibilities of the bioactive compounds isolated from the plant being used for their therapeutic effects. As anthocyanins revealed extremely high binding specificities toward the target proteins, they can be considered as natural negative regulators of leukemia signaling (Lin et al., 2017). This is in agreement with other studies done that showed that anthocyanin had anticancer activity (Zhao et al., 2023). The current experiment can therefore explain how the molecules engage with APL and CML. Such interaction includes several hydrogen bonding and hydrophobic interactions through which these compounds are capable of interacting with certain protein targets that are very vital in the growth and sustenance of the cancer cells (Zinzalla & Thurston, 2009). Also, the data from bioinformatics analysis provided enhanced comprehension of the behavior of enzymes and their targets, which is unconditionally necessary at the development of the drugs for specific diseases (Agu et al., 2023). The binding energies and interaction maps enhance the fact that natural anthocyanin compounds have the ability to be used for therapeutic applications (Jaiswal et al., 2019). To sum up, the synchronization of in silico strategies with in vitro screening forms a sound platform for natural product studies and constitutes a way of forecasting bioactive compounds. This research finding will be beneficial to food and pharma industries primarily because the bioactive compounds are not affected by the processing and storage conditions.

Conclusion

The study has established the groundwork for the extraction, characterization, and application of bioactive compounds from Ciravas Agra grape pomace. High UAE yields antioxidant activity and total phenolic content when subjected to the experimental conditions: molecular docking indicates therapeutic potential towards leukemia proteins. These results demonstrate the potential of further research focusing on applications of grape pomace in order to improve the health of consumers as well as increase the efficiency of bioactive molecules for pharmaceutical use.

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The authors declare no conflict of interest

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