

ENHANCING POLYPHENOL DELIVERY SYSTEMS FOR EFFECTIVE CHRONIC KIDNEY DISEASE MANAGEMENT

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Abstract. Nature provided the inspiration and the ingredients for therapeutic medicine. More than 50% of all current medicinal drugs are made from herbal plants, roots, and fruits. According to the World Health Organization, 75% of the world's population uses plant-based medicine. These products contain only purified ingredients that may reduce the final product's antioxidant, antibacterial, and antimicrobial properties. Therefore, we aim to develop a method of extraction that may stabilize the antioxidant properties of the product.

Polyphenols are organic compounds found in fruits and vegetables such as onions, grapes, berries, cherries, broccoli, and citrus. Polyphenols exhibit significant antibacterial and antimicrobial activity, antihypertensive and vasodilator effects, and antihypercholesterolemic and antiatherosclerotic activities. Conventional extraction methods, such as ultrasound-assisted extraction, solvent extraction, supercritical fluid extraction, or microwave-assisted extraction, use solvents with different polarities (ethanol, methanol, water, and chloroform) or elevated temperatures. Here, we developed a method of extraction and stabilization that involves an alcoholic solution and plant-based stabilizers.

Key words. Polyphenols, ultrasound-assisted extraction, chronic kidney disease, antioxidant activity, natural stabilizers, bioactive compounds, micelles,

agricultural waste valorization, plant-based medicine, drug delivery systems, red onions, grape skins, bioavailability, nanoparticles.

Introduction

The human body is a complex network of systems working synergistically to maintain balance and health, with the excretory system playing a crucial role in this process. Central to this system are the kidneys, vital organs that filter waste products and excess substances from the bloodstream, maintaining the body's chemical stability. Each kidney contains approximately 1-1.3 million per adult filtering units called nephrons [1, 2]. Each nephron houses a glomerulus, a tiny blood vessel knot encased in a cup-shaped structure known as Bowman's capsule. Blood enters the glomerulus through the afferent arteriole and exits through the efferent arteriole. The high blood pressure in the glomerulus forces water, salt, glucose, amino acids, and various waste products from the blood into Bowman's capsule, forming the glomerular filtrate. This filtration process is selective, primarily based on size, allowing only small molecules to pass through while larger molecules and blood cells are retained in the bloodstream. Following filtration, the glomerular filtrate travels through the renal tubule, which is composed of the proximal convoluted tubule, loop of Henle, distal convoluted tubule, and the collecting duct. As the filtrate moves, critical nutrients and a significant portion of the water are reabsorbed into the bloodstream. The proximal tubule reabsorbs around 65% of the filtered sodium, water, and nutrients critical for maintaining the pH balance. The loop of Henle, particularly its ascending limb, aids in the reabsorption of ions in a manner that helps create a concentration gradient in the kidney's medulla. While reabsorption is focused on pulling valuable substances back into the blood, secretion deals with the selective addition of ions, creatinine, drugs, and toxins from the blood into the tubular fluid. This process primarily occurs in the distal and collecting tubules, where substances such as potassium, hydrogen ions, and certain organic acids and bases are secreted. This mechanism not only disposes of waste substances but also assists in fine-tuning blood pH. Urine is the final modified solution remaining in the renal tubules after reabsorption and secretion. This fluid contains waste products like urea and creatinine, excess ions, toxins, and water that the body needs to eliminate. Urine flows from the collecting ducts into the renal pelvis, down the ureters, and into the bladder, where it is stored until elimination through the urethra.

Kidney function is regulated by a combination of hormonal and neural mechanisms [3-5]. For example, the antidiuretic hormone (ADH) plays a critical role in water balance by promoting water reabsorption in the distal tubules and collecting ducts of the nephrons. ADH effectively reduces urine output to conserve water during dehydration or increased plasma osmolality. Similarly, the renin-angiotensin-aldosterone system (RAAS) is essential in regulating both blood pressure and fluid balance, operating through mechanisms that adjust blood flow into and out of the glomerulus and modify sodium reabsorption. Additionally, atrial natriuretic peptide (ANP) serves as a counter-regulatory mechanism to the RAAS, promoting the excretion of sodium and water in response to excessive blood volume, thus maintaining equilibrium.

Despite these complicated regulatory mechanisms, the kidneys are vulnerable to a variety of diseases [6-10]. (a) Chronic kidney disease (CKD) represents a significant health concern, characterized by the progressive loss of kidney function over time. This deterioration can result in end-stage renal disease (ESRD), a critical condition that often necessitates dialysis or kidney transplantation for the patient's survival. The primary causes of CKD include diabetes, hypertension, and glomerulonephritis. (b) Polycystic kidney disease (PKD) is another incurable genetic disorder characterized by the growth of numerous cysts in the kidneys, managed primarily through symptomatic treatments. According to the World Health Organization, CKD is expected to become the fifth most common chronic condition globally by 2040. The principal risk factors contributing to CKD include diabetes, hypertension, obesity, smoking, and genetic predispositions. Among these, hypertension and diabetes are particularly significant, together accounting for approximately two-thirds of all CKD cases worldwide [11]. The progression of CKD varies widely and is often asymptomatic in its initial phases, making early detection difficult. As CKD progresses, the risks of developing end-stage renal disease (ESRD) and encountering serious cardiovascular issues increase. Therefore, implementing early intervention and preventive strategies is essential for effectively managing the long-term effects of the disease [12]. Pathophysiologically, CKD involves damage to the glomerular, tubular, or vascular components of the kidneys, stemming from genetic conditions like Alport syndrome, or acquired factors such as injuries or toxin exposure. Immune complexes can deposit in various parts of the glomerulus, leading to intense inflammatory responses that cause vascular and filtration barrier damage,

exacerbating kidney impairment. These immune responses, along with complement activation and non-immunological factors like hemodynamic stress and metabolic disturbances, increase the complexity and severity of CKD [13].

Current treatment strategies focus on slowing the progression of the disease through blood pressure control, blood sugar management, and cholesterol levels, along with a diet low in protein and salt. Recent advancements in biotechnology have opened new avenues for treating kidney diseases. One promising area is regenerative medicine, which involves the use of stem cells to regenerate damaged kidney tissues [14-16]. Clinical trials are currently exploring the potential of stem cells to halt the progression of CKD and repair kidney tissues, although this research is still in its early stages. Another emerging treatment is the development of artificial or bioartificial kidneys, which aim to mimic the kidney's filtration functions [17-19]. These bioartificial kidneys, grown from the patient's cells, could potentially eliminate the problems of organ rejection and donor shortages. However, these devices, still under development, hold the promise of supplementing or even replacing traditional dialysis. Additionally, advancements in immunotherapy and personalized medicine are being tailored to address autoimmune kidney diseases and genetic disorders, potentially leading to more effective and targeted treatments [20].

While traditional treatment strategies for kidney diseases are indispensable, they carry significant drawbacks that can impact patients' quality of life. For example, dialysis, a time-consuming procedure (several hours per session multiple times a week), can lead to complications such as infections, vascular access problems, and long-term issues like anemia and bone disease. Another example is kidney transplantation; while it offers a permanent solution compared to dialysis, the challenges here are the risk of organ rejection and the lifelong dependency on immunosuppressive medications to prevent rejection. These medications can have severe side effects, including an increased risk of infections and cancer.

Plant-based treatments offer a promising complementary strategy to managing kidney diseases, leveraging the therapeutic properties of plants, which have been utilized in medicinal practices for thousands of years [21]. The benefits of such treatments stem primarily from their natural origin, typically resulting in fewer side effects than synthetic medications. Rich in bioactive molecules; plants provide antioxidant,

anti-inflammatory, and diuretic benefits, which are particularly valuable in treating kidney-related ailments. Among these bioactive compounds, polyphenols and their derivatives have captured significant attention in the scientific community due to their protective roles in human health [22, 23]. These compounds, including flavonoids, are recognized for their potent antioxidant activities, which defend against oxidative stress induced by reactive oxygen species (ROS). This protection is crucial in preventing a range of pathological conditions and diseases associated with oxidative stress. Both preclinical and clinical studies have shown that a diet rich in polyphenols can help shield against the development of chronic diseases such as neurodegenerative disorders, cardiovascular diseases, kidney pathologies, cancer, diabetes, inflammatory conditions, and infectious diseases. The increased dietary intake of polyphenol-rich foods, such as quercetin, epigallocatechin-3-gallate, resveratrol, and apigenin and cyanidin, has been linked to reduced chronic oxidative cellular damage, DNA damage, tissue inflammation, and incidences of viral and bacterial infections, as well as neurodegenerative diseases.

Research by F. Vargas et al. has highlighted that quercetin significantly regulates renal epithelial Na⁺ channels (ENaC) and the Na⁺ + -K⁺ + -2Cl⁻ 1 [24] cotransporter (NKCC1) in salt-sensitive hypertensive Dahl rats, leading to reduced systolic blood pressure and enhanced sodium homeostasis [25]. Quercetin also reduces Na, K-ATPase activity, contributing to its diuretic, natriuretic, and potassium-sparing effects, which are further supported by increased prostanoid production and antioxidant properties. Similarly, polymethoxylated flavones from *Rubus rosaefolius* also offer diuretic benefits, enhancing their potential to manage hypertension [26]. Furthermore, polyphenols such as rutin and apigenin are vital in addressing renal fibrosis, a significant complication in nephropathies, by modulating fibrogenic pathways like the TGF- β 1 pathway. Rutin effectively reduces fibrosis by curbing macrophage infiltration and inflammation, while apigenin mitigates hypertension-induced renal injury by downregulating fibrogenic signaling [27-30].

In 2017, Malik et al. demonstrated that apigenin effectively attenuated renal dysfunction, oxidative stress, and fibrosis in STZ-induced diabetic rats and also prevented MAPK activation, which mediates inflammation and apoptosis [31]. Histopathological analysis of renal tissues from treated rats showed significant reductions in inflammation, collagen deposition, and glomerulosclerosis. Concurrently, Meng et al. (2017) discovered that apigenin-induced DNA damage, G2/M

cell cycle arrest, p53 accumulation, and apoptosis in renal cell carcinoma cells, inhibiting their proliferation both in vitro and in vivo, underscoring apigenin's potential as a low-toxicity agent against renal cell carcinoma [32]. These cellular effects collectively contributed to the inhibition of ACHN RCC cell proliferation both in vitro and in vivo, highlighting apigenin's potential as a low-toxicity therapeutic agent against renal cell carcinoma. Additionally, Han, et al. (2016) found that galanin, another flavonoid derived from *Alpinia officinarum* roots, suppressed the invasion of renal carcinoma cells by inducing apoptosis and generating reactive oxygen species (ROS) [33]. When combined with TRAIL, galangin significantly enhanced apoptosis in renal carcinoma cells but not in normal kidney and mesangial cells, suggesting its potential as adjunctive therapy in resistant cancer treatments. These comprehensive findings highlight the multifaceted therapeutic potential of flavonoids in both protecting renal function and treating renal carcinoma, demonstrating their dual role in mitigating renal diseases and combating renal carcinoma [34].

Polyphenols are found naturally in a diverse array of foods, including fruits, vegetables, grains, legumes, tea, and wine [35, 36]. They are classified into several groups based on the number of phenolic rings they contain and the structural elements that link these rings. These groups include phenolic acids, flavonoids, stilbenes, and lignans [37]. Flavonoids, for example, are further divided into subgroups such as flavan-3-ols, isoflavones, flavones, flavonones, flavonols, flavanones, and anthocyanidins. Non-flavonoid polyphenols include tannins, lignans, and stilbenes. The structural diversity of polyphenols, including their ability to bind to other organic molecules such as sugars, acids, and other phenolic compounds, contributes to their varied biological activities and health benefits. Notably, over 8,000 polyphenols have been identified in plant sources, with several hundred presents in common dietary items [34, 38]. These compounds are especially recognized for their strong antioxidant capacity, attributed to hydroxyl groups within their structures, enabling them to neutralize reactive oxygen species (ROS) effectively. This ability is essential in combating pathological conditions triggered by oxidative stress (OS), underlining the significant therapeutic potential of dietary polyphenols in preventing and alleviating oxidative damage associated with various chronic diseases. In addition, polyphenols have been shown to modulate the metabolism of carcinogens, exhibit anti-inflammatory properties, and enhance cellular antioxidant defenses,

among other effects [39, 40]. These actions make polyphenols integral components of a diet that may reduce the risk of chronic diseases, emphasizing the importance of consuming a wide variety of plant-based foods to gain the broadest range of health benefits from these compounds.

In the management of chronic kidney disease (CKD), polyphenols have shown promising potential as therapeutic agents [21, 41, 42]. These naturally occurring compounds, abundant in fruits, vegetables, and other plant-based foods, offer a range of bioactive properties that could be beneficial in treating CKD. Polyphenols possess potent antioxidant properties, which play a crucial role in mitigating oxidative stress, a common and detrimental factor in CKD progression. By neutralizing reactive oxygen species (ROS), polyphenols help protect renal cells from oxidative damage and subsequent dysfunction. Moreover, polyphenols exhibit significant anti-inflammatory effects. Chronic inflammation is a key driver in the progression of CKD, contributing to kidney tissue damage over time [43]. Polyphenols can modulate inflammatory pathways, reducing the production of pro-inflammatory cytokines and mediators, thus potentially slowing the deterioration of kidney function in CKD patients. Additionally, polyphenols have been shown to inhibit pathways that lead to fibrosis, a common complication of CKD where normal kidney tissue is gradually replaced with scar tissue. By interfering with fibrogenic signals, such as the TGF- β 1 pathway, polyphenols can help prevent or slow the fibrotic processes that impair kidney function. Importantly, the multifunctional nature of polyphenols extends to their ability to improve lipid profiles and reduce hypertension, both of which are risk factors that can exacerbate CKD. Managing these systemic conditions is crucial for slowing the progression of kidney disease and improving overall outcomes for patients.

Given these potential benefits, research into polyphenols as a component of drug treatment for CKD is expanding. Clinical trials and studies are needed to fully understand the therapeutic effects, appropriate dosages, and long-term safety of polyphenols in the context of kidney disease. If proven effective, polyphenols could become a valuable component of integrated treatment strategies for CKD, offering a natural, potentially less toxic alternative to conventional pharmacological treatments. As of now, there are no specific drugs based solely on polyphenols that are approved and marketed specifically for the treatment of chronic kidney disease (CKD). While polyphenols have shown potential in preclinical studies due to their anti-inflammatory, antioxidant,

and anti-fibrotic properties, the development of polyphenol-based pharmaceuticals for CKD is still in the research and experimental stages. Most of the evidence supporting the benefits of polyphenols for kidney health comes from dietary studies or animal models. For example, diets rich in polyphenols (such as those containing substantial amounts of fruits, vegetables, and teas) have been associated with better overall kidney health and slower progression of CKD in observational and clinical studies.

Researchers continue to explore the encapsulation and delivery of polyphenols in more bioavailable forms, such as nanoparticles or liposomes, which might one day overcome current limitations and lead to their development into formal drugs. Nanoparticles are one of the most promising delivery systems for polyphenols. They can protect polyphenols from degradation, enhance their absorption through the gastrointestinal tract, and allow for controlled release. Types of nanoparticles used include (a) lipid nanoparticles (made from solid lipids and can incorporate polyphenols in a stable matrix, protecting them from degradation and promoting better absorption) [44, 45], (b) nanoemulsions (oil-in-water emulsions with droplet sizes in the nanometer range) [46, 47], and (c) metallic nanoparticles (bind polyphenols on their surface and delivered them to specific sites within the body) [48, 49], (d) liposomes (vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer [50, 51], (e) micelles (colloidal particles with a hydrophobic core and a hydrophilic shell; this system is particularly useful for the oral delivery of hydrophobic compounds) [52, 53], (f) polymeric materials or dendrimers (highly branched, star-shaped macromolecules that can encapsulate polyphenols within their structure) [54, 55] etc. While these advanced delivery systems provide significant improvements in the stability and bioavailability of polyphenols, challenges remain in terms of their regulatory approvals and ensuring that the modifications do not affect the biological activities of the polyphenols.

In this study, we utilized ultrasound technology to extract polyphenols from grape skins and the skin of red onions. This technique leverages ultrasonic waves to effectively break down plant tissues, facilitating the release of polyphenolic compounds into solution. The extracted polyphenols are in the micelle formations that help preserve their stability and optical properties. Remarkably, these micelle-encapsulated polyphenols maintained their optical integrity over a period

of three years, preserving the functional qualities of these bioactive compounds for extended durations. This approach not only optimizes the yield and quality of the polyphenols extracted but also enhances their potential application in various industrial and health-related fields.

Results and Discussions

Waste materials from agricultural processes, such as grape skins from winemaking and onion skins from food processing, are rich sources of polyphenols, potent antioxidants known for their health benefits. Traditionally considered waste bioproducts, these materials can be valued for their high polyphenolic content, which can be harnessed using advanced extraction methods. One such method is ultrasound-assisted extraction, a technique that enhances the release of polyphenols by applying high-frequency sound waves [56-58]. This method is not only efficient but also environmentally friendly compared to conventional extraction techniques. In an industrial setting, solvent extraction is leading due to its scalability and relative simplicity. However, conventional techniques can alter food quality, so it's important to choose a method that is greener and more efficient [59]. By harnessing waste materials, we have designed and developed a method for extracting and stabilizing polyphenol molecules in micelle form. This approach not only contributes to waste minimization but also facilitates the development of functional products.

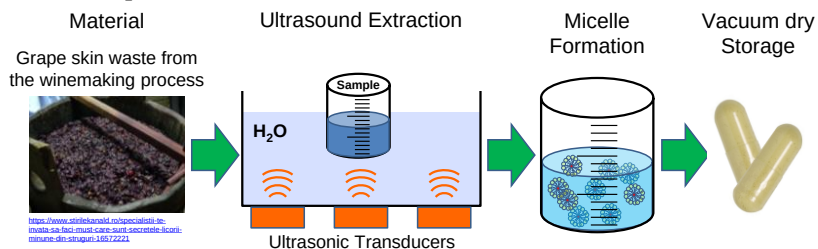


Figure 1. Schematic representation of the polyphenol's extraction process showing the multi-step process.

Initially, the grape skin (waste from the wine-making process) or red onion skin was finely chopped and mixed. The mixture was sonicated in the ultrasound machine for a determined time, then combined with the natural polar molecules and continued to sonicate for another 10 to 20 minutes. The micelles formed were dried with a vacuum and stored in the fridge until further use.

The schematic representation of the polyphenol's extraction process (Fig. 1) illustrates a detailed, multi-step procedure designed to efficiently extract valuable polyphenolic compounds from grapes skin. Initially, organic waste materials such as grape skins, leftover from the winemaking process, or red onion skins are finely chopped to increase the surface area for extraction. These chopped materials dispersed in alcoholic solutions are then subjected to sonication in an ultrasound machine for 10 to 20 minutes to begin the breakdown of plant cell structures and facilitate the release of polyphenols. During this phase, micelles, which are critical for encapsulating the extracted polyphenols, form within the solution. Once formed, these micelles are dried using a vacuum to remove any residual moisture and are subsequently stored in a refrigerator to preserve the integrity of the polyphenols until they are needed for further analysis or use. This method showcases an effective technique for utilizing agricultural waste to recover valuable antioxidants, which can be applied in various health and food industry applications.

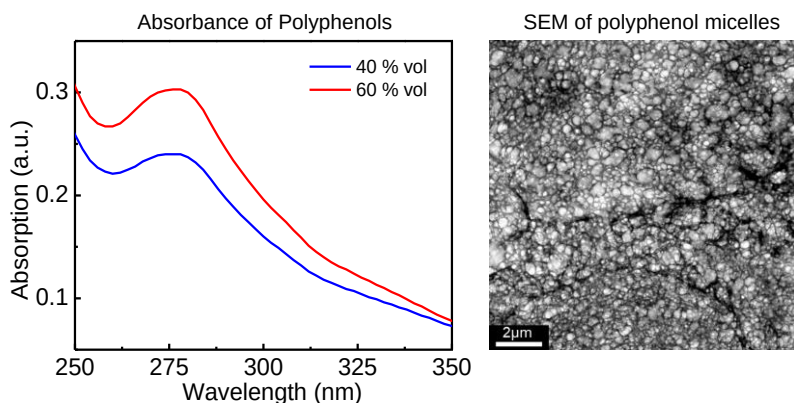


Figure 2. (Left) Extinction spectrum of polyphenols extracted solution from 2 g of skin grapes using ultrasound. (Right) SEM image showing the morphology of the polyphenol micelles.

The extinction spectrum (Fig. 2) of polyphenols extracted from 2 grams of grape skins using ultrasound technology reveals distinct peaks indicative of the presence and concentration of polyphenolic compounds. This spectrum is crucial for understanding the efficiency and completeness of the extraction process, as it provides insights into the

concentration of polyphenols that have been successfully extracted and their relative abundances. Additionally, the Scanning Electron Microscopy (SEM) image of the polyphenol micelles formed during the process offers a detailed visualization of their morphology. These micelles are typically in the nano to micron scale, enhancing their protection from oxidative degradation and improving their solubility in various media, highlighting the effectiveness of using ultrasound in the extraction process from grape skins. The extraction conditions of polyphenols from 2 g grapes skin are presented in Table 1.

Table 1. Ultrasound Extraction of Polyphenols from Ampelografic Skin Grapes

Extraction conditions	40 % vol.	60 % vol.
DPPH (2,2-diphenyl-1-picrylhydrazyl), inhibitor (%)	87.03	93.01
Cyanidin-3-glucoside (g/dm³)	62.858	66.4087
I.F.C.	80,943	85,445
I.P.T.	140,16	192
S.F.T. gallic acid (mg/dm³)	12429,73	13645,21
Malvidin 3-glucoside (mg/dm³)	8490,58	9652,95
The color intensity of the extract	4,3	5,6
The hue or tint of the color	0,850	0,769

In this study, grape skins, finely chopped to enhance surface area and extract release, were mixed with an alcoholic solution to facilitate the extraction of polyphenols. The resulting mixture was then subjected to centrifugation at 2000 rpm for 15 minutes to separate the extract from the solid particles. The absorbance of the extracted solution was measured at three wavelengths: 420 nm (yellow), 520 nm (red), and 620 nm (violet) using 1 mm cuvettes, with each wavelength reflecting a specific segment of the light spectrum. The chromatic parameters of the extracts were determined using a T-80 spectrophotometer based on the following formulas:

Color intensity: $I_c = A_{420} + A_{520} + A_{620}$ (1)

Hue color: $H_c = \frac{A_{420}}{A_{520}}$ (2)

Color purity: yellow (Y), red (R) and violet (V):

$Y = \frac{A_{420}}{I_c} * 100\%$ (3)

$R = \frac{A_{520}}{I_c} * 100\%$ (4)

$V = \frac{A_{620}}{I_c} * 100\%$ (5)

where: A_{420} – absorbance at 420 nm; A_{520} – absorbance at 520 nm; A_{620} – absorbance at 620 nm.

In addition to color analysis, the extracts' antioxidant capacity was assessed through the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. This involved mixing 0.1 mL of the extract with 3.9 mL of a 60 μ M DPPH solution and measuring the absorbance at 517 nm after 30 minutes [60]. The 50% methanol solution was used as a control. The inhibition ratio (IR) was calculated to quantify the extracts' antioxidant effectiveness.

$$IR = \frac{A_{control} - A_{sample}}{A_{control}} * 100 \% \quad (6)$$

where: $A_{control}$ – absorbance of the control (pure DPPH sample); A_{sample} – absorbance of the sample.

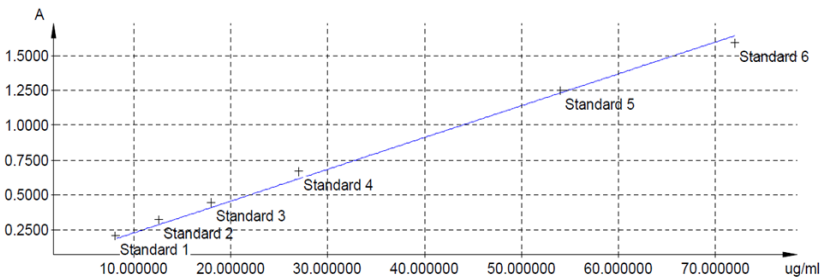


Figure 3. Calibration Curve of Polyphenols using Gallic Acid. Quality of regression ($R^2=0.9925$).

Furthermore, the total phenolic content was quantified using the Folin-Ciocalteu method. In a typical analysis, 1 cm^3 of the extract was diluted with 50 cm^3 of distilled water, 5 cm^3 Folin-Ciocalteu reagent, and 20 cm^3 of 20 % Na_2CO_3 solution. The mixture was shaken thoroughly, and after 30 minutes, the absorbance was measured at 750 nm. The results were calculated from a calibration curve (Fig. 3) using gallic acid (0–500 mg/L) and expressed in equivalents of gallic acid per gram of dried weight (DW) of grape marc.

The anthocyanin content was determined by adding 3 cm^3 of clear extract to a 25 cm^3 pycnometer, 12.5 cm^3 of rectified ethyl alcohol acidified to pH 1.2, and 3 drops of concentrated hydrochloric acid. The mixture was shaken vigorously and centrifuged for 15 min at 1500 rpm, and the absorbance was measured at 520 nm. The anthocyanin content was calculated using the following expression:

$$C_{ant} = 1056.7 \cdot A \quad (7)$$

where: A – absorbance of sample and 1056.7 is the transfer coefficient, established for malvidin-3-glucoside isolated from grape skins.

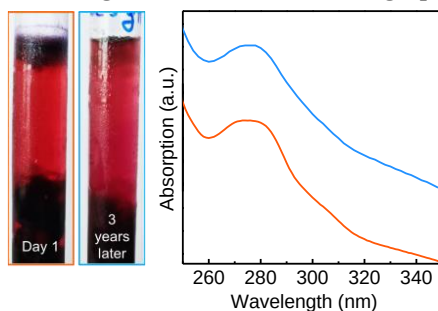


Figure 4. Long-Term Stability of Polyphenolic Compounds.

Pictures showing the time-dependent color stability and UV-Vis absorbance data of polyphenolic compounds extracted from grapes skin. The initial color stability on day 1 is represented in red, and the color stability after three years is shown in blue.

The post-vacuum drying process has proven effective in preserving the integrity and efficacy of the bioactive compounds extracted from grape skins and red onion samples. This preservation method ensures that the beneficial properties of the polyphenols are maintained over extended periods. The time-dependent color stability and UV-Vis spectroscopy data further support the long-term bio-stability of these compounds. The UV-Vis spectra (Fig. 4) depict how the polyphenolic compounds have maintained their structural integrity and color over a prolonged period, highlighting their long-term stability and potential for use in various applications. This long-term stability is crucial for their potential application in pharmaceuticals and nutraceuticals, where consistent and reliable bioactivity is essential. The data conclusively demonstrate that the vacuum drying process is an efficient method for stabilizing bioactive compounds, thus facilitating their storage and usage in various health-related applications.

The integration of polyphenols into advanced delivery systems presents a transformative potential in medical applications, particularly for the prevention and treatment of chronic conditions such as CKD and PKD. By converting what would otherwise be discarded as agricultural or industrial waste, such as grape skins from winemaking or onion skins from food processing, into valuable polyphenolic compounds, we are able to capture their potent antioxidant properties in a stable, bioavailable format. Through innovative drug delivery technologies, the bioavailability and therapeutic efficacy of polyphenols can be

significantly enhanced, allowing for more precise and effective management of CKD and PKD. These developments not only promise to improve patient outcomes by slowing disease progression but also contribute to a broader strategy that incorporates these potent compounds into daily diets.

Conclusions

In conclusion, our study successfully extracted a range of polyphenols from grape skins and red onion samples, utilizing advanced extraction techniques to optimize yield and purity. We generated specific calibration curves for each identified polyphenol, leveraging UV-Vis spectroscopy data to ensure precise quantification. Additionally, evaluations of the post-vacuum drying process confirmed that the integrity and efficacy of these bioactive compounds were maintained, indicating the process's effectiveness. Comprehensive assessments will be conducted to determine the antioxidant, anti-inflammatory, antibacterial, and antimicrobial properties of the extracted polyphenols to provide a detailed profile of their functional capabilities. Further, we will explore other naturally extracted polar molecules that facilitated the formation of micelles, enhancing the bioavailability and stability of these compounds. This research not only underscores the potential of using agricultural waste as a resource for valuable bioactive compounds but also highlights the feasibility of integrating these extracts into various applications, promising significant advancements in health-related sectors.

Materials and Methods

Materials. Fresh black grapes (Pinot Noir, Cabernet Sauvignon, and Merlot) were obtained from approximately 30-35 square kilometers away from Chişinău. Ethanol was purchased from Sigma-Aldrich. Water was deionized and filtered by a Milli-Q water system (18.2 M Ω ·cm at 25 °C, Millipore).

Methods. Absorption measurements were performed on a Cary 5000 UV/Vis/NIR Varian spectrophotometer. SEM measurements were performed using an FEI Quanta 400 field emission SEM at a 20 kV scanning electron microscope acceleration voltage. The extraction of polyphenols was conducted using an ultrasonic bath instrument (Fisher Scientific IsoTemp 205 Water Bath) from The Lab World Group.

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