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ORIGINAL ARTICLE

Optimization of Phenolic Compound Extraction from Pistachio Green Hulls: Influence of Blanching, Harvest Time, Solvent Concentration, and Extraction Method

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K E Y W O R D S

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A B S T R A C T

The processing of pistachios results in the generation of a substantial byproduct in the form of underutilized pistachio green hulls. This study aims to identify the most effective strategies for extracting beneficial phenolic compounds from these hulls. The impact of blanching, harvest timing, solvent concentration, and extraction methods on the extraction process was meticulously investigated. The pistachio green hulls were harvested at varying maturation stages during July, August, and September, with blanching achieved through heating the hulls at 80°C for 6 minutes. Extraction procedures involved the use of 100%, 75%, and 50% methanol concentrations, employing techniques such as percolation, Soxhlet, ultrasonic-assisted, and microwave-assisted extraction methods. Quantification of the total phenolic content was conducted using the Folin-Ciocalteu assay. The highest phenolic content was observed in green hulls harvested pre-maturity in July (1887.75mg GAE per 100g), surpassing the content from August and September harvests significantly. Blanching led to a 12% increase in phenolic yield compared to unblanched green hulls. Extraction using 50% methanol resulted in a 46% higher phenolic content compared to 100% methanol extraction. Among the extraction techniques, microwave-assisted extraction demonstrated the highest phenolic yield (3085.30mg GAE per 100g), outperforming other methods significantly. Percolation duration of 5 hours was identified as optimal. These results underscore the significant influence of early harvesting, blanching pretreatment, moderate solvent concentrations, and microwave irradiation in optimizing phenolic extraction from pistachio green hulls. The findings put forth novel insights into the sustainable utilization of abundant pistachio byproducts for the extraction of health-promoting antioxidants.

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Introduction

The cultivation of pistachio (*Pistachia vera L.*) is notably significant in the USA, which has been confirmed by the quantitative data from 2022. The USA holds a prominent position as the global leader in the production and exportation of this particular nut, with an aggregate yield approximating 250,000 metric tons (Zamani Bahramabadi *et al*., 2018; Bielecka *et al*., 2021; Nazoori *et al*., 2022; Shakerardekani *et al*., 2022; Abdollahi Ezatabadi *et al*., 2023). A consequential aspect of the pistachio processing procedure is the generation of a substantial quantity of pistachio green hull, a byproduct that has recently garnered scientific interest (Mandalari *et al*., 2021; Toghiani *et al*., 2022; Nejatian *et al*., 2023).

Explorations into the pistachio green hull have divulged that its extracts are rich in phenolic compounds, showcasing significant concentrations of various phenolic entities. These compounds are acknowledged for their antioxidant, antimutagenic, and antimicrobial efficacies, thereby broadening the scope of potential applications and benefits associated with pistachio byproducts (Ghandahari Yazdi *et al*., 2019; Nazoori *et al*., 2022). Polyphenolic compounds are bioactive molecules found in the green hulls of pistachios and have been recognized for their antioxidant properties (Arjeh *et al*., 2020). In the phytochemical analysis of pistachio, the following polyphenolic constituents were elucidated and quantified: thirteen derivatives of hydroxybenzoic acid, ten galloyl esters, five hydroxycinnamic acid derivatives, four flavone analogues, nine flavonol moieties, nine flavan-3-ol isomers, four flavanone structures, one flavanonol variant, and one stilbenoid compound (Hassan *et al*., 2022; Sharifkhah *et al*., 2020). In a recent investigation, 34 polyphenolic constituents were characterized in the extract derived from pistachio hulls, of which eight were reported for the first time in pistachio hulls (Seifzadeh *et al*., 2019). In this research, a biphasic membrane separation technique was applied to an aqueous solution derived from the green hull of pistachios to isolate a fraction rich in polyphenols. Subsequent analyses led to the identification of 34 distinct compounds, with predominant constituents such as pyrogallol, quercetin-O-hexoside, galloyl-O-hexoside, theogallin, gallic acid, and galloylshikimic acids. Besides polyphenols, pistachio hulls also contain other bio-active compounds such as tocopherols, dietary fibers, essential oils, and unsaturated fatty acids which together contribute to the antioxidant properties and health-promoting effects of pistachio hulls (Özdikicierler and Öztürk-Kerimoğlu, 2022).

Phytochemicals, particularly phenolic compounds, are renowned for their multifaceted advantageous attributes inclusive of anti-radical properties, mitigation of LDL oxidation and atherosclerosis, anti-carcinogenic traits, antioxidative potential, and antimicrobial efficacy, hence, signifying substantial implications for human health (Li *et al*., 2014; Khodadadi *et al.*, 2016; Jahanbani *et al.*, 2018 and 2021; Ranjha *et al*., 2023). These plant-derived polyphenolic compounds primarily function as reductive agents, with some molecular entities exhibiting antioxidant capabilities through the chelation of metal ions such as ferric (Fe^{3+}) and cupric ions (Cu^{2+}), thereby inhibiting metal-mediated generation of reactive free radicals (Khodadadi *et al.*, 2020; Akbari *et al*., 2022).

Research indicates that numerous determinants influence the concentration and quantity of phenolic compounds in extracts derived from the pistachio green hull (Rafiee *et al*., 2017; Ozay *et al*., 2021). The phenolic compound content in plants is modulated by an array of factors including genetic determinants, environmental conditions, and post-harvest storage conditions (Ghirardello *et al*., 2016; Habibie *et al.*, 2019; Pan *et al*., 2021; Jia *et al*., 2023; Yan *et al*., 2023). Furthermore, this content exhibits variability even among cultivars within the same species. Additionally, the maturation stage and the timing of harvest exert a significant influence on the phenolic profile (Ghirardello *et al*., 2016).

In our study, we delved into the role of blanching, harvest time, solvent concentration, and various extraction methods on the yield of phenolic compounds from the pistachio green hull. Our primary aim was to identify the most effective conditions for maximizing the extraction of these compounds. The determinants we explored included: the specific times of harvest during the months of July, August, and September, the intricacies of the blanching process, and the impact of different solvent concentrations and extraction techniques.

Material and Methods

The pistachio green hulls, specifically sourced from the Ahmad Aghaei variety, were procured from the Kerman Pistachio Research Station. To explore the impact of harvest timing on the phenolic compound content within the extract of pistachio green hulls, harvesting occurred at three distinct stages on the seventh day of July, August, and September of the year. All specimens were collected prior to sunrise. The pistachio trees exhibited a life of approximately a decade, and pruning interventions were employed on an as-needed basis. Furthermore, it is noteworthy that no chemical pesticides were employed in the maintenance of these trees. At all developmental phases, meticulously harvested, unblemished pistachios were manually collected, ensuring the preservation of the green hulls' pristine condition in a wholly random manner. Subsequently, the gathered samples were stored at a temperature of 5°C, from the point of harvest up to the culmination of the green hull separation process.

In the blanching process, whole pistachios, inclusive of their green hulls, were subjected to a thermal treatment in water maintained at a temperature of 80±5°C for duration of 6 min. The time subsequent to the evaluation of peroxidase enzyme activity was ascertained as an indicator of blanching efficacy (Bhat *et al*., 2019). Immediately upon removal from the heated aqueous medium, the specimens were relocated to a vessel containing an ice-water mixture, maintained at a temperature of 0°C. In a systematic procedure, the pistachio green hulls, were meticulously removed. Following this, the separated hulls were processed in a laboratory-grade vessel to obtain a representative sample. Subsequently, a quantity ranging between 15 and 30 g of this sample was transferred to a standard test tube. To this, 30 ml of purified distilled water, 1 ml of a 1.5% guaiacol solution, and 2 ml of a peroxide solution were added in sequence. The mixture within the test tube was then homogenized thoroughly. An emergent pale pink hue served as an indicator of the residual activity of the peroxidase enzyme, categorizing the observation as a positive reaction. Conversely, the absence of any chromatic transition within duration of 5 min was indicative of a negative reaction, signifying the deactivation of the peroxidase enzyme.

In the experiment, pistachios from both categories, blanched and non-blanched, were subjected to a decortication process. Subsequently, the isolated green hulls were desiccated in an environment shielded from photonic exposure, maintaining ambient temperature, facilitated by a fan. Upon achieving complete desiccation, they were meticulously pulverized utilizing an electric chopper, followed by a sieving procedure (particle size of 0.5–2 mm) to ensure particle size uniformity. The resultant powder (1 g) was stored in amber-colored glass containers and refrigerated at approximately 5°C until the culmination of the experimental procedures.

Upon preparation of the specimens, extraction was conducted utilizing the percolation technique over durations of 20 min, 1 h, 5 h, 10 h, and 24 h on a standardized specimen (initially sampled and subsequently blanched). Following the quantification of the extraction yield and the concentration of phenolic compounds, the optimal extraction duration using the percolation method was ascertained. To elucidate the impact of blanching on the yield of extraction and phenolic compounds, extractions were conducted utilizing the immersion technique during

an optimal duration for both blanched and non-blanched specimens. The influence of harvest time was subsequently examined by comparing the phenolic compounds derived from samples collected during varying months (July, August, and September) – all of which were blanched, following the aforementioned immersion procedure during its optimal time frame. Furthermore, the effect of solvent concentration variations was ascertained by employing pure methanol, 75% methanol, and 50% methanol respectively. Subsequent to identifying the specimen with the paramount quantity of phenolic compounds, extractions were carried out using the Soxhlet, ultrasonic, and microwave techniques with the most efficacious solvent. Ultimately, a comparative analysis of the total phenol yield from each method facilitated the selection of the most optimal extraction technique. In assessing the impact of solvent concentration and extraction methodology, it should be noted that, owing to the existence of water in the solvent and the potential lack of thorough desiccation of the extract (at temperatures not exceeding 45°C), only the quantification of phenolic compounds was undertaken.

The percolation extraction was conducted by introducing 30 ml of methanol to a 1 g sample, facilitated by a magnetic stirrer. Upon completion of the predetermined duration, the mixture was filtered using filter paper (Whatman No. 1) to isolate the contents of Arlene from a standard-weight Arlene, enabling the calculation of the extract's percentage. The residual matter retained on the filter was reintroduced to its initial vessel, amalgamated with an additional 20 ml of methanol for a duration of 30 seconds, and combined with the formerly filtered extract using the identical filter paper. This rinsing procedure was executed twice in succession. Subsequently, the filtered extract was desiccated utilizing a rotary evaporator under reduced pressure, ensuring the temperature did not exceed 45°C. Finally, evaluations were made to determine the percentage of extraction and quantify the phenolic compounds present in the extract (Ghirardello *et al*., 2016).

In the Soxhlet extraction procedure, approximately 8 g of the specimen were situated in the cartridge and subsequently affixed to the Soxhlet apparatus. Following the addition of the requisite volume of solvent (approximately 150 ml) to the specimen, the extraction was conducted over a duration of 5 h. Subsequent to this process, the solvent was meticulously evaporated under vacuum using a rotary evaporator. Thereafter, the extraction efficiency and the concentration of phenolic compounds within the extract were quantified.

In the ultrasonic extraction procedure, 30 ml of solvent were introduced to 1 g of the sample, subsequently subjected to sonication in an ultrasonic bath. The device parameters were precisely adjusted to 20% power, a frequency of 145 kHz, and an extraction duration of 20 min (Osorio Arias *et al*., 2023). Following the extraction process, the resultant extract was meticulously filtered using filter paper (Whatman No. 1) into a pre-weighed Erlenmeyer flask. The solvent was subsequently fully evaporated under vacuum using a rotary evaporator. Subsequent analyses determined the extraction efficiency and quantified the phenolic compound content present in the extract.

In the microwave-assisted extraction procedure, precisely 1 g of the specimen was dispensed into a diminutive Erlenmeyer flask. Upon the addition of 20 ml of an appropriate solvent, the flask was sequestered in an environment devoid of light for duration of 90 min. Subsequent to this interval; extraction was executed utilizing a microwave apparatus. The power setting of the device was adjusted to 12% and the extraction duration was designated as 5 min. It is imperative to note that after each one-minute interval, to inhibit excessive temperature augmentation, the specimen was removed from the apparatus and stored in a refrigeration unit until the temperature descended to below 25°C (Kalogiouri *et al*., 2022).

To ascertain the extraction yield of the specimen, an Erlenmeyer flask of consistent mass was initially measured. Subsequently, the methanolic extract underwent filtration and was subjected to vacuum-evaporation utilizing a rotary evaporator. Upon confirming the comprehensive evaporation of the solvent and the complete desiccation of the extract, the mass of the Erlenmeyer flask was determined once more. The extraction yield was then computed based on these measurements. For the quantification of total polyphenols present in the extract, 1 ml of the appropriately diluted extract was amalgamated with 1 ml of the Folin-Ciocalteu reagent. Following a 2-minute interval, 1 ml of a saturated solution of sodium carbonate was introduced to the amalgamation, and subsequently, the volume was adjusted to 20 ml using distilled water. The reaction was allowed to proceed in an environment devoid of light for a duration of 90 min, post which, the absorbance was measured at a wavelength of 760 nm using a spectrophotometer (Agilent Cary 60, USA) (Harandi *et al*., 2022). For the construction of the standard curve, gallic acid was employed at concentrations ranging from 0 to 0.075 mg ml^{-1}. The resultant data were articulated in terms of mg of gallic acid (GAE) per 100 g of the desiccated sample.

The results were obtained utilizing the analysis of variance (ANOVA) technique following a completely randomized design derived from three distinct replications of the experiments. This was facilitated by the employment of the SAS statistical software package (Version 9.4M8). The level of significance was established at $p<0.05$.

Results

In this section, we present our findings on the extraction of phenolic compounds from the pistachio green hull. The results are systematically organized based on the determinants studied: blanching process, specific harvest times during July, August, and September, solvent concentrations, and the various extraction methods employed. Detailed analyses, supported by quantitative data, shed light on the optimal conditions that maximize the yield of phenolic constituents from the pistachio green hull. To determine the optimal duration for phenolic compound extraction via percolation, the procedure was performed for intervals of 20 min, 1 h, 5 h, 10 h, and 24 h. The extraction efficiency was assessed by measuring the percentage extraction yield and the total phenolic content (Table 1). The results showed a positive correlation between extraction time and both the percentage yield and phenolic concentration. Statistical analysis indicated prolonging the extraction time increased the phenolic content. However, there were no significant differences in percentage yield or phenolic concentration between the 5-, 10-, and 24 h intervals. Additionally, the total phenols extracted after 20 min and 1 hour were not significantly different, although the 20 min extraction gave a substantially lower yield than 1 h. Given these findings, an extraction time of 5 h was deemed optimal for subsequent experiments. Table 2 shows the ANOVA analysis of the data for the extraction time treatments.

Table 1. The influence of extraction time on the extraction percentage and amounts of phenolic compounds in pistachio green hull extracts

Extraction time	20 min	1 h	5 h	10 h	24 h
Extraction percentage	29.97±0.27c	32.40±0.00b	35.10±0.45a	34.74±0.27a	35.64±0.54a
Total phenol (mg per 100 g)	630.30±19.08b	640.49±18.18b	1887.75±49.68a	1891.92±43.20a	1960.77±52.74a

Note: Similar letters in each row indicate no significant differences

Table 2. ANOVA assessing the influence of extraction time on the amounts of phenolic compounds in pistachio green hull extracts

Source of variation	Degrees of freedom (DF)	Sum of squares (SS)	Mean of squares (MS)	F-value	p-value
Treatment	4	6545338.02	1636334.50	344.92	<0.001
Error	11	52187.76	4744.34		
Total	15	6597525.87			

It is evident from the data that the proportion of extraction and the concentration of total phenolic compounds in blanched samples surpass that of the non-blanched samples, as demonstrated in Table 3. Subsequent analysis of variance (ANOVA) presents the results derived from the blanching treatments, delineated in Table 4.

Table 3. The influence of blanching on the extraction percentage and amounts of phenolic compounds in pistachio green hull extracts

Sample	Blanched	Non-Blanched
Extraction percentage	35.10±0.45a	32.40±0.00b
Total phenol (mg per 100 g)	1887.75±49.68a	1684.35±45.36b

Note: Similar letters in each row indicate no significant differences

Table 4. ANOVA assessing the influence of blanching on the amounts phenolic compound in pistachio green hull extracts.

Source of variation	Degrees of freedom (DF)	Sum of squares (SS)	Mean of squares (MS)	F-value	p-value
Treatment	1	68952.60	68952.60	8.22	0.03
Error	5	30222.98	6044.60		
Total	6	99175.58			

The influence of the harvest time of pistachio harvest on the concentration of phenolic compounds in the pistachio green hull is elucidated in Table 5. The data indicate that the timing of harvest significantly impacts both the extraction efficiency and the total phenolic content in the pistachio green hull. In a comprehensive analysis of the compounds contained within the pistachio green hull, it was observed that the extraction percentage notably declined from July through August, concomitant with the escalating rate of fruit maturation. The temporal factor of the harvest, in particular, played a salient role in determining the concentration of phenolic compounds. Remarkably, the zenith of these compounds was documented during the initial harvest period in July. It is noteworthy to mention that during this juncture, the pistachio fruit has yet to achieve full maturation and its woody pericarp is in the phase of hardening. When comparing the secondary harvest in August to the subsequent harvest in September, a greater prevalence of phenolic compounds was discerned in the former. However, the disparities in phenolic compound concentrations between these two harvest periods were not statistically significant. Subjoined is the variance analysis table elucidating the data procured from the distinct harvest timings (Table 6).

Table 5. The influence of harvest time on the extraction percentage and amounts of phenolic compounds in pistachio green hull extracts

Sample	July	August	September
Extraction percentage	35.10±0.45a	30.90±0.54b	23.09±0.27c
Total phenol (mg per 100 g)	1887.75±49.68a	1397.28±42.48b	1241.14±98.19b

Note: Similar letters in each row indicate no significant differences

Table 6. ANOVA assessing the influence of harvest time on the amounts phenolic compound in pistachio green hull extracts

Source of variation	Degrees of freedom (DF)	Sum of squares (SS)	Mean of squares (MS)	F-value	p-value
Treatment	2	758939.85	379469.92	28.61	<0.001
Error	7	92851.65	13264.52		
Total	9	851791.59			

In Table 7, the outcomes of the treatment employing various methanol concentrations on the quantity of phenolic compounds extracted from the green hull of pistachio vera are delineated. The data suggests a notable discrepancy in the quantities of phenolic compounds extracted using 100%, 75%, and 50% methanol concentrations, with the phenolic yield increasing inversely with a decrease in methanol

concentration from 100% to 50%. Subsequently, an ANOVA table elucidating the data derived from solvent concentration treatments is presented in Table 8.

Table 7. The influence of solvent concentration on the amounts of phenolic compounds in pistachio green hull extracts

Solvent concentration	Methanol 50%	Methanol 75%	Methanol 100%
Total phenol (mg per 100 g)	2503.49±31.59a	2081.93±35.55b	1887.75±49.68c

Note: Similar letters in each row indicate no significant differences

Table 8. ANOVA assessing the influence of solvent concentration on the amounts phenolic compound in pistachio green hull extracts

Source of variation	Degrees of freedom (DF)	Sum of squares (SS)	Mean of squares (MS)	F-value	p-value
Treatment	2	660636.72	330318.36	73.08	<0.001
Error	7	31624.92	4517.84		
Total	9	692261.64			

To elucidate the impact of various extraction methodologies on the yield of phenolic compounds from the pistachio green hull, techniques such as percolation, Soxhlet extraction, ultrasonic-assisted extraction, and microwave-assisted extraction were employed (Table 9). Regarding the yield of phenolic compounds from the pistachio green hull, a marked disparity is evident when utilizing microwave-assisted extraction in contrast to the other aforementioned methods. Employing microwave irradiation leads to a superior extraction yield of phenolic compounds from the pistachio green hull. Subsequent to microwave-assisted extraction, the ultrasonic-assisted, Soxhlet, and percolation techniques extract elevated quantities of phenolic compounds; however, the disparity amongst these three methodologies is not statistically significant. The subsequent variance analysis of the data derived from the extraction methodological treatment is presented in Table 10.

Table 9. The influence of extraction method on the amounts of phenolic compounds in pistachio green hull extracts

Extraction method	Microwave	Ultrasonic	Soxhlet	Percolation
Total phenol (mg per 100 g)	3085.30±122.22a	2707.45±71.10b	2697.57±54.09b	2503.49±31.77b

Note: Similar letters in each row indicate no significant differences

Table 10. ANOVA assessing the influence of extraction method on the amounts phenolic compound in pistachio green hull extracts

Source of variation	Degrees of freedom (DF)	Sum of squares (SS)	Mean of squares (MS)	F-value	p-value
Treatment	3	592466.43	197488.81	11.14	0.004
Error	9	159532.27	17725.81		
Total	12	751999.51			

Discussion

The extraction of phenolic compounds from pistachio green hulls has become an area of significant interest, primarily because of the myriad health benefits and potential applications associated with these compounds.

Blanching significantly increased the extraction yield and total phenolic content compared to unblanched samples. This aligns with previous research showing blanching enhances the extractability of phenolics in plant materials by inactivating polyphenol oxidases and cell wall degrading enzymes, increasing cell membrane permeability, and improving solvent diffusion (Bhat *et al*., 2019).

Our results also indicated harvest time significantly influences phenolic levels, with the highest concentrations obtained from hulls harvested early in July before full maturation. This agrees with past studies demonstrating phenolic content in plants decreases during maturation due to utilization in

metabolic and oxidative processes (Ghirardello *et al*., 2016). The phenolic levels were substantially higher in our July-harvested hulls compared to August and September, congruent with findings by Mandalari *et al*. (2021) showing immature pistachio hulls possess greater phenolic content.

Regarding solvent extraction, 50% methanol gave considerably higher phenolic yields than 75% and 100% methanol. This corroborates research by Ghandahari Yazdi *et al*. (2019) and Ozay *et al*. (2021) indicating diluted methanol is optimal for extracting pistachio hull phenolics. The lower dielectric constant of diluted methanol likely improves solvent penetration into the plant matrix.

Finally, microwave-assisted extraction produced markedly higher phenolic yields compared to ultrasonic, Soxhlet, and percolation methods. These results concur with Kalogiouri *et al*. (2022) demonstrating the superiority of microwave extraction for recovering phenolics from pistachio hulls. Microwaves likely rupture cell walls more effectively, enabling greater solvent access and mass transfer.

The results of our investigation offer pivotal insights into optimizing phenolic compound extraction from pistachio green hulls, presenting notable implications for both agricultural sustainability and the burgeoning nutraceutical industry. Through strategic harvest timing, innovative extraction techniques, and solvent optimization, we delineate a path toward enhancing the value extracted from agro-industrial byproducts. This approach not only fosters the circular economy within agriculture but also underscores the dual benefits of environmental stewardship and economic gain through the valorization of otherwise underutilized resources.

Moreover, the enhanced extraction yields spotlight the feasibility of integrating such methodologies into existing agricultural practices, potentially revolutionizing the production of phenolic-rich extracts. These extracts, given their bioactive properties, hold profound potential for nutraceutical applications, promising to elevate the health and wellness sector with scientifically backed, sustainable ingredients.

Future endeavors should pivot towards scaling these methodologies, assessing their commercial viability, and exploring the holistic benefits of such extracts in diet and health. Furthermore, the environmental footprint of scaled-up operations merits thorough evaluation to ensure that the benefits of these innovations extend comprehensively, reinforcing the commitment to sustainability.

Conclusions

The current investigation systematically analyzed the effects of various factors on the extraction of phenolic compounds from pistachio green hulls. Blanching, early harvest timing, moderate methanol dilution, and microwave-assisted extraction were found to significantly increase the phenolic yields. The results demonstrate that pistachio green hulls harvested pre-maturity in July contain substantially higher phenolic levels compared to those harvested later in August and September. Blanching is an effective pretreatment to enhance extractability. A 50% methanol solvent proves optimal, likely due to improved penetration into the plant matrix compared to higher concentrations. Additionally, microwave-assisted extraction markedly outperforms ultrasonic, Soxhlet, and percolation methods. Overall, the findings provide important insights into ideal protocols for maximizing phenolic extraction from pistachio byproducts. Early harvesting, blanching, moderate solvent dilution, and microwave irradiation stand out as key factors. The phenolic-rich extracts obtained exhibit excellent potential for applications as natural antioxidants and nutraceuticals. Further research could entail examining other variables such as temperature, pH, solid-solvent ratios, and phenolic characterization.

In conclusion, this study makes a valuable contribution towards the sustainable utilization of pistachio green hulls. The optimal extraction protocols

delineated can facilitate the efficient recovery of health-promoting phenolic compounds from this abundant agricultural waste. The bioactive extracts can promote environmental and economic sustainability through the development of plant-based functional foods, pharmaceuticals and bioproducts.

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Conflict of interest

The authors declare no conflict of interest.

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