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Algerian vine canes as a good source of phenolic compounds for several in vitro biological activities

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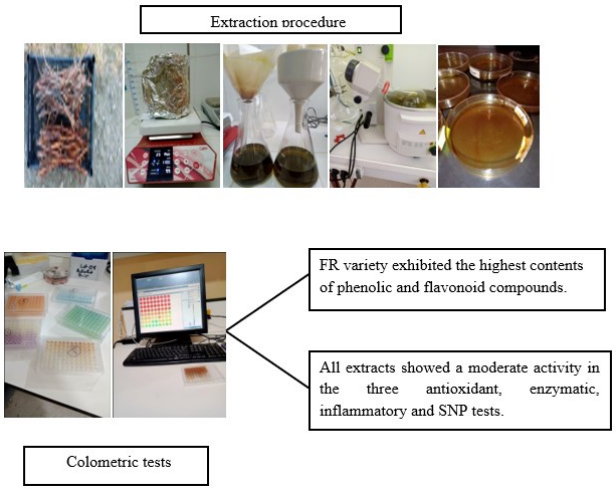
Graphical abstract



Abstract

One of the most widely cultivated crops in the world is grapevine knowing that about 77.8 million tons of grapes are produced worldwide each year. Both of vine growing, pruning, and winemaking generate a vast quantity of wastes and byproducts such as skin, seed, and canes. Grape canes include a wide range of phenolic profiles which have several biological effects. In the present study four vine Algerian grape cane cultivars were selected for the evaluation of their phenolic contents and biological activities. The objective of this study was to evaluate the total phenolic and flavonoid contents, in vitro antioxidant activity (using DPPH, ABTS, and reducing power methods), enzyme inhibitory activity (towards alpha amylase, and urease) anti-inflammatory activity, and sun protection factor of each extract. All grape extracts were compared for their TPC and TFC values which ranged from 123 to 288 mg of Gallic acid equivalent (GAE)/g extract to 24.5 to 55.6 mg of quercetin equivalent (QCE)/g extract respectively. The highest TPC and TFC were found in the grape cultivar FR (288.8 mg GAE/g extract, 55,625 mg QCE/g extract respectively). The results revealed also that all extracts showed high antioxidant with the same trend of phenolic composition and significant enzymatic activity in all tests. Additionally, all extracts showed a moderate anti-inflammatory activity by inhibiting the denaturation of BSA, and given a high sun protection factor. These findings imply that grape canes which considered as undervalued parts have a significant potential to be used as a natural treatment for many physiological diseases and for the pharmaceutical and cosmetic sector and medical research.

Keywords: vine, canes, polyphenols, flavonoids. Antioxidant, enzymatic activity, sun protection factor.

1. Introduction

One of the most widely cultivated crops in the world is grapevine, knowing that about 77.8 million tons of grapes are produced worldwide each year with around 10,000 varieties in the world. Wine making is considered the most significant usage of grapes (57%), especially in Germany, France, Italy, Canada, the USA, and New Zealand, followed by fresh fruits (36%) mostly in China, India, Iran, Egypt, Turkey, Brazil, and Mexico, while dried fruits and juice are coming in the latest list of use with a percentage of 7% (Baroi *et al.* 2022; Insanu *et al.* 2021). Both vine growing, pruning, and winemaking generate a vast quantity of waste and byproducts such as skin, cane, stalk, and seeds (Gharwalova *et al.* 2018; Squillaci *et al.* 2021). In the past, agro-industrial wastes were typically considered to be a residue with low value, but today there is increasing interest in their potential as a resource for the production of high-added-value chemicals and materials because of the information available on their content of apparently health-promoting phytochemicals (Squillaci *et al.* 2021; Ferreyra *et al.* 2019). Grapevine canes, also called stems, shoots, or stalks, are one of the most common viticulture by-products; their yield per hectare of vineyard is estimated to be between 2 and 5 tons (Aliaño-González *et al.* 2020). Grape canes are regarded as an undervalued waste because they are normally burned or integrated into the vineyard soil. They have the potential to be a high-value resource in terms of integrated biorefinery and circular

economy because of their important chemical composition and industrial applications (Escobar-Avello *et al.* 2021). Furthermore, studies have demonstrated that this raw material serves as an excellent source of dietary fiber, phenols, proteins, lipids, and hydrocolloids (Troilo *et al.* 2021). These byproducts are typically used for animal feed and organic fertilizer, as filler and a cosmetic ingredient, for lignin, hemicellulosic oligosaccharides, and cellulosic substrate regeneration, as enological additives to improve the wine's sensory characteristics, in the pharmaceutical, cosmetic, and food industries (Escobar-Avello *et al.* 2021; Kodeš *et al.* 2021). Grape canes include a wide range of phenolic profiles that make this by-product have key antioxidant, anti-microbial, anti-cancer, anti-inflammatory, and anti-aging effects, as well as a variety of potential applications (Escobar-Avello *et al.* 2021; Noviello *et al.* 2022; Squillaci *et al.* 2021).

In this case, total phenolic and flavonoid contents, antioxidant properties using various methods in vitro as free radical-scavenging and reducing power capabilities, anti enzymatique, anti-inflammatory activities, and sun protect factor of crude methanolic extracts from grape canes of four commonly Algerian grown V. labrusca and V. vinifera cultivars were evaluated, such, as part of our current study on the potential utilization of grape cane wastes.

2. Materials and Methods

2.1. Plant material

2.1.1. Information of grape varieties

2.1.1.1 Fragola Nera

Fregola Nera named also Isabella is an hybrid cultivar produced by crossing the American genotype *Vitis labrusca* (or 'fox' grape) and the European *Vitis vinifera*. It is more resistant to fungal diseases than other Vitis vinifera species. The variety is characterized by big fruits, dark purple berries, and green yellow fresh, with easy removal skin. The flavor of Isabella grapes has been described as foxy and as having special aromatic characteristics (Rodrigues. 2023; Aydemir et al. 2023).

2.1.1.2 Cardinal

The Vitis vinifera Cardinal is the most selected grape table in the world. It is produced by crossing 'Flame Tokay' (syn. 'Ahmer Bou Amer') with 'Ribier' (syn. 'Alphonse Lavalle´e') at the Horticultural Field Station of Fresno, California by E. Snyder and F. Harmon in 1939. It is characterized by its large berries, pleasant flavour, and early maturity (Akkak *et al.* 2007).

2.1.1.3 Red Globe

Vitis vinifera L. Red Globe (RG grape) is one of the most popular table grapes in the world da Silva et al. (2023). According to the National Research Centre for Grapes, Pune. (2009) it is obtained by Albert T. Koyama and Hardnold P. Olmo at the University of California at Davis in 1981. The berries are extremely in red, spherical, and have few fragile seeds. The pulp is quite firm and firm and the skin is light and thick, often peelable. The pruning is from 5-8 buds from base.

2.1.1.4 *Gros Noir*

Gros Noir named also Alphonse Lavallee is a Vitis Vinifera table and wine grape, created by crossing of Muscat Hamburg and Kharistvala Kolkhuri – an obscure variety from the Georgian Republic by Alphonse Lavallee in 1860. It is a dark-skinned grape variety. This variety produces medium to large and sometimes very voluminous bunches [Web1, Web 2]. The grapes have a thick, crisp, blue-black skin. The pulp is a little fleshy, firm, juicy and astringent. The Lavallée Alphonse has a particular and pleasant flavor with high acidity and low sugar content [Web 3].

2.1.2. Collection of canes and extraction of plyphenols

Late in the last January of 2022, in the area of Boumerdes (Baghlia) in the North of Algeria (36° 48′ 56″ N; 3° 51′ 40″ E), about 500 g of four different canes (Fragola Nera, Cardinale, Gros Noir, and Red Globe) were collected randomly from different plants grown under the same conditions and pruned at the same time. After collection, they were cut into small pieces and then dried in the shade for 4 weeks until their weight was stable. Additionally, grapevine canes were milled into fine powder using a MOLINEX grinder and stored until extraction. For extraction of phenolic compounds from the different cane extracts, the maceration technique was applied using a solid-to-solvent ratio of 1:10 and a solvent ratio of 60:10 (ethanol: water) during 24 hours in the dark, then filtered. This procedure was repeated three times. Supernatants were evaporated, and the average extraction yield was about 10% for all cultivars (Anna *et al.* 2020). The resultant extracts were used in the different in vitro assays.

2.2. Determination of Total Phenolic and Total Flavonoid Contents

Following the method described by Muller et al. (2010) Folin-Ciocalteu reagent was used in a colometric assay to determine the total phenolic content of grape canes. 1 mg of sample extracts was diluted with 1 ml of methanol to get the origin solution. 100 µl of FCR reagent (1:10) and 75 µl of 7,5% Na2CO3 were successively added to 20 µl of each origin solution. Then, an incubation of 2 hours in the dark was necessary before measuring the absorbance at 765 nm. Total phenolic content was estimated operating the calibration curve of Gallic acid (y = 0.0034x + 0.1044, R2 = 0.9972) and expressed as the equivalent to milligrams of Gallic acid per gram of dry extract (mg GAE/g).

Another colometric procedure was used to estimate the total flavonoid content according to Topçu et al. (2007) with some modifications. A mixture of 130 µl MeOH, 10 µl CH3COOK (1 M), and 10 µl of (10%) Al (NO3)2, 9H2O were added to 50 µl of samples in a triplicate. The mixture remained at room temperature for 40 min in the dark. The absorbance of resulting mixtures was measured at 415 nm and a calibration curve of quercetin (y = 0.0048x, R2 = 0.997) was used to estimate the TFC.

2.3. Evaluation in Vitro of Antioxidant Activity

Three different in vitro widely used assays were employed in the present study (DPPH, ABTS, and reducing power) to evaluate the antioxidant activity of grape cane extracts.

The fact that these techniques are based on several reaction mechanisms led to their selection. Antiradical activity was determined spectrophotometrically by measuring the absorbance of samples in micro plates of 96-well- using a micro plate reader (Perkin Elmer EnSpire, New York, NY, USA). Both of BHA and BHT was used in a linear regression analysis as standards to evaluate the results which represented as IC50 and A0.5 values ± SD of three measurements.

2.3.1. Determination of Free Radical Scavenging Activity

The method reported by Blois. (1958) was used to measure the free radical scavenging activity of cane extracts. 160 µl of DPPH diluted methanolique solution (0.1 M) with an absorbance of 0.9 was added to 40 µl of samples or positive controls (BHA and BHT) at different concentrations (12.5, 25, 50, 100, 200, 400, and 800 µg). The absorbance was recorded at 517 nm after 30 min of incubation at room temperature in the dark. The results were reported as the IC50 value.

Following the method described by Re et al. (1999) with some modifications the ABTS assay was evaluated after formation of ABTS radical cation (ABTS•+) by mixing 19.2 mg of ABTS (7mM) with 3.3 mg of potassium persulfate (K2S2O8). The mixture was then diluted after a stored period of 16 h in the dark to obtain an absorbance of 0.9 at 734 nm. A total of 40 µL of the diluted samples and positive controls (BHA and BHT) with concentrations of 12.5, 25, 50, 100, 200, 400, and 800 µg were added to 160 µL of (ABTS•+) solution and incubated at room temperature for 10 min. The results were reported as the IC50 value.

2.3.2. Reducing Power Assay

The antioxidant activity of the extracts was also confirmed through ferricyanide reduction method according to Oyaizu. (1986) with slight modifications. 10 µl of each samples and positive controls (BHA and BHT) at different concentrations (3.125, 6.25, 12.5, 25, 50, 100, and 200 µg) were mixed with 40 µl phosphate buffer (0.2 M, pH 6.6) and 50 µl of (1%) potassium ferricyanide solution ($K_3Fe(CN)_6$). A 20 min incubation at 50°C was provided for the reaction followed by an addition of 50 µl tri-chloroaceticacid (TCA) (10%), 40 µl distilled water, and 10 µl ferric chloride ($FeCl_3$ 0.1%). The absorbance was recorded at 700 nm and the results were expressed as A0.5 value.

2.4. Enzymatique activity

A 96-well micro plate reader (Perkin Elmer EnSpire, New York, NY, USA) was used to evaluate the spectrophotometric inhibition of such enzyme (α-amylase and urease) using a micro plate assays modified from different colorimetric techniques. Cane extract stock solutions were prepared at different concentrations in methanol. The concentration of plant extract that inhibited 50% of the enzyme activity (IC50) was calculated by linear regressions using Acarbose and Thiourea as standards respectively.

2.4.1. Urease inhibitory Activity

Measuring the ammonia produced during the reaction realized by Taha et al. (2018) allowed for the determination of anti-urease activity. Briefly, 10 µL of the extracts at different concentrations, 20 µL of urease solution (Urease from Canavalia ensiformis (Jack bean), Type IX, powder, 50,000-100,000 units/g solid (sigma-aldrich)), 20 µL of urea substrate (100 mM), 45 µL of phenol reagent and 70 µL of alkaline reagent were combined in a 96-well micro plate. The mixture was then incubated at 30°C in the dark during 2 hours and the absorbance was measured at 630 nm.

2.4.2. α-amylase inhibitory Activity

The revealing iodine/potassium iodide was used to estimate the efficacy of extracts to inhibit the activity of α-amylase according to Zengin et al. (2014) with slight modifications. In a microtitre plate a mixture containing 25 µl of each extract at different concentrations and 50 µl enzyme (α-Amylase from *Aspergillus oryzae*, ≥150 units/mg protein (biuret) (sigma-aldrich)) prepared in sodium phosphate buffer solution (pH = 6.9 with 6 Mm NaCl) were pre-incubated during 10 min at 37°C. An addition of 50 µl of (1%) starch was also followed by a second incubation at 37°C during 20 min in the dark. The reactions were stopped by adding 25 µl HCl (1 M) and the color change was noted after adding 100 µl of iodine reagent. The absorbance was measured at 630 nm and percentage inhibition of enzyme activity was calculated.

2.5. In vitro anti-inflammatory activity:

A slight modified version of the BSA assay established by Benmohamed et al. (2023) was used to determine the anti-inflammatory properties of the plant extracts. In this case, 100 µL of 0.2% BSA solution prepared with tris-HCl (pH = 6.6) was added to 100 µL of each concentration of extract or standard (diclofenac sodium) in a 96-well micro plate. The mixtures passed through a 15 minute incubation period at 37°C and a 5 minute incubation period at 72°C in an oven. A micro plate reader was used to measure the turbidity at 660 nm after cooling.

2.6. Sun Protection Factor

UV-visible spectrophotometer micro plate reader was used to measure the anti solar activity following the method described by Mansur et al. (1986) with minor modifications. All of the water and methanol extracts were tested with preliminary analysis. The extract was prepared in methanol at a concentration of 2 mg/mL, and spectrophotometric readings were taken using a range of 5 nm from 290 to 320 nm. The readings were all done in triplicates and the SPF factor was calculated using Mansur's mathematical equation.

2.7. Statistical analysis

Results were conducted in triplicate and expressed as means ± standard deviations (SD). Statistical analysis was carried out using SPSS 21.0 including one-way ANOVA test to evaluate the differences between samples. Post-hoc turkey test with a statically significance at a 5% level used to represent differences between means ($p \leq 0.05$). Whereas, Pearson's correlation coefficients (r) were used to determine the correlation between variables.

3. Results and Discussion

3.1. Total Phenolic and Flavonoid contents

In the present study, four vine Algerian grape cultivars were selected for the evaluation of their total phenolic (TPC) and total flavonoid contents (TFC). All grape extracts were compared for their TPC and TFC values, which ranged from 123 to 288 mg of gallic acid equivalent (GAE)/g extract to 24.5 to 55.6 mg of quercetin equivalent (QCE)/g extract, respectively. The highest TPC was found in the grape cultivar FR from the hybride (*Vitis vinifera/Vitis Labrusca*) with an amount of 309,8 ± 11.5mg GAE/g extract, followed by RED from Vitis Vinifera (205,07 ±16,4 mg GAE/g extract), while the lowest concentrations were found in GR (123 ± 11,7 mg GAE/g extract) and CR (198,3 ± 5.4 mg GAE/g extract). The FR variety also has the highest TFC value (55,6 ± 2,06 mg QCE/g extract), followed by CA, RE, and GRE with an average TFC of 48,5 ± 7,9, 40,5 ± 7,3, and 40,5 ± 0,2 mg QCE/g extract, respectively. As per the data represented in Table 1, the total phenolic contents in the cane grape extracts from 60% ethanol differed significantly ($p < 0.05$) in the most sample extracts of the different grape varieties. However, two samples of RED and CAR from Vitis vinifera showed no significant differencesA non-significant difference was also observed in the TFC between GR and RED, whereas the other extracts differed significantly ($p < 0.05$). the results of our study proving that the TPC and TFC values of the four Algerian vine-cane types produced under the same conditions showed significant differences, suggesting that these differences may be caused by the vine-cane variety. et al. (2016) also studied the TPC and TFC contents of the methanolic cane extracts from 11 genotypes and their fractions. The results revealed that the ethyl acetate fraction demonstrated the highest TPC and TFC contents with an amount of 586 mg/g of gallic acid equivalent and 320 mg/g of quercetin equivalent, respectively. Additionally, lower amounts of TPC and TFC were detected in the grape wastes (skin and seeds) of four grape cultivars studied by Nedelkovski et al. (2017), with the highest concentrations in the seed extracts (82.7 mg/g of gallic acid equivalentontents and 44.3 mg/g of quercetin equivalent, respectively).

According to Šikuten et al. (2020), the most important factor that contributes to the variation of the phenolic compounds in grape wastes is the genotype (cultivar), whereas other factors can also play a key role in this variation, such as the extraction method, harvesting time, and environmental conditions in which the cultivar is grown, like temperature, soil, and water availability. Regarding the same context, total phenolic compounds obtained by ultrasonic extraction from 8 Vitis Vinifera cane varieties from different locations in Argentina were also demonstrated using 50% acetone as solvent at 60 °C during 60 min. The results showed levels ranging from 36 to 20 mg GAE g-1 DW and 32 to 22 mg GAE g-1 DW of TPC both by FC and 280 nm lecture, respectively, which found to be very low in comparison with our results (Ferreyra *et al.* 2020). Other evaluations of both total phenolic (TPC) and total flavonoid (TFC) contents from different spices and varieties of grape canes cultivated in China were also carried out using an acidified methanol solution as a solvent for 24 h at 20 °C in a shaking incubator.

Table 1. Antioxidant potentials with total phenolic and flavonoid contents of four Algerian cane extracts

Extract	DPPH	ABTS	Reducing power	TPC	TFC
	IC50(µg/mL)	IC50(µg/m)	A0.5(µg/mL)	(mg GAE/g)	(mg QE/g)
GR *	128b ± 3.24	11,6a ± 0,3	74.33a ± 0.4	123c ± 11,7	40,5c ± 0,2
FR *	41.58d ± 0.7	3.13c ± 0.4	40.12c ± 3.41	309,8a ± 11.5	55,6a ± 2,06
RED *	184a ± 14.22	9,18b ± 0,2	68.80a ± 5.50	205,07b ±16,4	40,5c ± 7,3
CAR *	70.74c ± 1	8.56b ± 0.7	41.33c ± 0.77	198,3b ± 5.4	48,5b ± 7,9
BHA **	5.73f ± 0.4	1.81d ± 0.1	8.41d ± 0.67	NT	NT
BHT **	22.32e ± 1.2	1.29d ± 0.1	50.1b ± 1.53	NT	NT

*A0.5: the concentration at the 0.50 absorption and IC50: the concentration at the fifty of inhibition. A0.5 and IC50 values represent the means ± SEM of three measures. **Standard compounds, *extracts from the different varieties. BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene. The values in identical columns with various superscripts (a, b, c, d, e, or f) differ significantly ($p < 0.05$). NT: not treated, TPC (mg gallic acid/g extract), TFC (mg quercetin/g extract).*

Table 2. Correlation coefficient between phenolic and flavonoid contents (mg gallic acid/g extract, mg quercetin/g extract) and the IC50 of different activities. ** Correlation significative at a level of 0.01.* Correlation significative at a level of 0.05.

	DPPH	ABTS	Reducing Power	Urease	Amylase	flavonoids
polyphenols	-0,980**	-0,584*	-0,707*	0,604*	0,544*	0.830**
flavonoids	-0,894**	-0,911**	-0,914**	0,203	0,850**	1

The results of this study were found to be, in some cases, lower and in others, higher compared to our results. Significant differences were observed both between species and between varieties of the same species, with values varying from 76.4 to 224.5 mg (GAE)/g for TPC and from 33.1 to 146.6 mg (QCE)/g for TFC (Zhang *et al.* 2011). The results obtained by these studies are in accordance with our suggestion regarding the influence of variety. Moreover, another study conducted by Esparza et al. (2021) found that climatic conditions and the variety influenced the phenolic content of *Vitis vinifera* stems harvested in two different years, 2016 and 2018. The findings revealed substantial variances in terms of total phenolic content (TPC) and total flavonoid content (TF) among the grape stems originating from different varieties within each harvest year, as well as among the stems from

the same variety across the two vintage years. It was noticed that the higher temperatures and water stress may affect final yields and production through the reduction of grapevine metabolism during the active growth season (Shah *et al.* 2021). Regarding the influence of extraction technique, Moreira et al. (2018) studied the effect of three different extraction techniques, namely: microwave-assisted extraction (MAE), subcritical water extraction (SWE), and conventional extraction (CE), on the TPC and TFC contents of two Portugal vine cane extracts, and the results showed that the most friendly two techniques (MAE and SWE) extracted the highest content of phenolic and flavonoid compounds. These two green extraction procedures break down more easily the phenolic compounds in samples, releasing them to the extracellular medium and dissolving them in solvents.

Furthermore, Esparza et al. (2020) followed the changes in the grape stem total phenolic compounds during three times of storage (2, 4, and 6 months) and demonstrated a decrease trend in the TPC content, especially after six months of storage.

On the other hand, the statistical analysis represented a high correlation between TPC and TFC ($r = 0.830$, $p < 0.01$), which revealed that flavonoids are the major compound contributing to total phenolics in grape cane extracts. Zhang et al. (2011) also mentioned this relation.

3.2. Antioxidant activity

In the present study, we used a total of three in vitro assays (DPPH, ABT, and reducing power) to evaluate the antioxidant properties of the different cane extracts, knowing that each assay has a different mode of action and reaction mechanism where the hydrogen donation and electron transfer are the main ones (Srief *et al.* 2022). Furthermore, the fact that free radicals exert effects on biological systems, especially human tissues, made radical scavenging activity a crucial trait (Moreira *et al.* 2020). The results show that most extracts demonstrate moderate antioxidant abilities by all of the investigated methods.

3.2.1. DPPH Scavenging Activity

The most common free radical used to evaluate in vitro antioxidant activity is DPPH. The DPPH antioxidant assay is based on the fact that antioxidant molecules, after reacting with the stable free radical DPPH, change their color from purple to pale yellow. It's classified as both an electron or hydrogen atom transfer mechanism (Barchan *et al.* 2014; Christodoulou *et al.* 2022). The results of the inhibition assay represented in **Figure 1** showed that the FR variety attained a maximum percentage of inhibition (89.67%) at a concentration of 25 µg/ml; both CR and GR reached it at 50 µg/ml (88.85%, 88.80%, respectively), whereas the RED variety (88.80%) at 100 µg/ml. The FR extract showed the best scavenging (DPPH) potential in comparison with the other extracts with the lowest IC50 value (41.58 ± 0.70 µg/ml), which is found to be higher than that of BHA and BHT (5.73 µg/ml, 22.32 µg/ml). The IC50 values of the others ranked as follows: CAR<GR<RED and was: 70.74 ± 1.09, 128 ± 3.24, and 184 ± 14.22 µg/ml, respectively. It is well known that the differences between grape varieties and the diversity of extraction procedures and measurement methods used make it difficult to directly compare the antioxidant activities of cane extracts with those reported in the literature. However, these DPPH scavenging results are higher than those from an earlier study by Zhang et al. (2011), who predicted this value in the range of (IC50: 21.97-60.88 g/mL). Furthermore, Moreira et al. (2020) estimated the DPPH scavenging activity of two Portugal vine shoots named TN and TR extracted by three different methods: CE, MAE, and SWE, where the extracts from CE showed the higher antioxidant effect; these findings were found to be lower than our results. It is observed that extracts with an abundance of polyphenols and flavonoids exert significant antioxidant activity. Therefore, it can be assumed that the plant's phenolic content may contribute to their antioxidant power due to their ability to act as reducing agents, hydrogen donors, and singlet oxygen quenchers via their redox characteristics (Noviello *et al.* 2022). That is what can be presented by the high correlation between TPC, TFC, and the AC activity of the different extracts with the different methods. In the same context, the correlation coefficients (Table 2) were found to be strongly and highly negative when comparing the phenolic and flavonoid contents with the IC50 values of the DPPH scavenging assay ($r = -0.584$ and -0.911 for TPC and TFC, respectively, $p < 0.01$) with the highest correlation with TFC, which can be explained by the fact that flavonoids are the main group of polyphenols that contribute to the antioxidant activity against DPPH. Other authors also reported the correlation between TPC, TFC, and antioxidant activity by DPPH (Zhang *et al.* 2011; Balík *et al.* 2008; Gharwalova *et al.* 2018).

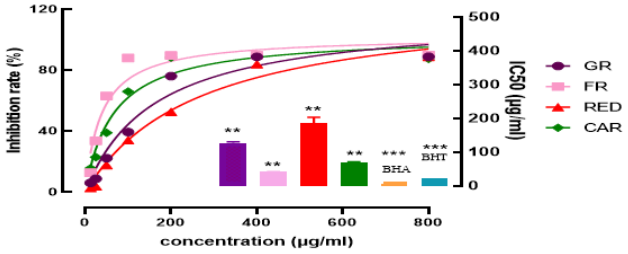


Figure 1. Inhibitory activity and IC50 values of the studied extracts and standard determined using DPPH inhibitory assay. (**) for comparison inter-compounds and (***) with standard.

3.2.2. ABTS Scavenging Activity

In this test, the antioxidant capacity of extracts to neutralize the stable radical cation ABTS•+ is assessed. When ABTS is oxidized by a potent oxidant (K2S2O8), an ABTS•+ radical is produced and scavenged by a potent antioxidant, reducing its blue-green color (Christodoulou *et al.* 2022). The obtained values for the ABTS assay were found to be much lower and more effective than those of DPPH in terms of IC50; that can be explained by the reduced form of ABTS, which is absent in the DPPH solution, and which is consistent with the different kinetics of the two reactions (Benmohamed *et al.* 2023). Moreover, certain monoterpene alcohols, ethers, ketones, and

aldehydes, as well as several nonpolar bioactive substances, including pigments that may be extracted with phenolic compounds, were thought to be responsible for the ABTS scavenging activity, which makes the ABTS assay more adaptable due to its ability to detect the scavenging activity of both polar and non-polar compounds (Srief *et al.* 2022; Zhang *et al.* 2011). The results of the antioxidant power of the tested extracts presented in **Figure 2** showed that the inhibition percentage of all extracts reached 93% at concentrations of 25µg/ml. FR, CR, RED, and GR extracts (12.5–800 µg/ml) showed a percentage of ABTS radical inhibition ranging from 47.2% to 93.80%, 26.60% to 93.64, 26.23% to 93.80%, and 21.29% to 93.91%, respectively. According to the recorded results, all extracts have strong antioxidant activity in the term of IC50 (table 1), which is found to be higher than that recorded for both BHA and BHT (1.81 and 1.29 µg/ml). As for the DPPH test, the lowest IC50 value was detected in the FR variety (3.13 ± 0.4 µg/ml), which is found to be 2.73 folders lower than the CR one (8.56 ± 0.7 µg/ml). A significantly higher value was obtained for the RED variety (9,18 ± 0,2 µg/ml) and an even higher value in the case of the GR (11,61 ± 0,3 µg/ml). In this context, the ability of different white Portuguese grape stem extracts to quench free ABTS radicals was measured and reported to be lower than our results (Leal *et al.* 2020). Another study carried out by Noviello et al. (2022) estimated higher values in the range of (79.2-136.5 µmol TE g−1 DW) for 23 Italian varieties. Another evaluation of the ability of eight grape stem extracts from Argentina to scavenge ABTS was examined by Moreira et al. (2020), showing less potent results in the range of (108–221 µmol TE g−1 DW).

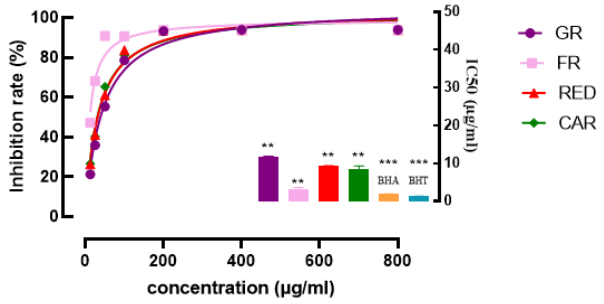


Figure 2. Inhibitory activity and IC50 values of the studied extracts and standard determined using ABTS inhibitory assay. (**) for comparison inter-compounds and (***) with standard.

For all cultivars, a correlation analysis between phenolic and flavonoid content and IC50 values were conducted (Table 2). The IC50 values strongly and negatively correlated with both TPC ($r = -0.980$, $p < 0.01$) and TFC ($r = -0.894$, $p < 0.01$), showing a high correlation between the total phenolic and flavonoid content and the ABTS scavenging activity that was confirmed by Zhang et al. (2011) and Ferreyra et al. (2019).

3.2.3. Reducing Power Activity

This method is based on the fact that when a single electron is transferred from an antioxidant to the ferric ion presented in the Fe3+-TPTZ (ferric 2,4,6-tripyridyl-s-triazine) complex, it turns it into the Fe2+-TPTZ (ferric 2,4,6-tripyridyl-s-triazine) complex and changes its color from yellow to various shades of green. The Fe2+ concentration is followed by measuring the production of Perl's Prussian blue at 700 nm, where a higher reducing power is indicated by a higher absorbance (Christodoulou *et al.* 2022; Derradji-Benmeziane *et al.* 2014). The evaluation of the reducing power of vine cane extracts also showed the same tendency, with the best activity of FR in relation to that of the other varieties. At the lowest concentration tested (3.125 µg/ml), the FR extract showed an inhibition percentage of 12% against 11%, 11%, and 9% for CR, RED, and GR, while at 200 µg/ml The PI of the extracts are ranked as 74%, 61%, 65%, and 54%, respectively (**Figure 3**). With regard to A0.5, those of the extracts and standards BHA, FR, CR, BHT, RED, and GR correspond to 8.41 ± 0.67 µg/ml, 40.12 ± 3.41 µg/ml, 41.33 ± 0.77 µg/ml, 50.1 ± 1.53µg/ml, 68.80 ± 5.50 µg/ml, and 74.33 ± 0.44 µg/ml, respectively. Both FR and CR were found to be better than the BHT standard in terms of A 0.5 values.

The results of the reducing power assay of our study are close to that evaluated by Zhang et al. (2011) for some varieties and better than others. According to the findings provided by Balík et al. (2008), the power of grape berries, stems, and leaves of three white and three blue varieties from Vitis vinifera L to reduce ferric ions was averagely lower than our results, and these values were oscillated between 6.25 and 12.4 mg/g for stem extracts. Regarding grape cane extracts from typical cultivars of Southern Italy extracted at different ph values, the IC50 was in the range of (3.649 to 39.482 µg (AAE)/mg DE) with the best reduction of ferric ions at ph 13.00; these findings are lower than our results (Squillaci *et al.* 2021). The A0.5 values of grape cane extracts were shown to be considerably and significantly correlated with both TPC and TFC (table 2) in the current analysis ($r = -0.707$ and -0.914, respectively, $p < 0.01$). Other studies have also demonstrated that the reducing power of grape cane extracts is well correlated with their phenolic contents (Zhang *et al.* 2011; Balík *et al.* 2008). A high correlation was represented between the TPC and TFC, and the three antioxidant tests confirmed that these molecules are the main contributors to the AC activity. Furthermore, according to Castro-López et al. (2019), the content of antioxidants in grapes is directly related to the content of different polyphenols, which contained one or more aromatic benzene rings linked by mono- or poly-hydroxyl groups, which allowed them to donate electrons or hydrogens (DPPH and ABTS), as well as reduce and chelate metals (FRAP) (Munthe *et al.* 2023). In the case of flavonoids, the presence of high reactive hydroxyl groups in their structure enabled them to stabilize reactive oxygen species through reactions with free radical compounds (Munthe *et al.* 2023). It is well noticed that ROS and free radicals can cause oxidative damage to cell membranes, DNA, and proteins, contributing to degenerative processes like aging, cancer, and atherosclerosis (De Freitas Araújo *et al.* 2012). Because of that, grape canes, a neglected agricultural pruning waste from the grape and wine industries, have potential to be used as nutraceutical supplements or antioxidants (Zhang *et al.* 2011).

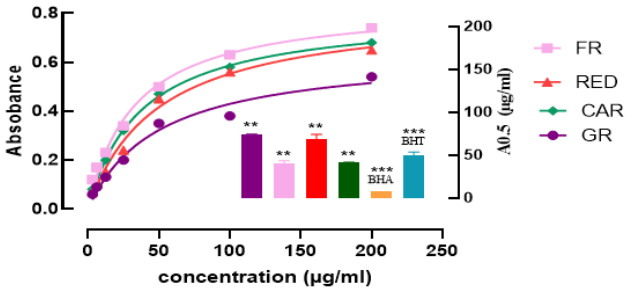


Figure 3. Inhibitory activity and A0.5 values of the studied extracts and standard determined using reducing power inhibitory assay. (**) for comparison inter-compounds and (***) with standard.

3.3. Enzymatic Activity

3.3.1. Anti α-Amylase Activity

One of the primary enzymes responsible for digesting dietary starch is a-amylase. It produces oligosaccharides, which can then be further broken down into absorbable monosaccharides at the brush border of the gastrointestinal tract. Therefore, it is thought that inhibiting this enzyme is a preventive diabetes management method resulting in reduced postprandial hyperglycemia (Oluwagunwa *et al.* 2021; Oyedemi *et al.* 2017). This study investigated the effects of ethanolique cane extracts on α-Amylase inhibition. As demonstrated by the results of different extracts (**Figure 4**), there is a dose-dependent effect, increasing inhibitory efficacy with the increase of concentration. The results revealed a maximum of 82.84, 75.49, 71.10, 66.10, and 20,05% inhibition of α-amylase activity at the highest concentration of 200 µg/ml for the extracts from CR, FR, GR, RED, and at 300 µg/ml for acarbose, respectively. All of these natural extracts were found to be more effective than acarbose at inhibiting α-amylase. The IC50 was found to be in the following order: 10.30, 12.60, 16.50, 40.20, and 298.5 µg/mL for CAR, RED, GR, FR, and Acarbose, respectively. These results are in accordance with the findings of Ben Khadher et al. (2022), who similarly noted the inhibitory effects of macerated ethanolic extract from grape stem plant, which exerted a high inhibition activity against amylase represented by a low value of IC50 13.4 µg/ml. Additionally, Moreira et al. (2018) also assessed the inhibition of α-amylase by natural stem extracts from two different varieties, which showed values with an IC50 of 60.37 ± 5.55 and 73.28 ± 6.77 µg/ml for the MAE technique; these findings are higher than our ones. In addition, some in vivo and in vitro investigations have suggested that phenolic compounds from grape stems may also have antidiabetic effects by demonstrating insulinotropic effects, giving the possibility that grape stems have the ability to control insulin production and supporting their use for type II diabetic treatment (Barros *et al.* 2015; Baroi *et al.* 2022).

A positive correlation between the total phenolic ($r = 0.544$, $p < 0.01$) and flavonoid content ($r = 0.855$, $p < 0.01$) and the IC50 values of anti-α-amylase activity was evaluated, showing that the inhibition of the enzyme is more correlated with flavonoids; in the same case, Cisneros et al. (2023) mentioned the ability of flavonoids to inhibit the action of α-amylase by establishing the covalent bonds with starch during cooking and in the stomach, reducing its availability as an enzyme substrate.

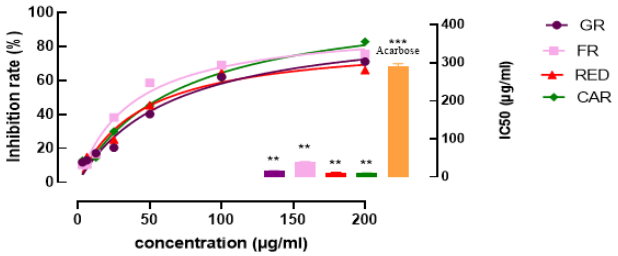


Figure 4. Inhibitory activity and IC50 values of the studied extracts and standard determined using α-amylase inhibitory assay. (**) for comparison inter-compounds and (***) with standard.

3.3.2. Anti Urease Activity

The multi-subunit nickel-containing enzyme urease (also known as urea amidohydrolase; EC 3.5.1.5) is found in plants, fungi, and bacteria and catalyzes the hydrolysis of urea to ammonia. It also plays a crucial role in seed germination and microbial development. In a pathological sense, urease's production of ammonia leads to an increase in environmental Ph; this encourages the survival of harmful bacteria like H. pylori, which could ultimately induce gastrointestinal problems like duodenal, peptic ulcers, and gastric cancer (Al-Rooqi *et al.* 2023; Rauf *et al.* 2020). Using a range concentration from 3.125 µg/ml to 200 µg/ml, the ability of the different extracts to inhibit urease enzyme activity was evaluated, showing a dose-dependent activity manner. All the extracts except the RED variety reached their 50% inhibition of the enzyme activity at a concentration of 100 µg/ml. A maximum of 63.74, 70.5, 71.32, 79.35, and 98.90% inhibition of urease activity was observed at the highest concentration for the cane extracts from FR, GR, RED, CAR, and Thiouria, respectively. The standard inhibitor used in the current study with an IC50 value of 11.57 ± 0.68 µg/ml was found to be more potent, whereas the extract from the CAR variety exhibited the best urease inhibitory activity regarding the other extracts with an IC50 value of 25.88 ± 2.26 µg/ml. The IC50 values moderately correlated with TPC ($r = 0.604$, $p < 0.05$) and weakly with TFC ($r = 0.203$, $p < 0.05$), showing a positive correlation between flavonoid content and the urease inhibitory activity.

The inhibition of urease activity by grape canes has not previously been treated, despite the fact that various authors have characterized their different biological effects. However, novel urease inhibitors with agriculture interest have been investigated using red grape pomace polyphenols from winery byproducts extracted by Deep Eutectic Solvents, where the DES-polyphenol formulation demonstrated the best urease inhibition among the others with an inhibition percentage of 60–90% (Samorì *et al.* 2019). Another study carried out with various resveratrol concentrations contained in two commercial red wines revealed the remarkable inhibition of H. pylori urease with more potency in extracts with higher contents of

resveratrol (Paulo *et al.* 2011). The use of plant-based enzyme inhibitors is becoming popular in the pharmaceutical sector as an essential part of the current prescription drug to treat numerous human diseases (Dwibedi *et al.* 2022), giving the possibility to use grape cane extracts in the medical field.

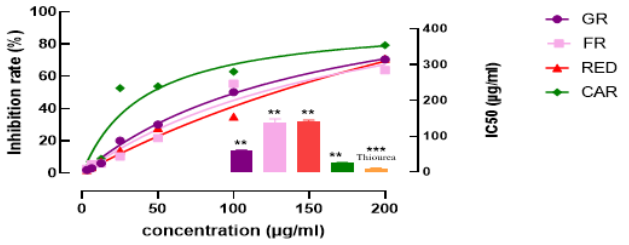


Figure 5. Inhibitory activity and IC50 values of the studied extracts and standard determined using urease inhibitory assay. (**) for comparison inter-compounds and (***) with standard.

3.4. Anti-inflammatory Activity by BSA

One of the factors that contribute to inflammation is the denaturation of proteins (Bailey-Shaw *et al.* 2017). It is a pathological process that involves electrostatic hydrogen, hydrophobic, and disulfide bonding modification, which could result in loss of both configuration and functionality (Dharmadeva *et al.* 2018). In diseases like rheumatoid arthritis, cancer, and diabetes, which are inflammatory disorders, denaturation of proteins induces the generation of autoantigens. Therefore, potential anti-inflammatory medication prospects may come from medicinal plant extracts that prevent protein denaturation and maintain cell membranes against lyses (Anyasor *et al.* 2019). It was found that the inhibition rate of BSA denaturation by both cane extracts and standard gradually increases with the increase in the concentration (table 3). The four extracts exhibited more than 40% inhibition of BSA denaturation at 1000 µg/ml, whereas both RED and FR varieties displayed 60% inhibition at 2000 µg/ml, showing a moderate activity against BSA denaturation. These results were found to be lower than that of dichlofenac, which gave a 100% inhibition at the highest concentration.

The anti-inflammatory effect of vine cane and stems has been identified by other authors using different methods. However, the use of BSA has not been reported in the literature. Pop et al. (2022) tested the anti-inflammatory effect of two pomace extracts, white and red, a cane extract, and their combination, all from the Vitis vinifera by-products. The results showed that the four samples exhibited a potent dose-dependent anti-inflammatory effect by reducing the levels of proinflammatory cytokines (IL-6, IL-8, and IL-1) induced by exposing cells to non-cytotoxic doses of lipopolysaccharides. Using a lipoxygenase inhibition assay, another evaluation of the anti-inflammatory activity of white Sauvignon stem was carried out, showing that the macerated ethyl acetate extract represented the highest inhibition percentage of 64.5% and the lowest IC50 of 26.6 µg/ml (Ben Khadher *et al.* 2022).

Table 3. Percentage inhibition of BSA by both extracts and standard. (*) for comparison inter-compounds and (**) with standard.

Concentration µg/ml	GR*	FR*	RED *	CAR*	Dichlofenac Sodium**
2000 µg/ml	48 ± 0.5	60 ± 0.55	61 ± 0.95	46 ± 0.82	100 ± 0.18
1000 µg/ml	40 ± 0.7	59 ± 0.45	42 ± 0.65	42 ± 0.97	92 ± 0.15
500 µg/ml	28 ± 0.8	47 ± 0.47	38 ± 0.43	37 ± 0.22	61 ± 0.15
250 µg/ml	22 ± 0.20	46 ± 0.32	35 ± 0.12	30 ± 0.18	37 ± 0.18

3.5. Sun Protect Factor

People were recently exposed to another type of radiation (ultraviolet B radiation) reaching the earth's surface as a result of the destruction of the ozone layer. The UVB spectrum (280-320 nm) penetrates the skin and is the main contributor to a number of skin disorders (Che *et al.* 2017). Recently, due to their comparable structures to chemical UV filters, which show the same mechanism of action, natural phenols have been proposed as active agents in cosmetic formulations as constituents for sunscreens (Nunes *et al.* 2017). The results demonstrated that CAR, GR, and RED were given the highest SPF index with no significant differences between the samples, whereas FR showed the lowest SPF value. It can be suggested that all extracts have a photoprotective effect with high SPF values. However, the use of grapevine stem extracts as a raw material in cosmetics in order to fight skin damage is increasing recently. In the same context, Vitis vinifera L. was tested for safety and clinical efficacy against UVB radiation. Two of the nine emulsions developed were clinically examined, and the results showed that the formulation with 10% w/w grape pomace extract (seed, skin, stem) exhibited the highest SPF value with the best antioxidant activity and UVB protection (Hübner *et al.* 2020). Che et al. (2017) et al. also assessed the preventive effect of grape stem extract against UVB-induced oxidative damage in C57BL mice. The results confirmed the repaired effect of extracts through the inhibition of several damages, such as collagen breakdown and pigmentation induced by UVB. From that, grapes and their derivatives provide potential photoprotective properties against UV radiation, making them appropriate for herbal cosmetic compositions (Soto *et al.* 2015).

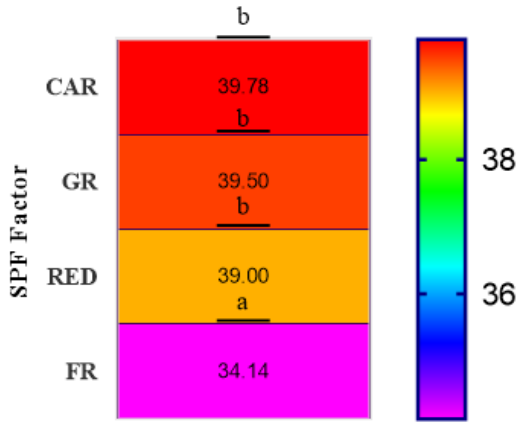


Figure 6. SPF value of each extracts, the values in identical columns with various superscripts (a, b) differ significantly ($p < 0.05$).

Table 4. Sun products categories according to the Recommendation of the Commission of the European Communities (2006).

Indicated category	Indicated protection	Sun protection factor measured
Low protection	6	6 - 9,9
	10	10 - 14,9
Average protection	15	15 - 19,9
	20	20 - 24,9
	25	25 - 29,9
High protection	30	30 - 49,9
	50	50 - 59,9
Very high protection	50+	60 ≤

4. Conclusion

The total phenolic and flavonoid contents and biological activities of four Algerian vine cane cultivars were reported for the first time in this work. All the cultivar extracts demonstrated a good source of bioactive compounds, with a priority to the FR variety, which represented the highest content of both phenolic and flavonoid compounds. The same variety exhibited the highest antioxidant activity towards all methods tested (DPPH, ABTS, Reducing Power), which can be explained by the relation between the phenol composition of the different extracts and their antioxidant activity. This relation was confirmed by a high correlation coefficient. The different extracts demonstrated also good anti-amylase and urease activity, a potent protective agent against denaturation of protein (BSA), and a high protective effect against solar radiation. Results encourage the use of grape canes as a source of bioactive phytochemicals in the pharmaceutical, cosmetic, and food industries as antioxidants, providing an effective, economical, and eco-friendly solution for generated wastes. Moreover, the remarkable activity registered towards BSA may facilitate and guide further studies to develop new anti-inflammatory compounds, besides the possible use of these wastes as sunscreen ingredients. To achieve these goals, more research is ongoing to identify the phytochemical composition of grape cane extracts and to assess their in vivo activities.

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References

Al-Rooqi, M. M., Mughal, E. U., Raja, Q. A., Hussein, E. M., Naeem, N., Sadiq, A., & Ahmed, S. A. (2023). Flavonoids and related privileged scaffolds as potential urease inhibitors: a review. *RSC advances*, *13*(5), 3210-3233.

Akkak, A., Boccacci, P., & Botta, R. (2007). 'Cardinal'grape parentage: a case of a breeding mistake. *Genome*, *50*(3), 325-328.

Anna Malinowska, M., Billet, K., Drouet, S., Munsch, T., Unlubayir, M., Tungmunnithum, D., ... & Lanoue, A. (2020). Grape cane extracts as multifunctional rejuvenating cosmetic ingredient: Evaluation of sirtuin activity, tyrosinase inhibition and bioavailability potential. *Molecules*, *25*(9), 2203.

Anyasor, G. N., Okanlawon, A. A., & Ogunbiyi, B. (2019). Evaluation of anti-inflammatory activity of Justicia secunda Vahl leaf extract using in vitro and in vivo inflammation models. *Clinical Phytoscience*, *5*, 1-13.

Aydemir, B. Ç., & Göksu, B. (2023). Phenological, morphological and genetic characterization of local grapevine (Vitis labrusca L.) genotypes grown in the Black Sea Region in Northern Turkey.

Barchan, A., Bakkali, M., Arakrak, A., Pagán, R., & Laglaoui, A. (2014). The effects of solvents polarity on the phenolic contents and antioxidant activity of three Mentha species extracts. *Int. J. Curr. Microbiol. Appl. Sci*, *3*(11), 399-412.

Bailey-Shaw, Y. A., Williams, L. A., Green, C. E., Rodney, S., & Smith, A. M. (2017). In-vitro evaluation of the anti-inflammatory potential of selected jamaican plant extracts using the bovine serum albumin protein denaturation assay. *International Journal of Pharmaceutical Sciences Review and Research*, *47*(1), 145-153.

Balík, J., Kyseláková, M., Vrchotová, N., Tříska, J., Kumšta, M., Veverka, J., ... & Lefnerová, D. (2009). Relations between polyphenols content and antioxidant activity in vine grapes and leaves. *Czech Journal of Food Sciences*, *26*(Special Issue), S25-S32.

Baroi, A. M., Popitiu, M., Fierascu, I., Sărdărescu, I. D., & Fierascu, R. C. (2022). Grapevine wastes: A rich source of antioxidants and other biologically active compounds. *Antioxidants*, *11*(2), 393.

Barros, A., Gironés-Vilaplana, A., Texeira, A., Baenas, N., & Domínguez-Perles, R. (2015). Grape stems as a source of bioactive compounds: application towards added-value commodities and significance for human health. *Phytochemistry reviews*, *14*, 921-931.

Ben Khadher, T., Aydi, S., Mars, M., & Bouajila, J. (2022). Study on the chemical composition and the biological activities of Vitis vinifera stem extracts. *Molecules*, *27*(10), 3109.

Benmohamed, M., Guenane, H., Messaoudi, M., Zahnit, W., Egbuna, C., Sharifi-Rad, M., ... & Yousfi, M. (2023). Mineral profile, antioxidant, anti-inflammatory, antibacterial, anti-urease and anti-α-amylase activities of the unripe fruit extracts of pistacia atlantica. *Molecules*, *28*(1), 349.

Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, *181*(4617), 1199-1200.

Castro-López, L., Castillo-Sánchez, G., Díaz-Rubio, L., & Córdova-Guerrero, I. (2019). Total content of phenols and antioxidant activity of grape skins and seeds cabernet sauvignon cultivated in Valle de Guadalupe, Baja California, México. *BIO Web of Conferences*, *15*, 04001.

Che, D. N., Xie, G. H., Cho, B. O., Shin, J. Y., Kang, H. J., & Jang, S. I. (2017). Protective effects of grape stem extract against UVB-induced damage in C57BL mice skin. *Journal of Photochemistry and Photobiology B: Biology*, *173*, 551-559.

Christodoulou, M. C., Orellana Palacios, J. C., Hesami, G., Jafarzadeh, S., Lorenzo, J. M., Domínguez, R., ... & Hadidi, M. (2022). Spectrophotometric methods for measurement of antioxidant activity in food and pharmaceuticals. *Antioxidants*, *11*(11), 2213.

Cisneros-Yupanqui, M., Lante, A., Mihaylova, D., Krastanov, A. I., & Rizzi, C. (2023). The α-amylase and α-glucosidase inhibition capacity of grape pomace: a review. *Food and Bioprocess Technology*, *16*(4), 691-703.

da Silva, C. E. R., de Oliveira, M. G., Baesso, M. L., Agra, K. L., Suassuna Filho, J., & da Silva Lima, R. J. (2023). Vitis vinifera Red Globe grape: In natura investigations on skin pigmentation using phase-resolved photoacoustic and TDDFT methods. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 298, 122761.

Derradji-Benmeziane, F., Djamai, R., & Cadot, Y. (2014). Antioxidant capacity, total phenolic, carotenoid, and vitamin C contents of five table grape varieties from Algeria and their correlations. *OENO One*, *48*(2), 153-162.

De Freitas Araújo, M. G., Hilário, F., Vilegas, W., Dos Santos, L. C., Brunetti, I. L., Sotomayor, C. E., & Bauab, T. M. (2012). Correlation among Antioxidant, Antimicrobial, Hemolytic, and Antiproliferative Properties of Leiothrix spiralis Leaves Extract. *International Journal of Molecular Sciences*, *13*(7), 9260-9277.

Dharmadeva, S., Galgamuwa, L. S., Prasadinie, C., & Kumarasinghe, N. (2018). In vitro anti-inflammatory activity of Ficus racemosa L bark using albumin denaturation method. *Ayu*, *39*(4), 239.

Dorosh, O., Moreira, M. M., Rodrigues, F., Peixoto, A. F., Freire, C., Morais, S., & Delerue-Matos, C. (2020). Vine-canes valorisation: Ultrasound-assisted extraction from lab to pilot scale. *Molecules*, *25*(7), 1739.

Dwibedi, V., Jain, S., Singhal, D., Mittal, A., Rath, S. K., & Saxena, S. (2022). Inhibitory activities of grape bioactive compounds against enzymes linked with human diseases. *Applied Microbiology and Biotechnology*, *106*(4), 1399-1417.

Escobar-Avello, D., Mardones, C., Saez, V., Riquelme, S., von Baer, D., Lamuela-Raventos, R. M., & Vallverdu-Queralt, A. (2021). Pilot-plant scale extraction of phenolic compounds from grape canes: Comprehensive characterization by LC-ESI-LTQ-Orbitrap-MS. *Food Research International*, *143*, 110265.

Esparza, I., Moler, J. A., Arteta, M., Jiménez-Moreno, N., & Ancín-Azpilicueta, C. (2021). Phenolic composition of grape stems from different Spanish varieties and vintages. *Biomolecules*, *11*(8), 1221.

Ferreyra, S., Bottini, R., & Fontana, A. (2019). Assessment of grapevine stems as source of phenolics with antioxidant properties. *Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo*, *51*(2), 417-426.

Ju, Y., Zhang, A., Fang, Y., Liu, M., Zhao, X., Wang, H., & Zhang, Z. (2016). Phenolic compounds and antioxidant activities of grape canes extracts from vineyards. *Spanish Journal of Agricultural Research*, *14*(3), e0805.

Gharwalova, L., Hutar, D., Masak, J., & Kolouchova, I. (2018). Antioxidant activity and phenolic content of organic and conventional vine cane extracts. *Czech Journal of Food Sciences*, *36*(4), 289-295.

Hübner, A. A., Sarruf, F. D., Oliveira, C. A., Neto, A. V., Fischer, D. C., Kato, E. T., ... & Bacchi, E. M. (2020). Safety and photoprotective efficacy of a sunscreen system based on grape pomace (Vitis vinifera L.) phenolics from winemaking. *Pharmaceutics*, *12*(12), 1148.

Insanu, M., Karimah, H., Pramastya, H., & Fidrianny, I. (2021). Phytochemical compounds and pharmacological activities of Vitis vinifera L.: An updated review. *Biointerface Res. Appl. Chem*, *11*(5), 13829-49.

Kodeš, Z., Vrublevskaya, M., Kulišová, M., Jaroš, P., Paldrychová, M., Pádrová, K., ... & Kolouchová, I. (2021). Composition and biological activity of vitis vinifera winter cane extract on candida biofilm. *Microorganisms*, *9*(11), 2391.

Leal, C., Santos, R. A., Pinto, R., Queiroz, M., Rodrigues, M., Saavedra, M. J., ... & Gouvinhas, I. (2020). Recovery of bioactive compounds from white grape (Vitis vinifera L.) stems as potential antimicrobial agents for human health. *Saudi journal of biological sciences*, *27*(4), 1009-1015.

Mansur, J. D. S., Breder, M. N. R., Mansur, M. C. D. A., & Azulay, R. D. (1986). Determinação do fator de proteção solar por espectrofotometria. *An. Bras. Dermatol*, 121-4.

Moreira, M. M., Barroso, M. F., Porto, J. V., Ramalhosa, M. J., Švarc-Gajić, J., Estevinho, L., ... & Delerue-Matos, C. (2018). Potential of Portuguese vine shoot wastes as natural resources of bioactive compounds. *Science of the Total Environment*, *634*, 831-842.

Moreira, M. M., Barroso, M. F., Porto, J. V., Ramalhosa, M. J., Švarc-Gajić, J., Estevinho, L., Morais, S., & Delerue-Matos, C. (2018). Potential of Portuguese vine shoot wastes as natural resources of bioactive compounds. *Science of The Total Environment*, *634*, 831-842.

Moreira, M. M., Rodrigues, F., Dorosh, O., Pinto, D., Costa, P. C., Švarc-Gajić, J., & Delerue-Matos, C. (2020). Vine-canes as a source of value-added compounds for cosmetic formulations. *Molecules*, *25*(13), 2969.

Müller, L., Gnoyke, S., Popken, A. M., & Böhm, V. (2010). Antioxidant capacity and related parameters of different fruit formulations. *LWT-Food Science and Technology*, *43*(6), 992-999.

Munthe, S. W. N., Riskianto, R., Juvi, D., & Novia, J. (2023). Antioxidant, Total Phenolic, and Total Flavonoid of 70% Ethanol Extract of Avocado Seeds (Persea americana Mill.). *Pharmacognosy Journal*, *15*(4), 599-605.Nedelkovski, D., J. Cvetković, K. Beleski and H. Poposka, 2017. Phenolic composition of Vranec grapevine cultivar (Vitis vinifera L.) grafted on different rootstock. Bulg. J. Agric. Sci., 23 (3): 389–395.

Noviello, M., Caputi, A. F., Squeo, G., Paradiso, V. M., Gambacorta, G., & Caponio, F. (2022). Vine shoots as a source of trans-Resveratrol and ε-Viniferin: A study of 23 Italian varieties. *Foods*, *11*(4), 553.

Nunes, M. A., Rodrigues, F., & Oliveira, M. B. P. (2017). Grape processing by-products as active ingredients for cosmetic proposes. In *Handbook of grape processing by-products* (pp. 267-292). Academic Press.

Oyedemi, S. O., Oyedemi, B. O., Ijeh, I. I., Ohanyerem, P. E., Coopoosamy, R. M., & Aiyegoro, O. A. (2017). Alpha-amylase inhibition and antioxidative capacity of some antidiabetic plants used by the traditional healers in Southeastern Nigeria. *The scientific world Journal*, *2017*.

Oluwagunwa, O. A., Alashi, A. M., & Aluko, R. E. (2021). Inhibition of the in vitro Activities of α-Amylase and Pancreatic Lipase by Aqueous Extracts of Amaranthus viridis, Solanum macrocarpon and Telfairia occidentalis Leaves. *Frontiers in Nutrition*, *8*, 772903.

Oyaizu, M. (1986). Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. *The Japanese journal of nutrition and dietetics*, *44*(6), 307-315.

Paulo, L., Oleastro, M., Gallardo, E., Queiroz, J. A., & Domingues, F. (2011). Anti-Helicobacter pylori and urease inhibitory activities of resveratrol and red wine. *Food Research International*, *44*(4), 964-969.

Pop, A., Bogdan, C., Fizesan, I., Iurian, S., Carpa, R., Bacali, C., ... & Moldovan, M. L. (2022). In vitro evaluation of biological activities of canes and pomace extracts from several varieties of Vitis vinifera L. for inclusion in freeze-drying mouthwashes. *Antioxidants*, *11*(2), 218.

Rätsep, R., Karp, K., Maante-Kuljus, M., Aluvee, A., Kaldmäe, H., & Bhat, R. (2021). Recovery of polyphenols from vineyard pruning wastes—shoots and cane of hybrid grapevine (Vitis sp.) cultivars. *Antioxidants*, *10*(7), 1059.

Rauf, A., Bawazeer, S., Naseer, M., Alhumaydhi, F. A., Aljohani, A. S., Habib, A., ... & Shariati, M. A. (2021). In vitro α-glycosidase and urease enzyme inhibition profile of some selected medicinal plants of Pakistan. *Natural Product Research*, *35*(23), 5434-5439.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, *26*(9-10), 1231-1237.

Rodrigues, J. A. (2023). Microsatellite DNA fingerprinting has revealed the identities of both parents of the hybrid grape cultivar, Isabella-a 200 year-old mystery finally solved.

Samorì, C., Mazzei, L., Ciurli, S., Cravotto, G., Grillo, G., Guidi, E., & Galletti, P. (2019). Urease inhibitory potential and soil ecotoxicity of novel “polyphenols–deep eutectic solvents” formulations. *ACS Sustainable Chemistry & Engineering*, *7*(18), 15558-15567.

Šikuten, I., Štambuk, P., Andabaka, Ž., Tomaz, I., Marković, Z., Stupić, D., ... & Preiner, D. (2020). Grapevine as a rich source of polyphenolic com Soto, M., Falqué, E., & Domínguez, H. (2015). Relevance of Natural Phenolics from Grape and Derivative Products in the Formulation of Cosmetics. *Cosmetics*, *2*(3), 259-276. pounds. *Molecules*, *25*(23), 5604.

Squillaci, G., Zannella, C., Carbone, V., Minasi, P., Folliero, V., Stelitano, D., ... & Morana, A. (2021). Grape Canes from Typical Cultivars of Campania (Southern Italy) as a Source of High-Value Bioactive Compounds: Phenolic Profile, Antioxidant and Antimicrobial Activities. *Molecules*, *26*(9), 2746.

Srief, M., Bani, M., Mokrani, E. H., Mennai, I., Hamdi, M., Boumechhour, A., ... & Akkal, S. (2023). Evaluation of In Vitro and In Silico Anti-Alzheimer Potential of Nonpolar Extracts and Essential Oil from Mentha piperita. *Foods*, *12*(1), 190.

Taha, M., Ullah, H., Al Muqarrabun, L. M. R., Khan, M. N., Rahim, F., Ahmat, N., ... & Khan, K. M. (2018). Bisindolylmethane thiosemicarbazides as potential inhibitors of urease: Synthesis and molecular modeling studies. *Bioorganic & medicinal chemistry*, *26*(1), 152-160.

Topçu, G., Ay, M., Bilici, A., Sarıkürkcü, C., Öztürk, M., & Ulubelen, A. (2007). A new flavone from antioxidant extracts of Pistacia terebinthus. *Food chemistry*, *103*(3), 816-822.

Troilo, M., Difonzo, G., Paradiso, V. M., Summo, C., & Caponio, F. (2021). Bioactive compounds from vine shoots, grape stalks, and wine lees: Their potential use in agro-food chains. *Foods*, *10*(2), 342.

Zengin, G., Sarikurkcu, C., Aktumsek, A., Ceylan, R., & Ceylan, O. (2014). A comprehensive study on phytochemical characterization of Haplophyllum myrtifolium Boiss. endemic to Turkey and its inhibitory potential against key enzymes involved in Alzheimer, skin diseases and type II diabetes. *Industrial Crops and Products*, *53*, 244-251.

Zhang, A., Fang, Y., Wang, H., Li, H., & Zhang, Z. (2011). Free-radical scavenging properties and reducing power of grape cane extracts from 11 selected grape cultivars widely grown in China. *Molecules*, *16*(12), 10104-10122.

Web sites

National Research Centre for Grapes (2009) https://nrcgrapes.icar.gov.in/Technical%20or%20Extension%20folders/Red-Globe.pdf. Consulté 29 sep. 2024.

Web 1: wine searcher: https://www.wine-searcher.com/grape-2008-alphonse-lavallee.

Web 2: weins.plus: https://glossary.wein.plus/alphonse-lavallee

Web 3: https://www.lecepdevigne.fr/raisins-de-table/61-alphonse-lavallee.html