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Evolution during the maturity and biological properties of phenolic compounds of the vine in Algeria

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Abstract

The aim of the present study is the evaluation of phytochemical composition, *in vitro* biological activities, and *in silico* anti-urease activity of four Algerian grape cane cultivars (Fragola Nera from the hybrid *Vitis vinifera-Vitis labrusca*, and Cardinal, Red glob, Gros noir from *Vitis vinifera*) and three seed varieties (Sabel, Cardinal, and Red glob from *Vitis vinifera*) at different repining stages (before veraison, veraison, and repining). The used methods include: the quantification of phenolic compounds using Folin-Ciocalteu reagent, flavonoids and flavonols using aluminum chloride method, phytochemical analysis using LCMS/MS, colorimetric assays to evaluate *in vitro* antioxidant and enzymatic activities, molecular docking and ADME/Tox investigation, determination of SPF factor, denaturation of BSA, and antimicrobial activity using disk diffusion method and determination of minimum inhibitory concentration (MIC). All grape cane extracts were compared for their TPC, TFC, and TF-OL values. The highest TPC and TFC were found in the grape cultivar FR (309,8 ± 11.5 mg GAE/g extract and 55,6 ± 2,06 mg QE/g extract, respectively). The highest TF-OL content was recorded in the RG extract with an amount of 15.98 ±1.20 mg QCE/g extract. Three different classes of phenolic compounds were detected: flavonoids, phenolic acids, and stilbenes, with flavonoid being the major class quantified followed by phenolic acid and stilbenes. Catechin was the highest molecule detected with a concentration of 1440.45 mg/kg D.W in the FR cultivar. The results revealed also that all the extracts showed high antioxidant capacity. The FR extract exhibited the most potent antioxidant activity with the lowest IC50 value in the ABTS test (3.13 ± 0.4 µg/mL). Significant enzymatic activity was also detected against all the studied enzymes. Molecular docking of the phenolic compounds against urease enzyme demonstrated the highest binding affinity with gallic acid (a binding energy value of -28.8802 kJ/mol). Additionally, all extracts showed a moderate anti-inflammatory activity, given a high sun protection factor, and exhibiting potent antimicrobial activity. The total phenolic compounds and the phenolic profile of grape seed was found to be changed with the changes of maturation stage. These changes contribute to different potential in the biological activities with the green stage being the most effective one. Catechin was detected in higher amounts in all cultivars giving the extracts high antioxidant potential, whereas, gallic acid was detected as the second highest molecule. These findings imply that grape canes and seeds have a significant potential to be used in pharmaceutical industry.

Key words: vine, grapes, cane, seed, polyphenols, antioxidant and enzymatic activities.

Résumé

Le but de la présente étude est l'évaluation de la composition phytochimique, des activités biologiques *in vitro*, et de l'activité anti-uréase *in silico* de quatre variétés de sarments de raisin Algérien (Fragola Nera de l'hybride *Vitis vinifera-Vitis labrusca*, et Cardinal, Red glob, Gros noir de *Vitis vinifera*) ainsi que trois variétés de pépin (Sabel, Cardinal et Red glob de *Vitis vinifera*) à différents stades de maturation (avant la véraison, durant la véraison, et durant la maturation). Les méthodes utilisées comprennent : la quantification des composés phénoliques à l'aide du réactif de Folin-Ciocalteu, les flavonoïdes et les flavonols à l'aide de la méthode du chlorure d'aluminium, l'analyse phytochimique à l'aide de LCMS/MS, les dosages colorimétriques pour évaluer les activités antioxydantes et enzymatiques *in vitro*, docking moléculaire et l'étude de l'ADME/Tox, la détermination du SPF facteur, la dénaturation de la BSA, et l'activité antimicrobienne par diffusion des disques et la détermination de la concentration minimale inhibitrice (CMI).Tous les extraits des sarments ont été comparés pour leurs valeurs TPC, TFC et TF-OL. Les plus hauts TPC et TFC ont été trouvés dans le cultivar de raisin FR (309,8±11,5 mg GAE/g extrait et 55,6±2,06 mg QE/g extrait, respectivement). Trois classes différentes de composés phénoliques ont été détectées : les flavonoïdes, les acides phénoliques et les stilbènes, la classe principale étant les flavonoïdes suivis des acides phénoliques et des stilbènes. La catéchine était la molécule la plus élevée détectée, avec une concentration de 1440,45 mg/kg D.W dans la variété FR. Les résultats ont également révélé que tous les extraits présentaient une forte capacitée antioxydante. L'extrait de FR a présenté l'activité antioxydante la plus puissante avec la plus faible valeur d'IC50 dans le test ABTS (3,13±0,4 µg/mL). Une activité enzymatique a été également détectée contre toutes les enzymes étudiées. Le Docking moléculaire des composés détectés contre l'enzyme uréase a démontré la plus haute affinité de liaison avec l'acide gallique (une valeur d'énergie de liaison de -28,8802 kJ/mol). De plus, tous les extraits ont montré une activité anti-inflammatoire modérée, un SPF élevé et une activité antimicrobienne puissante. Le profil phénolique des pépins de raisin sont trouvés modifiés avec les changements du stade de maturation. Ces changements contribuent à un potentiel différent dans les activités biologiques, avec l'étape verte étant la plus efficace. La catéchine a été détectée en plus grande quantité dans toutes les variétés, ce qui donne aux extraits un fort potentiel antioxydant, tandis que l'acide gallique était détecté comme deuxième molécule la plus élevée. Ces résultats impliquent que les sarments et les pépins de raisins ont un potentiel important pour être utilisés dans l'industrie pharmaceutique.

Mots clés : vigne, raisin, sarment, pépin, polyphénols, activité antioxydante et enzymatique.

الملخص

هدف الدراسة الحالية هو تقييم التركيب الفيتو كيميائي، والأنشطة البيولوجية في المختبر، والنشاط المضاد لإنزيم اليورياز باستخدام الحوسبة للأنواع الأربعة من أغصان العنب الجزائري (Fragola Nera من الهجين -*Vitis vinifera* *Vitis labrusca* و Red glob ,Cardinal, و Gros noir من *Vitis vinifera*) بالإضافة إلى ثلاثة أصناف من البذور (Sabel و Cardinal و Red glob من *Vitis vinifera*) في مراحل نضج مختلفة (قبل فترة التلون، خلال فترة التلون، وأثناء النضج). تشمل الطرق المستخدمة: تقدير كمية المركبات الفينولية باستخدام كاشف Folin-Ciocalteu، والفلافونويدات والفلافونول باستخدام طريقة كلوريد الألومنيوم، التحليل الكيميائي النباتي باستخدام LCMS/MS، فحوصات القياس اللوني لتقييم الأنشطة المضادة للأكسدة والأنشطة الأنزيمية في المختبر، تحديد عامل SPF وتخريب بنية (BSA)، والنشاط المضاد للميكروبات عن طريق الانتشارعلى القرص, وتحديد التركيز الحد الأدنى المثبط (MIC). تمت مقارنة جميع مستخلصات الكرمة من حيث قيم TPC وTFC وTF-OL. تم العثور على أعلى TPC و TFC في صنف العنب FR (309.8 ± 11.5 g /GAE mg مستخلص و 55.6 ± 2.06 g/QE mg مستخلص على التوالي). تم اكتشاف أعلى محتوى TF-OL في مستخلص RG بكمية 15.98 ± 1.20 ملغ من g/QCE مستخلص. تم الكشف عن ثلاث فئات مختلفة من المركبات الفينولية: الفلافونويدات، والأحماض الفينولية، والاستلبينات، والمركبات الرئيسية هي الفلافونويدات تليها الأحماض الفينولية والستيلبينات. كان الكاتشين هو أعلى جزيء تم اكتشافه، بتركيز 1440.45 ملجم/كجم وزن جاف في صنف FR. كشفت النتائج أيضًا أن جميع المستخلصات أظهرت قدرة قوية مضادة للأكسدة. أظهر مستخلص FR أقوى نشاط مضاد للأكسدة مع أدنى قيمة IC50 في اختبار ABTS (3.13 ± 0.4 ميكروغرام / مل). كما تم اكتشاف نشاط أنزيمي كبير ضد جميع الإنزيمات التي تمت دراستها. أظهر الالتحام الجزيئي للمركبات المكتشفة ضد إنزيم اليورياز أعلى درجة تقارب لحمض الغاليك (قيمة طاقة ربط تبلغ -28.8802 كيلوجول/مول). بالإضافة إلى ذلك، أظهرت جميع المستخلصات نشاطًا معتدلًا مضادًا للالتهابات، وعامل حماية من الشمس (SPF) مرتفعًا، ونشاطًا قويًا مضادًا للميكروبات. وجد أن المركبات الفينولية الكلية والصورة الفينولية لبذور العنب تتغير مع تغيرات مرحلة النضج. تساهم هذه التغييرات في إمكانات مختلفة في الأنشطة البيولوجية حيث تكون المرحلة الخضراء هي الأكثر فعالية. تم اكتشاف الكاتشين بكميات أعلى في جميع الأصناف مما أعطى المستخلصات قدرة عالية على مقاومة الأكسدة، وتم اكتشاف حمض الغاليك باعتباره ثاني أعلى جزيء. وتشير هذه النتائج إلى أن أغصان العنب وبذورها تتمتع بإمكانات كبيرة للاستخدام في صناعة الأدوية.

الكلمات المفتاحية: الكرمة، العنب، أغصان، البذور، البوليفينول، نشاط مضادات الأكسدة والأنزيمات.

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Abbreviations list

ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

AChE: Acetylcholinesterase

AD: Alzheimer's disease

BChE: Butyrylcholinesterase

BHA: Butylated hydroxyanisole

BHT: butylated hydroxytoluene

BSA: Bovine serum albumin

BV: Befor veraison

C.A: Cojik Acid

CAT: Catechin

CR: Cardinal

DPPH: 2,2-diphenyl-1-picrylhydrazyl

D.W: Dry weight

EGC: Epigallocatechin

FCR: Folin Ciocalteu reagent

FR: Fragola Nera

FRAP: Ferric reducing power

GA: Gallic acid

GAE/g extract: gallic acid equivalent/g extract

GR: Gros noir

GSE: Grape seed extract

Hesp: Hesperetin

HY-B: Hydroxybenzaldehyde

Iso: Isoquercetin

K-3-G: Kamperol-3-glucoside

MIC: Minimal inhibition concentration

OR: orlistat

O-CA: O-coumaric acid

PA: Protocatechuic acid

PCA: principal component analysis

Pol: Polydatin

QCE/g extract: Quercetin equivalent/g extract

R: Repining

Res: Resveratrol

RG: Red Globe

ROS: Reactive oxygen species

SA: Salicylic acid

SB: Sabel

SNP: Silver nanoparticles

SPF: Sun protection factor

TCT: total condensed tannin content

TFC: Total flavonoid compound

TF-OL: Total flavonol content

TPC: Total phenolic compound

TYT: Total hydrolysable tannin

UVB: ultraviolet B radiation

V: Veraison

Introduction

One of the most fundamental human necessities is food, which maintains our body's health. The quantity of edible food and byproducts that are being wasted at an alarming rate is a problem that affects the entire planet, resulting in a major pollution problem (Fărcaş *et al*., 2019). According to the Food and Agriculture Organization, food loss and waste (FLW) in food value chains is expected to be 250 kg per person and surpass USD 60 billion per year in the Near East and North Africa (NENA). Therefore, in order to solve these issues, it's important to maximize and control the utilization of food by-products (Abdel-Khalek and Mattar, 2022).

Grapevine is one of the most widely cultivated crops in the world; 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 as 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 percentage of 7% (Insanu *et al*., 2021; Baroi *et al*., 2022).

Both of vine growing, pruning and winemaking generate a vast quantity of wastes 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 Phyto-chemicals (Ferreyra *et al*., 2019; Squillaci *et al*., 2021). From that, the search for bioactive compounds derived from plant wastes has gained a lot of attention in recent years because of their potential applications in a wide range of industries, including food additives, agrochemicals, pharmaceuticals, flavors, fragrances, and colors, as well as their protective effects on human health against a variety of degenerative diseases. These chemicals are divided in three groups: mostly polyphenols, terpenoids, and alkaloids (Ben Khadher *et al*., 2022).

Naturally occurring substances polyphenols are secondary metabolites mostly present in fruits, vegetables, cereals, and drinks. It was noticed that Fruits like grapes, apple, pear, cherries and berries contains up to 200–300 mg polyphenols per 100 grams fresh weight and that these chemicals are mostly involved in defense against pathogen aggression or UV radiation (Pandey and Rizvi, 2009).

Grapevine canes named also 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, this raw material has been shown to be a good 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 cosmetic ingredient, for lignin, hemicellulose, oligosaccharides, and cellulosic substrate regeneration, as an enological additive to improve the wine's sensory characteristics, in pharmaceutical, cosmetic, and food industries (Escobar-Avello *et al*., 2021; Kodeš *et al*., 2021). Grape canes include a wide range of phenolic profiles which have key antioxidant, anti-microbial, anti-cancerous, anti-inflammatory, and anti-aging effects, as well as a variety of potential applications (Escobar-Avello *et al*., 2021; Squillaci *et al*., 2021; Noviello *et al*., 2022).

From quantitative point of view, grape seeds are considered as he important part in the whole berry with a roughly 60-70% of the total soluble phenolic contents where Proanthocyanidins and flavan-3-ols are the main phenolic compounds detected. The amount of these phenols can be affected by various factors such as: grape variety, seasonal variation and climatic conditions (Pantelić *et al*., 2016). Furthermore, due to their phenolic composition which exhibited high antioxidant proprieties and other biological activities, grape seed have gained an increase attention in the last decades especially as dietary supplements (Ma and Zhang, 2017; Padilla-González *et al*., 2022). The group of catechins ((+)-catechin, (-)-epicatechin and(-)-epigallocatechin-3-gallate), Proanthocyanidins, flavonols quercetin and quercetin-3-glucoside, gallic acid, and resveratrol presented in grape seeds are considered potent antioxidant molecules (Chengolova *et al*., 2023). Additionally, the use of plant-based enzyme inhibitors is becoming popular in the pharmaceutical sector as essential part of the current prescription drug to treat numerous human disease (Dwibedi *et al*., 2022).

In the same context, total phenolic and their sub-groups content, LCMS/MS analysis, antioxidant properties using various methods *in vitro* as free radical-scavenging and reducing power capabilities, inhibition of enzymatic activity, anti-inflammatory activities, sun protection factor, and antimicrobial activity of crude extracts from vine pruned canes and seeds at three

different stages (before veraision, veraison, and repining) of commonly Algerian grown *V. labrusca* (Fragola Nera) and *V. vinifera* (Cardinal, Red glob, Gros noir, and Sabel) cultivars were evaluated, such, as part of our current study on the potential utilization of grape wastes.

The first chapter presents the literature review containing brief information on the botany of the vine, the biosynthesis and structure of grape polyphenols, the evolution of phenolic composition during the maturation in the grape berry, as well as their biological activities (antioxidant, anti-microbial, neuroprotective, anti-inflammatory and anti-diabetic) and possible use of grape waste.

the second part represents the material used for this work and the different spectrophotometric methods used for the determination of the phenolic composition and biological activities.

The third part reports the results and discussion of the study and divided in two parts

- The first part represents the phenolic profile and biological activities of the pruned canes.
- The second part consist of studying the evolution of the phenolic profile of grape seed during three different repining stages and the evolution of biological activities during these stages.

The writing of this manuscript ended with a general conclusion and suggested perspectives.

Literature review

1. Taxonomical classification and general structure of the grape vine

The woody lianas, or grapevines, are extremely hardy plants that are deciduous (shedding their leaves annually), polycarpic (flowering frequently), and perennial (living more than two years). In order to spread their foliage over the tree canopy, wild vines use their flexible trunks and tendrils to climb trees up to 30 meters or more (Keller, 2010).

The vine is part of the branch of the Dicotyledons and belonging to the order of the Vitals and the family of the Vitaceae's. in this family the vine belongs to the genus *Vitis* (Figure 1). The great botanical family of vines includes 1000 to 1200 species, grouped in seventeen genera. Within this family, only the genera *Vitis* and *Muscadinia* are used in agriculture. The genus *Vitis* includes about sixty species of interfertile, almost all wild, mainly distributed in the northern hemisphere: about thirty in Europe and Asia, and about thirty in America (Kremer, 2017).

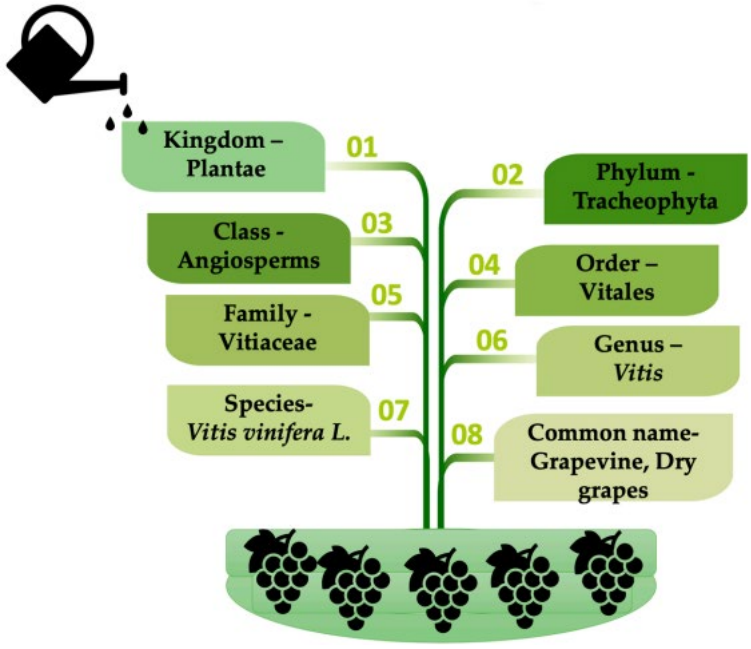


Figure 1: Taxonomical classification of *V. vinifera* L. (Gitea *et al*., 2023).

1.1. Structure of the vine

The vine is composed from: vegetative organs: (roots, trunk, shoots, leaves, and tendrils) (Figure 2) and reproductive organs: bunches of berries or flowers. the berry of the grapes contained three principal types of tissues: skin, seed, and pulp (Keller, 2010; Benbouguerra, 2020)

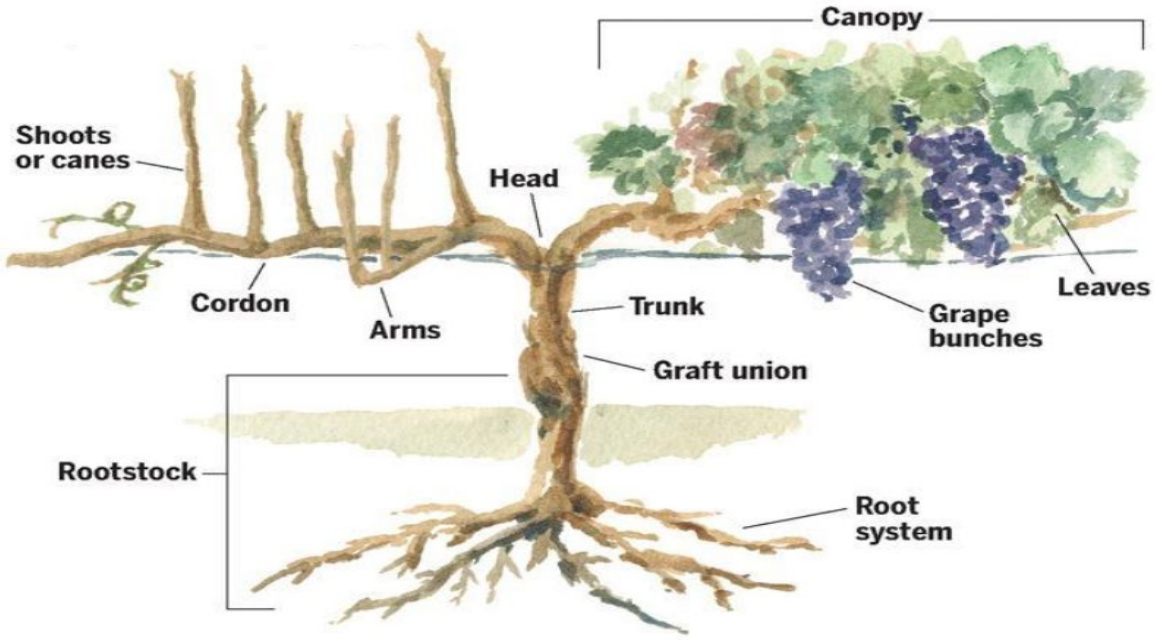


Figure 2: Vine anatomy (Web 1).

▪ **Roots**

Depending on the variety (rootstock), a vine plant's roots are multibranched structures that grow to different depths in the soil and serve as the plant's support (Goufo *et al*., 2020).

▪ **Woods**

Samples taken from the trunk and the cordons are referred to as the "wood" in the literature. Sleeves of conducting tissues, particularly the phloem and xylem, make up the trunk. Extensions of the trunk, cordons or "arms" are where canes (one-year-old wood with eight to fifteen buds) and spurs (one-year-old wood with two to three buds) begin (Goufo *et al*., 2020).

▪ **Canes (shoots)**

The main unit of vine growth and the main focus of many viticultural practices is the shoot. A shoot is a green growth that resembles a stem and emerges from a bud. Primary shoots, which often produce fruit on the vine, develop from primary buds. The shoot's main axis is made up of conducting tissues that carry nutrients, water, and photosynthetic products as well as structural support tissues which support early growth in spring and during stress periods. Leaves, tendrils, flower or fruit clusters, and buds are all arranged in regular patterns along the shoot. Canes are the mature form of shoots; the same terms are used to describe the two parts. Canes are the main structure in the dormant season; they typically get pruned back in the winter

(Figure 3) to either shorter spurs (also known as spur pruning, which often keeps one to three buds per spur) or longer canes (also known as cane pruning, which usually keeps eight or more buds per cane); once they have reached maturity and the leaves have fallen off to control vine size, shape, and the amount of potential harvest in the coming growing season. The majority of spur and cane pruning is performed manually, giving the vine the most control node number and arrangement (Hellman, 2003; Keller, 2010).

▪ **Leaves**

The most apparent components of the canopy are the leaves, which are made up of the petiole (the stem-like structure that joins the leaf to the shoot) and the blade (the broad, flat portion of the leaf intended to absorb sunlight and CO2) (Goufo *et al*., 2020).

Figure 3: Grape cane pruning (Hansse Gluszak, 2019).

1.2. Grape berry

The grape is a berry, classified in the group of fleshy fruits with seeds. The bunch of grapes is made up of two distinct parts: the stalk, which is its framework, and the fruit itself, the grape seed or berry. The stalk is made up of a central axis; the peduncle, on which the pedicels are attached (El Darra, 2013).

The grape berry is divided into three parts (Figure 4): the skin (or exocarp), the pulp (or mesocarp) and the seeds. The pericarp includes the exocarp, the mesocarp and the endocarp (Zouid, 2011).

▪ **Exocarp (skin)**

The exocarp or skin is an essential tissue of the grape berry. It is the outermost part of the fruit; it represents 5 to 18% of the total weight of the berry and constitutes the barrier between the external environment and the interior of the berry. It is also the place of synthesis and accumulation of a majority of compounds of oenological interest (Lacampagne, 2010), such as odorant and coloring matters and a large proportion of tannins (El Darra, 2013).

. The epicarp is composed of the cuticle, the epidermis and the hypodermis.

- The cuticle is the waxy external part of the berry in direct contact with external agents and thus plays an important role in the defense of the plant against yeast and bacteria.
- The epidermis which is the single layer of cells below the cuticle.
- The hypodermis, which is made up of several cell layers containing the granulations of coloring and odorous matter, responsible respectively for the color and fruitiness of the grape and which are generally only found in the skin (Kremer, 2017).

▪ **Mesocarp (pulp)**

The mesocarp, or pulp, is the most important part of the grape, accounting for 80 to 85% of the grape's weight. Once mature, it consists of cells with large vacuoles fully with complex chemicals (aqueous solution of sugar, nitrogenous and pectic components, organic acids, and mineral materials) (Kremer, 2017).

▪ **Endocarp (seed)**

The endocarp is a thin layer of cells separating the capillary caves which contain the seeds. The seed is the reproductive organ of the vine, comprise approximately 3% of the weight of the grape. They are the result of ovules fertilization. They have a thick, rigid, and leathery integument that protects an albumen and an embryo. Each single berry contained normally one to four seeds; their number changes due to non-fertilization. However, some varieties were found to be seedless (without seed) (El Darra, 2013; Kremer, 2017; Hansse Gluszak, 2019). The seed with the skin contained a diversity of phenolic compounds such as: stilbenes, flavonols, flavanols, anthocyanidins, and hydroxycinnamic acids, unlike grape pulp which only contains certain families of polyphenols (anthocyanidins, flavanols and hydroxycinnamic acids) and in lower concentrations (Figure 4) (Ferrier, 2018).

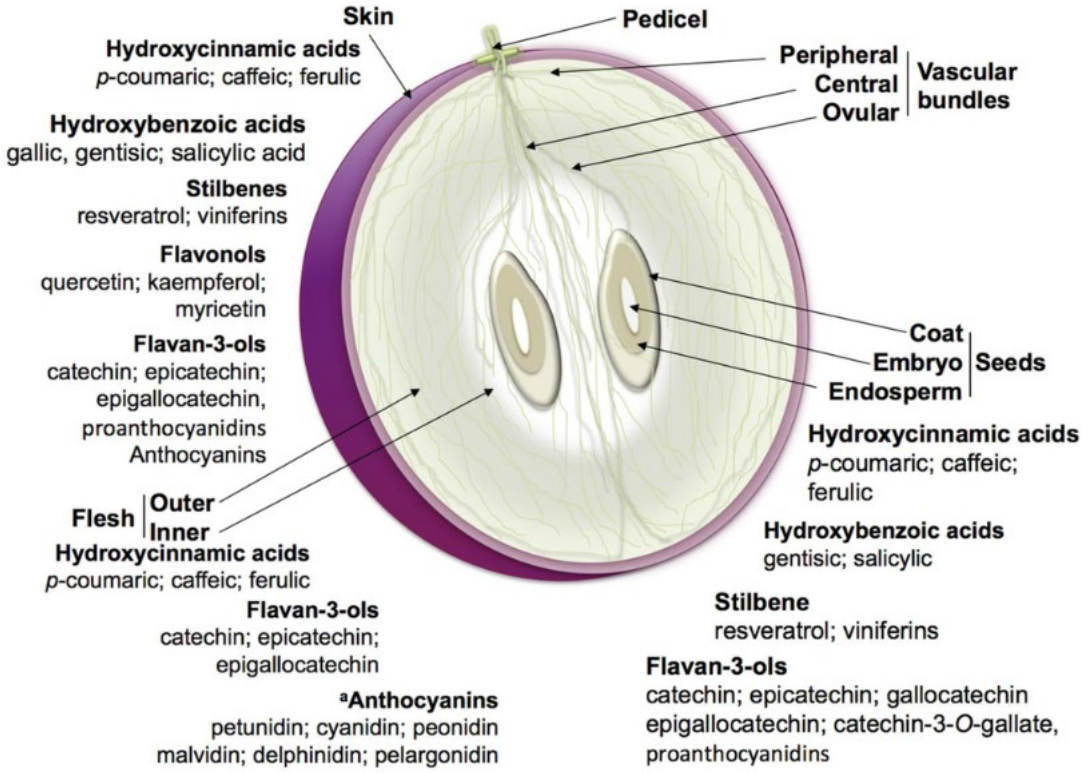


Figure 4: Grape berry parts and their phenolic composition. (Ferrier, 2018).

2. The viticulture in Algeria

In Algeria, there are several archeological texts demonstrating the development of viticulture during the first century of the Christian period, with an estimated 2,000 hectares of table grape plantations. The grape area increased significantly during French colonization, reaching 400,000 ha in 1935 through the colonial state which provided help to French farmers in order to feed metropolitan markets. After the country's independence, the place of viticulture has seen a considerable decline (58 720 ha in 2000) due to the interweaving of several unfavorable factors such as: political, economic, and social constraints of the country (Laiadi, 2013; Sahali, 2023).

In the beginning of the 2000s, the implementation of an agricultural policy based on both public and banking financing from the state for agricultural investment was perceived for the revival of the agricultural sector. However, this agriculture policy has gone through three distinct arrangements, namely: The national agricultural and rural development plan (PNDAR) from 2000 to 2009, Agricultural and Rural Renewal Policy (PRAR) from 2010 to 2014, and

Filaha plan (2014-2020) resulting in an increase in the area production which reached 70 000 ha in 2019 (Sahali, 2023).

According to Sahali (2023) the wilaya of Boumerdes consolidates its leading in table grape production and area on the national level with a production of 217,7 Qx/ha (Figure 5).

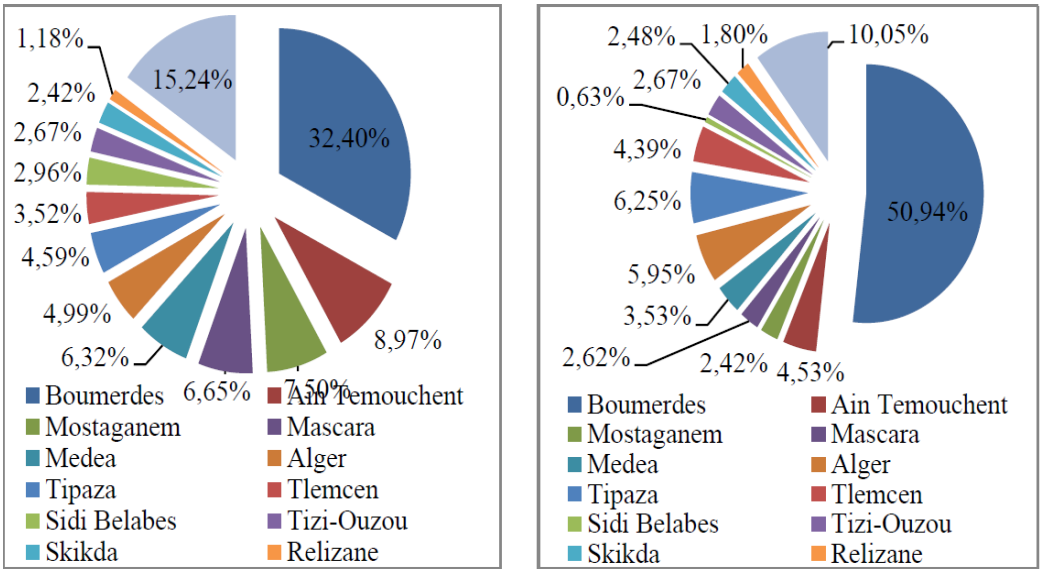


Figure 5: The area of production and the percentage production of table grape in different Algerian cities (Sahali, 2023).

These results are the product of the efforts of the wilaya's viticulturists, especially those of Baghlia, Dellys, Bordj Menail, and Isser, who developed modern working methods and introduced new varieties outperforming a group of centers wilaya constituted by the wilaya of Tizi-Ouzou 200.3 Qx/ha, the wilaya of Tipaza 174.4 Qx/ha and the wilaya of Algiers 151.0 Qx/ha.

2.1. Origins and characteristics of grape varieties

A total of five different cultivars were used in the current study, represented in Figure 6.

▪Fragola Nera

Fregola Nera (FR) named also Isabella is a 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 (Aydemir *et*

al., 2023; Rodrigues, 2023). It is harvested in September-October, depending on the season (Web 3).

▪Cardinal

The *Vitis vinifera* Cardinal (CR) 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 flavor, and early maturity (Akkak *et al*., 2007). The time of maturity is around medium-July which is considered as medium-early time (Web 4).

▪Red Globe

Vitis vinifera L. Red Globe (RG grape) is one of the most popular table grapes in the world according to da Silva et al. (2023). It is obtained by Albert T. Koyama and Hardnold P. Olmo at the University of California in 1981 by crossing « L 12-80 (Emperor x Hunisia L.I.) x S45-48 (L 12-80 x Nocera) ». The berries are extremely red, spherical, and have few fragile seeds. The pulp is quite firm and the skin is light and thick, often peelable. The pruning is from 5-8 buds from base. Maturation medium to late from September to the end of December if the berries covered with plastic film (Web 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 (Web 5, Web 6). 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 7). It is Harvested from mid-August until mid-October (Web 8).

▪Sabel

The *Vitis vinifera* Sabel named also malaga blanc, Khalili, dahouki. It was among the first fruits to be cultivated in the Middle East and the Mediterranean periphery (Web 8). It's can be found also in Jordan, Lebanon, Tunisia, Palestine, and Marrocco (Web 9). The grapes have green-yellow color. They ripen around the middle of September (Web 10).

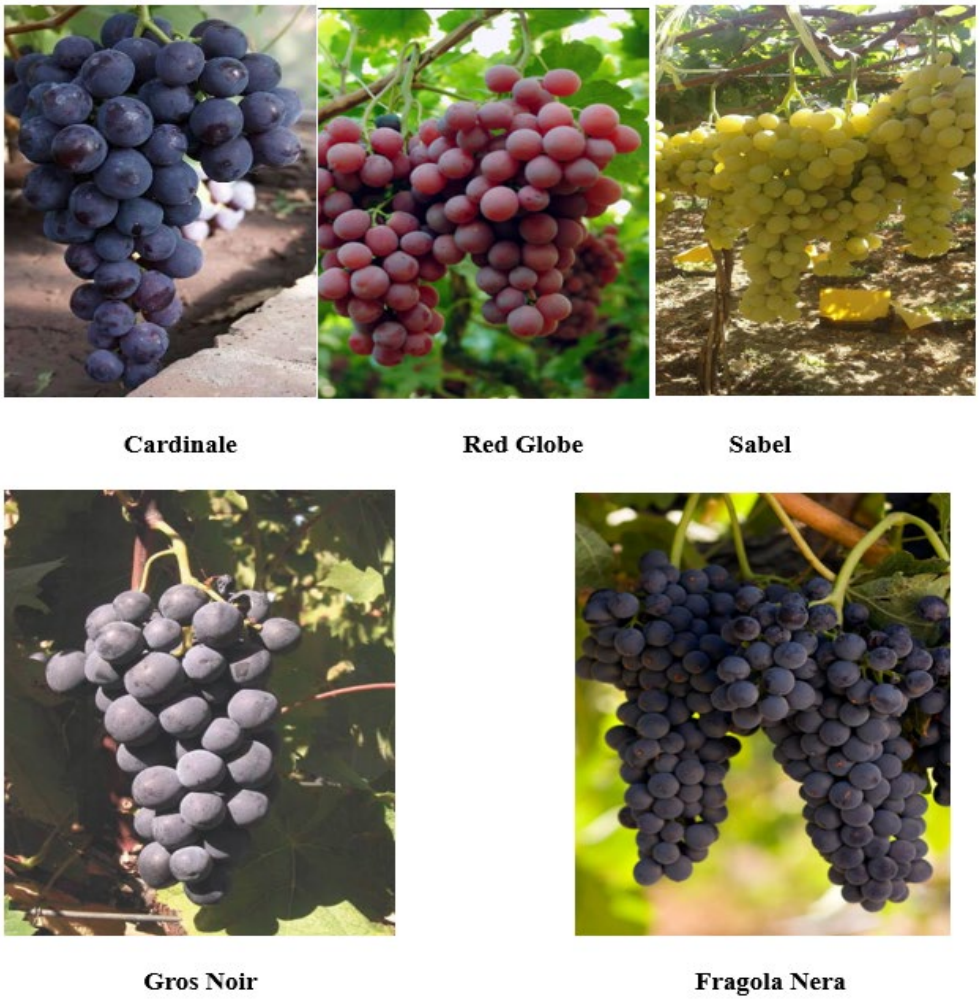


Figure 6: Grape cultivars used in the study.

3. Development cycle of grape vine

The two superposition cycles, the vegetative and the reproductive cycle, form the annual development cycle of the vine. The vegetative cycle is characterized by a growth phase in spring and summer, a reserve accumulation phase in the wood until late autumn, then a rest phase in winter (Figure 7). The reproductive cycle, on the other hand, leads to the development and maturation of grape berries (Hansse Gluszak, 2019).

The development cycle of the vine can be divided according to the changes in each month to:

▪ **In March-April**: the budburst; when buds remaining dormant in the spring start to growth after receiving enough heat. This normally happens when the daily average temperature reaches roughly 50 degrees Fahrenheit (Hellman, 2003; Hansse Gluszak, 2019).

▪ **In April-June**: the development of the branches, formation and exit of inflorescences and flowers of the current year. For latent buds, beginning of differentiation (mid-May) of the primordia inflorescences for the following year;

▪ **In June-July**: flowering (end of May-beginning of June). The fruit growing: growth and development of grape berries;

▪ **In August**: veraison (softening of the berries and color change for red grapes). The ripening of berries and early harvest. The beginning of the ageing of the branches;

▪ **In September-October**: Aging, ripening and harvesting;

▪ **In November-February**: vegetative rest, pruning of the plant (Hansse Gluszak, 2019).

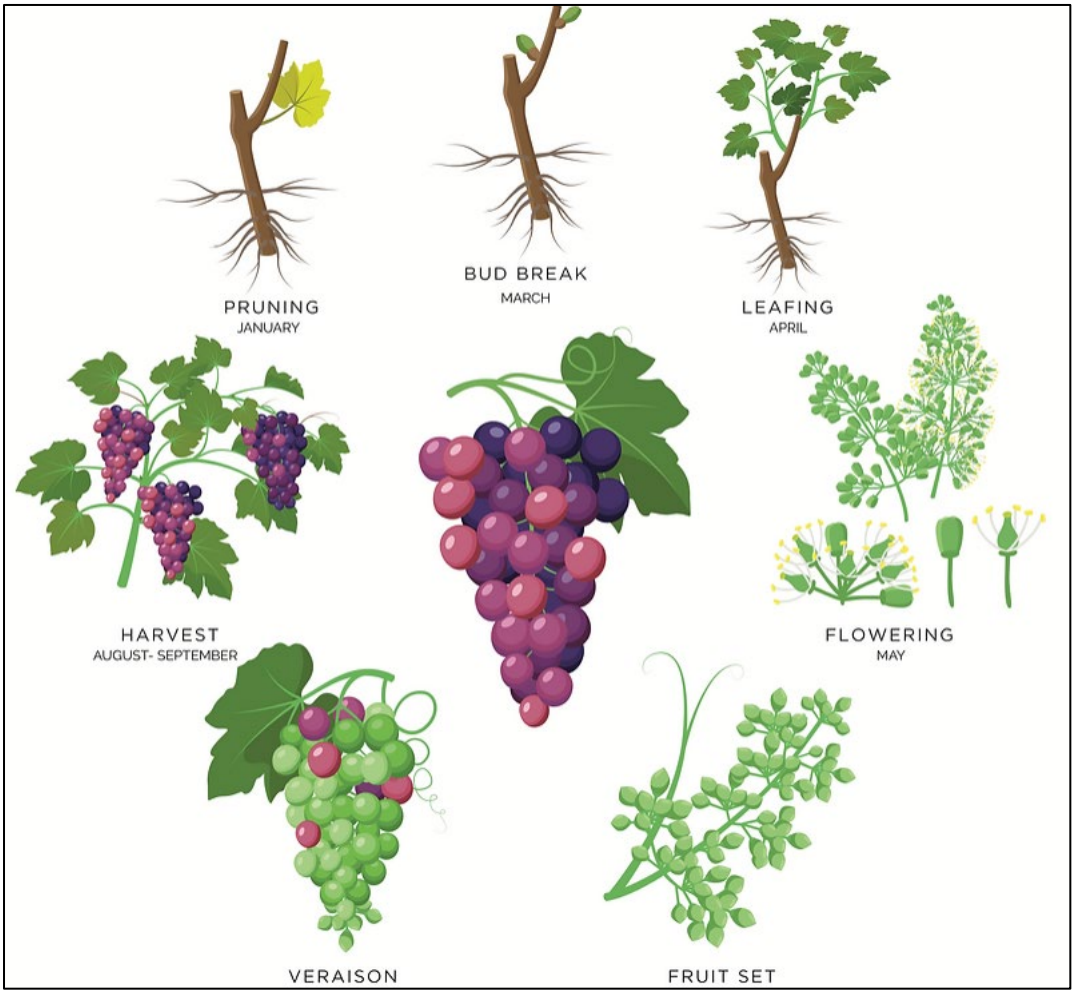


Figure 7: Development cycle of the vine (Web 2).

3.1. Development of grape Berry

The development of berries consists of two successive sigmoidal growth periods separated by a delay phase (Figure 8). The first growing period lasts from flowering to about

60 days after. It consists primarily of cell division and expansion, followed by a rapid growth phase during which the berry is formed and seed embryos are produced. During this period, several compounds are accumulated in the berries, especially tartaric and malic acids, giving acidity to the future wine. Several polyphenolic compounds have increased, such as hydroxycinnamic acids in grape pulp and skin, tannins and catechins in skin and seeds. There are other compounds that accumulate in the berry during the first phase of growth, such as minerals, amino acids, micronutrients and aromatic compounds (such as methoxypyrazines) (Kennedy, 2002; Hornedo-Ortega *et al*., 2020).

The most significant changes in the composition of grapes occur during the second phase of growth (the maturation stage). During the ripening phase of the grape, physiological and biochemical changes determine the quality of the grape. The grapes change from small, hard and acidic berries to larger, sweeter, less acidic, flavored and colored berries. The majority of solutes accumulated during the first phase of growth remain at harvest. During the second period, malic acid is metabolized and used as an energy source, its proportion decreasing towards the concentration of tartaric acid, which remains almost unchanged. In general, the chemical composition of the final product is much more complex than in the raw material, due to the formation of new compounds (Kennedy, 2002; Hornedo-Ortega *et al*., 2020).

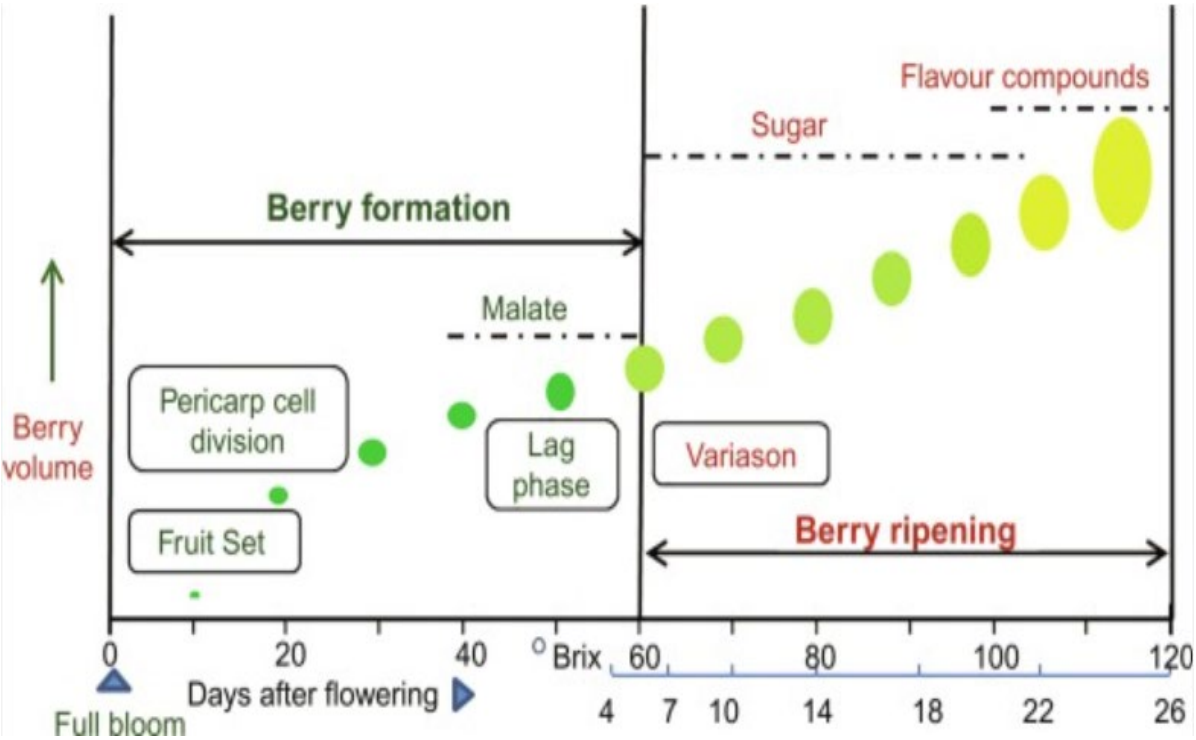


Figure 8: Grape berry development (Upadhyay *et al*., 2022).

4. Polyphenols of the vine

Several classes of phenolic compounds were identified in the grape wastes with clarification of their biosynthesis pathways.

4.1. Biosynthesis of phenolic compound

Polyphenol synthesis can occur through two biosynthetic pathways. The most common is that of shikimic acid. The other way, that of aceto-malonate. The shikimate pathways lead to the synthesis of aromatic amino acid phenylalanine, the precursor of phenylpropanoid compounds, through the action of phenylalanine amino lyase (LPA). The deamination of phenylalanine lead to the formation of trans-cinnamic acid (the earliest phenolic metabolite produced in plant cells), This compound undergoes a series of transformations resulting in the formation of precursors of several simple phenolic compounds, such as phenolic acids (Figure 9). Additionally, the ring B and the carbon bridge in the flavonoid structure are built on this principle (Chira *et al*., 2008; Zagoskina *et al*., 2023).

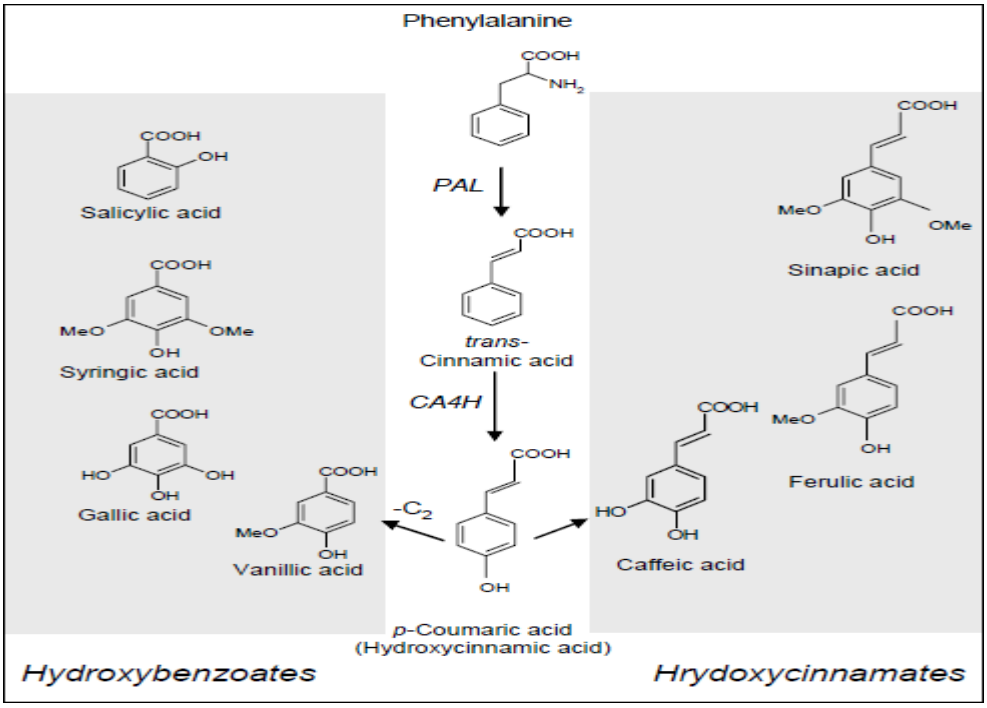


Figure 9: Phenylpropanoid pathway leading to hydroxycinnamates and hydroxybenzoates (Iriti and Faoro, 2009).

In the acetate malonate pathway, Chalcone synthase (CHS) condenses 3 molecules of malonyl-CoA (C2) and p-coumaroyl-CoA to create a chalcone, which then converts to naringenin through the action of chalcone isomerase, this flavanone is the precursor of flavones, flavonols, flavanols and anthocyanidins, the main flavonoids in grape (Figure 10). In the case

of stilbenes, the enzyme stilbene synthase (STS) is involved. This pathway is used to create a second ring Benzene (A) in higher plants for many compounds that already have an aromatic ring obtained by shikimate pathway. Thus, the two pathways responsible for the biosynthesis of phenolic compounds meet and goes through them the two main classes of metabolites flavonoids and stilbenes can be produced (Chira *et al*., 2008; Iriti and Faoro, 2009; Benbouguerra, 2020; Zagoskina *et al*., 2023).

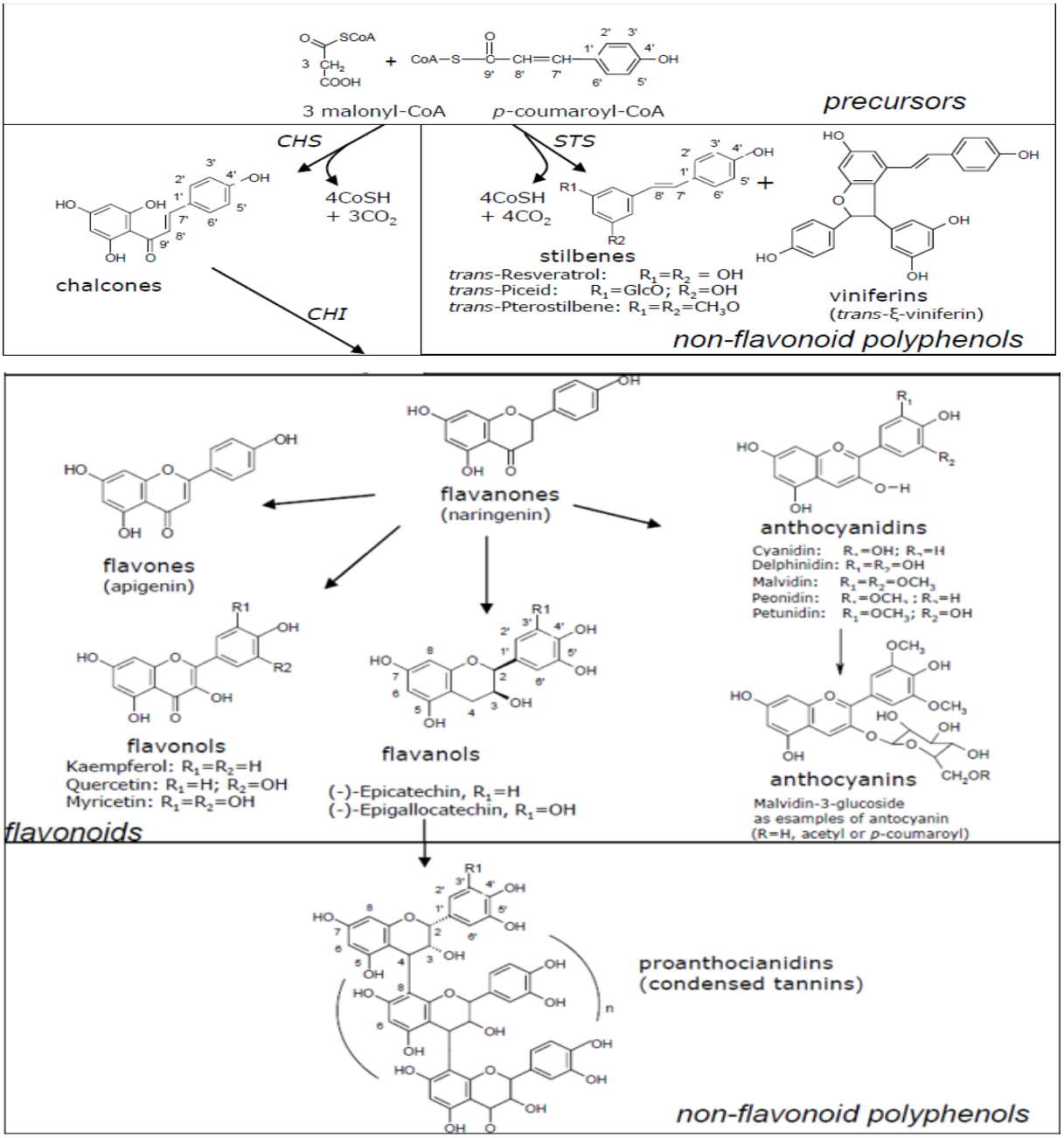


Figure 10: Malonate pathway leading to flavonoids and non-flavonoid polyphenols (Iriti and Faoro, 2009).

4.2. Non flavonoid class in grape berry

▪ Phenolic acids

The predominant phenolic acids present in grapes are hydroxybenzoic acids and hydroxycinnamic acids, this family is present in grape skins, pulp, seeds, and stem with skins frequently exhibiting the highest concentration (0.2–8.2 g/kg dm) (Barros *et al*., 2015; Hornedo-Ortega *et al*., 2020).

In the case of hydroxycinnamic acids, the product of the shikimate pathway, cinnamic acid, is hydroxylated by 4-hydroxylase to produce p-coumaric acid, which can then be methylated and hydroxylated again to produce ferulic, sinapic, and caffeic acids the main hydroxycinnamic acids. They characterized by a C6-C3 carbon skeleton and they frequently accumulate as tartaric acid esters, where the major compound of the hydroxycinnamic acids in grape is the caftaric acid (caffeoyl-tartaric ester) (Iriti and Faoro, 2009; Derradji-Benmeziane, 2014). These acids can be found in all grape tissues but predominantly in the vacuoles of pericarp cells, and they differ by the type and number of substituents on the benzene ring (Šikuten *et al*., 2020).

Regarding hydroxybenzoic acids, as well as benzoic acid derivatives such as salicylic, syringic, vanillic, and gallic acids, are produced when a C2 fragment from the aliphatic side chain of coumaric acid is broken to form a C6–C1 carbon skelton (Iriti and Faoro, 2009). Gallic acid is the most common phenolic acid that serves as a precursor to all hydrolysable tannins and as part of condensed tannins in grapes (Šikuten *et al*., 2020).

In general, hydroxycinnamic acids and phenolic acids can function as co-pigments. They are in fact linked to the synthesis of new, more stable pigments in wine called pyranoanthocyanins, and as a result, they are regarded as color stabilizers for young red wines. In addition, they are linked to the sensations of bitterness and astringency (Hornedo-Ortega *et al*., 2020).

▪ Stilbenes

Stilbenes are non-flavonoid compounds with low molecular weight present in different plant families such as *Papilionaceae Pinaceae, Myrtaceae*, *Moraceae, Liliaceae Fagaceae,* and *Vitaceae. Vitis vinifera* represent the most abundant specie of *Vitacea* concerning table grapes

(Bavaresco *et al*., 2007). Stilbenes have a structure from two aromatic rings connected with an ethylene bridge (Figure 11) which give the two main configurations cis and trans to the structure (Shazmeen *et al*., 2021). they are protective molecules and considered as phytoalexins produced during repones to biotic and abiotic stress (herbivore attack ultraviolet irradiation environmental stresses like cold frost salinity). They are found as free, glycosylated and methoxylated forms in plants and as oligomers like vineferin (Mekinić *et al*., 2016). Stilbenes have been shown in numerous studies to have positive health effects on people, including protection against diabetes, cancer (by preventing cell proliferation), neurological illnesses like Alzheimer's disease, and coronary heart disease (Noviello *et al*., 2022). In human nutrition grapes and wine are considered as the main dietary sources of stilbenes (Benbouguerra *et al*., 2021). In grape vine they are found in the woody parts of the vine such as seeds, stems, canes, and roots (Bavaresco *et al*., 2007).

Resveratrol is considered as the most representative molecule in subclass of stilbenes and other identified stilbenes are mostly derivatives of transresveratrol. resveratrol (3,5,4'-trihydroxystilbene), resveratrol-3-O-β-D-glucopyranoside (piceid), piceatannol (3,4,3',5'-tetrahydroxy-trans-stilbene) and resveratrol dimers (viniferins) are the most stilbenes in grape plants. Astringin (5,4',3-trihydroxystilbene-3-*O*-β-glucoside) isorhapontin (5,4′-dihydroxy-3′-methoxystilbene-3-β-Dglucoside) pterostilbene (*trans*-3,5-dimethoxy-4'-hydroxy-stilbene and pallidol (*trans*-resveratrol dimer) have also been identified (Bavaresco *et al*., 2007).

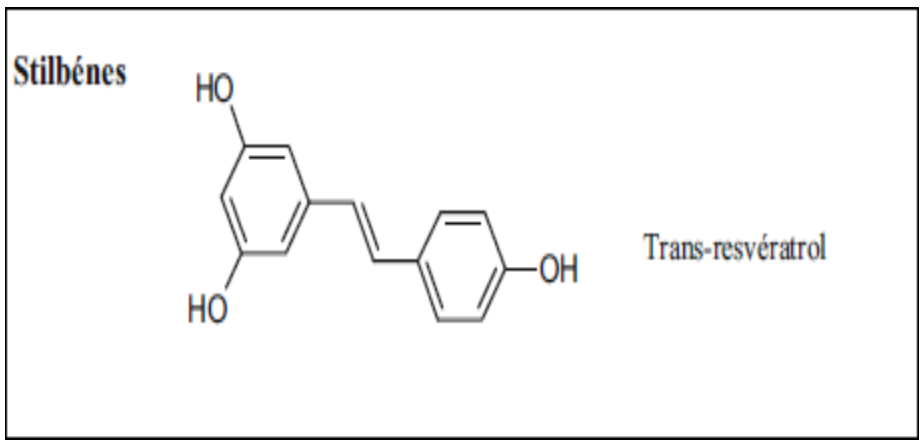


Figure 11: Structure of the principal stilbene (Chira *et al*., 2008).

The production of these phytochemicals in grapes and wine is in accordance with grape varieties viticultural and enological conditions such as location, climate conditions, temperature, light, maceration, pectolytic enzymes, and fining agents (Mekinić *et al*., 2016).

4.3. Flavonoid class in the grape berry

flavonoids are polyphenolic compounds comprising 15 carbon atoms with a structure of C6-C3-C6, forming two aromatic rings (A and B) connected by a bridge of 3 carbons (heterocyclic ring C) (Figure 12). These are the most abundant of all the phenolic compounds (Chira *et al*., 2008; Liu *et al*., 2021). Flavonoids are classified based on their heterocyclic ring oxidation and benzene ring hydroxyl or methyl group number into several sub-families with the predominant ones being flavones, flavonols, flavan-3-ols, isoflavones, flavanones and anthocyanidins. Additionally, various changes, including glycosylation and acylation, and molecular polymerization, result in the creation of many flavonoid compounds. It is well noticed that *Vitis vinifera* grapes include mostly anthocyanins, flavonols, and flavan-3-ols (Derradji-Benmeziane, 2014; Liu *et al*., 2021).

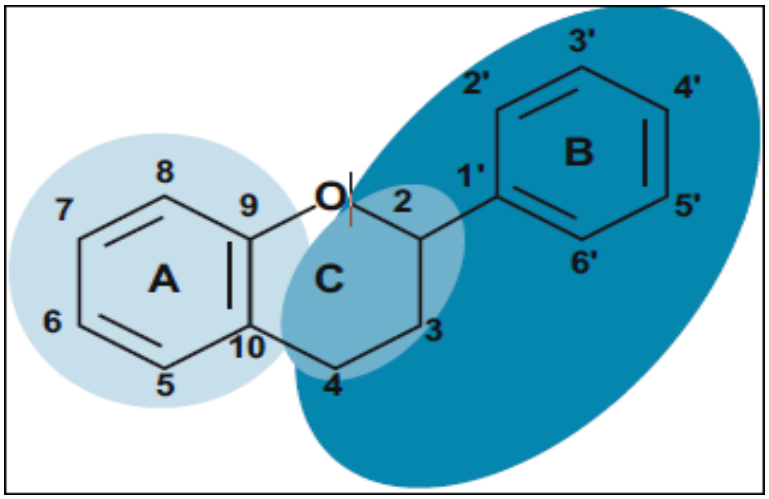


Figure 12: Basic structure of flavonoids (Chira *et al*., 2008).

▪ **Flavanols and condensed tannin**

Flavanols are the most abundant flavonoid class of phenolic compounds found in grape berries (Pérez-Navarro *et al*., 2019). They exist in all grape solid parts (skin, seed, and canes) and are responsible for the stabilization of the wine's color and sensations due to their astringent and bitter properties (Hornedo-Ortego *et al*., 2020). Flavanols represent 46% to 56% of total phenolics in white grape varieties, whereas in red grapes they represent between 13% and 30%

of total phenolic content. These compounds are distinguished by a three-ring C6-C3-C6 carbon skeleton. ("flavan") consists of a saturated central heterocycle ring with an hydroxyl group at the C3 position (-ol) which share with C2 a chiral center and give to this class numerous diasteriosomer (Figure 13), whereas the A-ring is generally hydroxylated in C5 and C7, and the B-ring in C4. (Pérez-Navarro *et al*., 2019; Goufo *et al*., 2020). Flavan-3-ols are generally divided into three groups according to their DP: monomers, oligomers and polymers (Liu *et al*., 2010). The main monomers found in grapes are (+)-catechin, (−)-epicatechin, (+)-gallocatechin, (−)-epigallocatechin, and (−)-epicatechin-3- O-gallate (Pérez-Navarro *et al*., 2019). A total of 45 flavan-3-ols have been reported in grape seeds (Padilla-González *et al*., 2022) whereas 8 flavanol and nine proanthocyanidins were reported in grape canes stems and leaves (Goufo *et al*., 2020).

The condensation of the oligomeric and polymeric units of flavan-3-ols through C4→C8 or C4→C6 bonds lead to the formation of the condensed tannin or Proanthocyanidins with a ranged polymerization degree between 3 and 11. They are located in hypodermal layers of the skin and the soft parenchyma of the seed coat between the cuticle and the hard seed coat, with crucial role in defense against herbivores and pathogens (Padilla-González *et al*., 2022).

Proanthocyanidins are considered the higher class of phenolic compound detected in grape seed with less polymerization degree than those of the skin. However, the localization of skin proanthocyanidin in the vacuoles and cell wall make them easily extracted during winemaking, thus conferring important organoleptic properties to wine, such as astringency, bitterness, browning, turbidity and color stability (Iriti and Faoro, 2009).

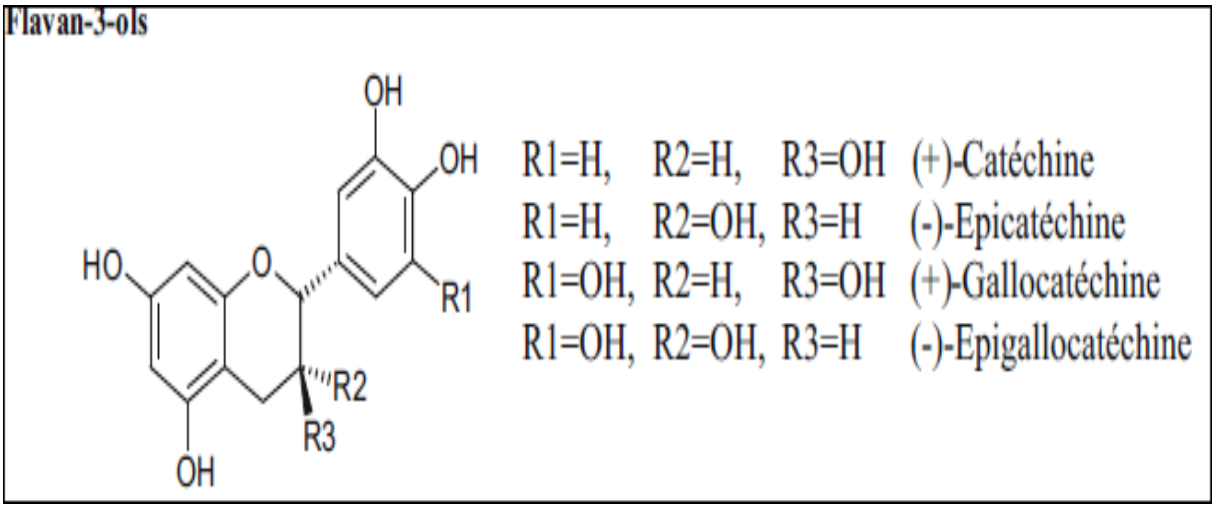


Figure 13: Structure of the principal grape flavanol (Chira *et al*., 2008).

▪ **Flavonols**

Flavonols are a class of flavonoid with a ketone group in position 4 of the heterocycle, while position 3 contain an hydroxyl and form a double bond with the C2 (Figure 14) (Šikuten *et al*., 2020). The glycosylation can have place at position 3 of the C ring, thus conferring to the molecule a high structural diversity and high potential of activity with the diversity of the hydroxy and methoxy groups in the aglycone part. the predominant form of flavonols is the glycosylated one, glucoside, glucuronide, and galactoside derivatives (Gouot *et al*., 2019), but it can be found as aglycon as a result of hydrolysis process during storage of skins, juice and wine (Georgiev *et al*., 2014).

Flavonols play the role of copigmentation and they confer a color stability for red wine with anthocyanin, because of that flavonols and anthocyanin are related in the time of biosynthesis which is around flowering and during the repining stage of grape berries. Flavonols found in outer epidemic of grape berry skin, their color varies from white to yellow (Šikuten *et al*., 2020). kaempferol, quercetin, myricetin, and isorhamnetin are the Four flavonol mostly Presence in the grape with laricitrin and syringetin in red varieties (Benmeziane, 2018). Only derivatives of Quercetin, kaempferol and isorhamnetin are found in both red and white grapes while derivatives of myricetin are found only in red grapes (Georgiev *et al*., 2014). According to Chira et al. (2008), The average maximum level of flavonols in grapes is around 50 mg/kg, but can vary between 10 and 285 mg/kg.

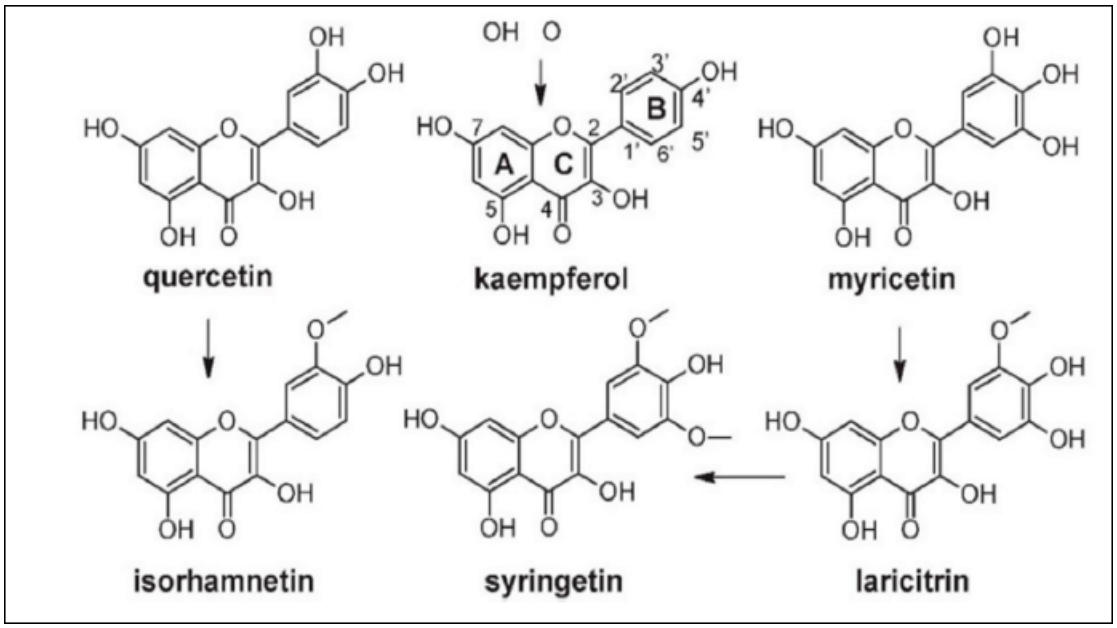


Figure 14: Structure of aglycone flavonols found in grapes (Šikuten *et al*., 2020).

▪Anthocyanins

Compounds that act as natural colorants in the red grape skins. These compounds are co-located with the tannins in the thick-walled hypodermic cells of the skin and in the pulp of some dye varieties and are a glycosidic form of anthocyanidins. Anthocyanins are synthesized in cytosol and delivered to vacuoles, where they are stored as colored coalescences. In most *V. vinifera* grape berries, glycosylation of anthocyanidins occurs exclusively at position 3 by the activity of 3-O-glucosyltransferase, whereas in non-*V. vinifera* and their hybrids, positions 3 and 5 are glycosylated (Figure 15). Acylated anthocyanins are also present in most red grapes, probably due to the presence of the enzyme anthocyanin acyl-transferase. Delphinidin, peonidin, cyanidin, petunidin and malvidin are usually the main anthocyanins found in most red grapes (Flamini *et al*., 2013; Šikuten *et al*., 2020).

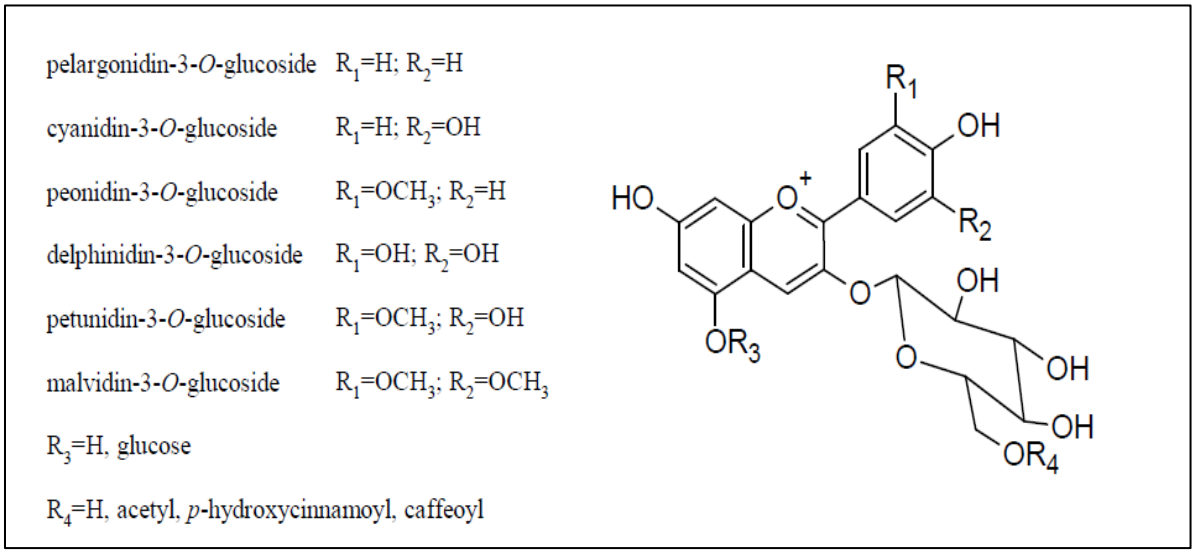


Figure 15: Structure of principal anthocyanins detected in grapes (Flamini *et al*., 2013).

5. Development of berry polyphenols during the maturation

The polyphenolic composition of grapes is influenced not only by genetic (varietal) factors, but also by the location of the vineyard, environmental conditions, cultural practices, as well as the growing season. During maturation, the berries of grapes can undergo significant changes in their composition, and the last stages (weeks) of maturation are particularly important because, during this period, Changes in polyphenolic content play a critical role (Roufas *et al*., 2023).

- The composition of flavonoids changes throughout the seed maturation process, with macroscopic changes in tissues such as color and hardness. The highest concentration

of flavanol-3-ols (condensed monomers and tannins) is present at veraison, where accumulation reaches maximum, but then slowly decreases as maturity approaches, with a 90% decrease in monomers and a 60% decrease in PA (Braidot *et al*., 2008).

- In the skins, the highest level of flavan-3-ol was measured at the first stage of maturation and before veraison, and then significantly decreased from veraison to maturity (Dudoit *et al*., 2020). the oxidation of flavan-3-ols as well as the diversion of intermediate metabolites (cyanidins and delphinidins) towards synthesis of anthocyanins results in a decrease in the content of flavan-3-ol (Benmeziane, 2018). Qualitative changes, such as increased degree of polymerization, may also occur from maturation to harvest (Benbouguerra, 2021).
- The content of stilbene is influenced by several factors such as varieties, years, viticulture, and climatic conditions. In addition, depending on the stage of maturation, stilbene molecules can be found at varying concentrations; with an increase in the content from veraison to maturation. (Dudoit *et al*., 2020; Benbouguerra, 2021).
- According to the available data in the literature, the evolution of flavonol does not show any specific pattern during maturation. In contrast, other studies on *V. vinifera* showed that there was a steady increase for several flavonols during the later stages of maturation. On the other hand, for other grape varieties a decrease was observed from veraison to maturity (Roufas *et al*., 2023).
- With respect to phenolic acids, more recent studies have indicated that there may be significant variations in the content of hydroxycinnamates in grapes near harvest, characterized by a gradual decrease as one approaches the harvest period. However, it has been shown that hydroxycinnamates such as trans-caftaric acid generally peak in their content before veraison, while as the berries mature, they stabilize (Roufas *et al*., 2023).
- Regarding anthocyanins, they are also missing in the green stages (first stage before veraison) of grape skins. Anthocyanin synthesis started at veraison and accumulated during maturation (Dudoit *et al*., 2020) followed by a decrease just before harvest and or during overmaturation (Benbouguerra, 2021). The accumulation of sugars during maturation can play a major role in anthocyanin synthesis. It has been considered as a substrate for the formation of anthocyanins and as a regulator in synthesis. Anthocyanin levels were mainly affected by altitude and environmental factors, such as temperature, which stimulates expression and varieties of regulatory genes. It found that higher

temperatures, such as 35°C, promote the degradation of anthocyanins, however lower temperatures, around 25°C, induce synthesis of anthocyanins (Dudoit *et al*., 2020).

6. Biological activities of grape wastes

Grape wastes are well known in the literature by their biological properties (Figure 16).

6.1. Antioxidant activity

Oxidative stress causes cell damage and degenerative processes. Severe pathologies such as neurological disorders, diabetes, cancer, liver disease, cardiovascular disease, and fast aging are probable results. Antioxidant therapy is widely regarded as the most efficient strategy to manage oxidative stress and prevent oxidative damage (Georgiev *et al*., 2014).

Grape phenolic compounds have been extensively studied for their antioxidative properties, such as scavenging free radicals, inhibiting lipid oxidation, and reducing hydroperoxide production. Several methods were used to assess the antioxidant capabilities of phenolic compounds isolated from various grapes or distinct parts of grapes (Xia *et al*., 2010). Grape skin and seeds include phytochemicals such as gallic acid, catechin, resveratrol, and epicatechin, enabling them to be ideal raw materials for producing antioxidant dietary supplements (Yadav *et al*., 2009). Additionally, Scientific studies have demonstrated that the antioxidant power of seed proanthocyanidins is 20 times greater than vitamin E and 50 times greater than vitamin C (Bhise *et al*., 2014). According to Yadav et al. (2009), Grape juice flavonoids are powerful antioxidants that prevent oxidative stress, free radical damage, and the development of chronic diseases. Moreover, when *Vitis vinifera* shoot extract was added to cultured human keratinocytes, it demonstrated greater antioxidant activity than Vitamins E and C. Fluorometric analysis *in vivo* experiments found a decrease in ROS levels compared to controls, whereas dermatologic evaluation after four weeks demonstrated improvements in photoaged skin clinical symptoms (Soto *et al*., 2015). In addition, grape Anthocyanins' antioxidant properties have been thoroughly explored and evaluated elsewhere besides to their other biological activities. Based on these findings, grape fruit and/or its contents may be used in therapeutic regimens to combat oxidative stress-related problems (Yadav *et al*., 2009).

6.2. Antimicrobial activity

The antimicrobial properties of grapes, wine, and grape-derived products have been extensively studied. their action is related to the presence of phenolic compounds, which are mainly composed by hydroxycinnamic acids, gallic acid, flavonols, favan-3-ols and trans resveratrol. Phenolic compounds interact with the microbial cell membrane. They aggregate in

the lipid bilayer, disrupting membrane structure and function, and then penetrate the bacterial cell, exerting inhibitory activity in the cytoplasm, resulting in lysis and the release of intracellular ATP. They can also lead to cell component loss by increasing cytoplasmic membrane permeability (Mattos *et al*., 2017).

Grapes contain antimicrobial compounds that can decrease the oral cavity microorganisms linked to caries and periodontal disease (Lung *et al*., 2015). Winery by-products (seed, skin, stem, shoot, pomace) contain significant bioactive substances that are effective in inhibiting food-borne bacteria such as *Bacillus cereus*, *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica, Vibrio cholera*, *Vibrio vulnifcus*, and *Yersinia enterocolitica*, as well as pathological bacteria like *Helicobacter pylori* and *Klebsiella. pneumonia*, viruses like hepatitis, cytomegalo, rota, noro, fungi like *Candida albicans*, *Botrytis cinerea*; parasites *Trichomonas vagilanis,* and microbial toxins like Shiga toxin (Kalli *et al*., 2018). According to Soto et al. (2015), the seed extracts showed dose-dependent antibacterial activity against pathogenic and spoilage bacteria such as *Aeromonas hydrophila, Bacillus cereus, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Mycobacterium smegmatis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescens, Salmonella enteritidis,* and *Salmonella. typhimurium.* Additionally, Grape skin is rich in phenolic compounds such as flavan-3-ols, phenolic acids, (+)-catechins, proanthocyanidins, flavonols, and anthocyanins. Its antimicrobial properties are due to its high phenolic content. Several studies have reported the use of grape skin extracts as antibacterial agents (Mattos *et al*., 2017).

6.3. Anti-inflammatory activity

Inflammation protects tissues from injury, irritation, and pathogens, and destroys damaged and necrotic cells. Several stresses from the environment may induce inflammation**.** Acute inflammation can be beneficial for injured tissue under normal conditions. However, prolonged inflammation can lead to chronic inflammation and resulting in the development of chronic diseases such as cancer, Alzheimer's, neurodegenerative diseases, cardiovascular diseases, diabetes, arthritis, autoimmune and pulmonary diseases (Georgiev *et al*., 2014).

Grape polyphenols reduce chronic inflammation by modulating inflammatory pathways or decreasing ROS levels. Grape flavonoids and seed proanthocyanidins are more effective than synthetic drugs to combat chronic inflammation by targeting many pathways simultaneously (Soto *et al*., 2015). Besides to numerous biological activities of grape Syrah stem extracts, they

exhibit anti-inflammatory activity by inhibiting the nitrite production at non-toxic cell concentrations (Leal *et al*., 2020). *V. vinifera* skin and seed extracts from two variety demonstrated anti-inflammatory activity by inhibiting IL-8 on TNF-α-induced released with low IC50, the anti-inflammatory activity is related to high levels of anthocyanins in Albarossa skin, flavonols in Exalt seeds, and procyanidins in both varieties (Colombo *et al*., 2019). Resveratrol another phenolic compound present in grapes demonstrated potent anti-inflammatory activity by reducing the production and expression of numerous inflammatory substances (Martin *et al*., 2003).

6.4. Anti-diabetic activity

Polyphenols in grapes and grape products may protect metabolic syndrome, obesity, and type 2 diabetes by functioning as multi-target modulators with antioxidant and anti-inflammatory properties (Tsuda, 2012). The anti-diabetic activity of grape seed, skin, and flesh from *V. vinifera* L. varieties was confirmed in the study of Tkacz et al. (2019) through inhibiting the action of α-amylase and α-glycosidase by IC50 ranging from 0.27 to 1.13 mg dry sample/mL. Grape seed extracts contain procyanidin, which functions as an insulinomimetic drug by phosphorylating insulin receptors, leading to improved glucose absorption. grape seeds and stems may be a new source of insulin secretagogues, suggesting their application in the treatment of type II diabetes by increasing the insulin secretion in the mice pancreatic islets (Baroi *et al*., 2023). Flavonoids, tannin, anthocyanins, catechin, resveratrol, and quercetin are the main phenolic compounds present in grape pomace responsible of the diabetic enzyme inhibition (Cisneros-Yupanqui *et al*., 2023).

6.5. Other activities and utilizations of grape wastes

Several studies have shown that consumption of grape products may have beneficial effect on cardiovascular system by enhancing endothelial function, decreasing LDL oxidation, improving vascular function, altering blood lipids, and modulating inflammatory process (Georgiev *et al*., 2014). Anticancer properties of grapes and grape products have been widely discussed in the scientific literature. The remarkable anticancer effect of grape products is considered to be due to their unique mixture of polyphenolic compounds with various biological activities. Flavonoids are the main group of active anticancer constituents in grape products, and are concentrated mainly in grape skins and seeds (Zhou *et al*., 2012).

The grape polyphenolic compounds can delay the initiation and/or hamper the progression of cognitive decline and AD. They can neutralize accumulated neurotoxins and

inhibit apoptosis of neural cells caused by oxidative stressors and pro-inflammatory factors (Šikuten *et al*., 2020). Polyphenols derived from grape seed extract resveratrol has found to protect from neurodegenerative diseases such as Alzheimer's disease (Kalli *et al*., 2018). Consumption of flavonoid-rich grape products may have a significant beneficial effect on brain function and central nervous system. Grape flavonoids, specifically anthocyanins, can prevent neurodegenerative processes both by inhibition of neuro-inflammation and by reducing oxidative stress (Georgiev *et al*., 2014).

In the last few years, there has been a wide range of food additives and nutritional products originating from grapes, distributed in the worldwide market. Most of these commercialized products are obtained during processing of pomace from wine or grape juice production. This includes several grape skin or seed extracts, grape skin powder, dry seed powder (capsulated or bulk), pomace powder, and anthocyanin colorants (Georgiev *et al*., 2014). A dietary supplement with grape seed extracts and other natural extracts was found to improve face condition, structure, and firmness in healthy post-menopausal females compared to a placebo (Skovgaard *et al*., 2006). Some commercial products contain pure natural compounds from grape, such as procyanidins, quercetin, or resveratrol, and melatonin, which are effective in delaying the onset of a variety of age-related diseases (Soto *et al*., 2015). The Swiss company "Mibelle Biochemistry" commercialized the biomass from grape cell suspension of *V. vinifera* L. "Gamay Fréaux" var. to create a natural additive "PhytoCellTec™ Solar *Vitis*" for use in skin-care and cosmetic products (Georgiev *et al*., 2014). The grape pomace was used in the synthesis of antioxidant food packaging and the results showed improved mechanical, antioxidant, and antibacterial properties, demonstrating their potential for significant effects under real conditions. Additionally, Grape pomace includes both insoluble (cellulose, hemicellulose, lignin) and soluble (pectin) fibers that can enhance the texture, stability, and nutritional value of baked products, cereals, and meat products. The use of anthocyanins as food colors in beverages, jams, sweets, ice creams, and medicines is now permitted by the European Food Safety Authority (EFSA) (Abreu *et al*., 2024).

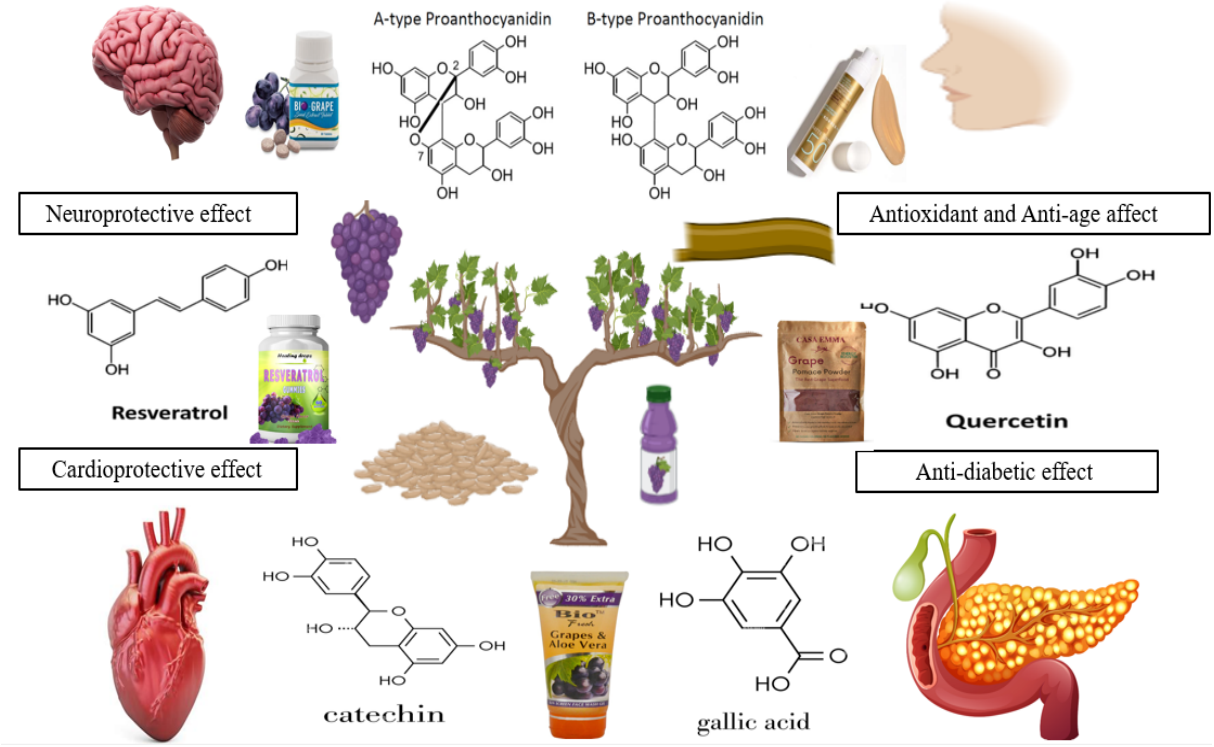


Figure 16: Grape waste biological effects and utilizations (Biorender).

Materials and methods

1. Collection of grape wastes and extraction of polyphenols

Vine canes and grape (seeds), used as experimental material, were collected randomly from the region of Baghlia, Boumerdes in the north of Algeria (Figure 17), in the winter (last of January) and summer (from July to August) of 2022, respectively.

In the case of canes (Red Globe (RG), Cardinale (CR), Fragola Nera (FR), and Gros Noir (GR)), they were pruned according to the pruning time in Algeria which started from last January to February. Regarding grapes, they were handpicked three times (before veraison, veraison, and repining) according to the maturation date of each variety (Sabel (SB), Red Glob, and Cardinale) (Figure 18). Maceration technique was used to extract the phenolic compound of the two wastes with different solvent (Figure 19).

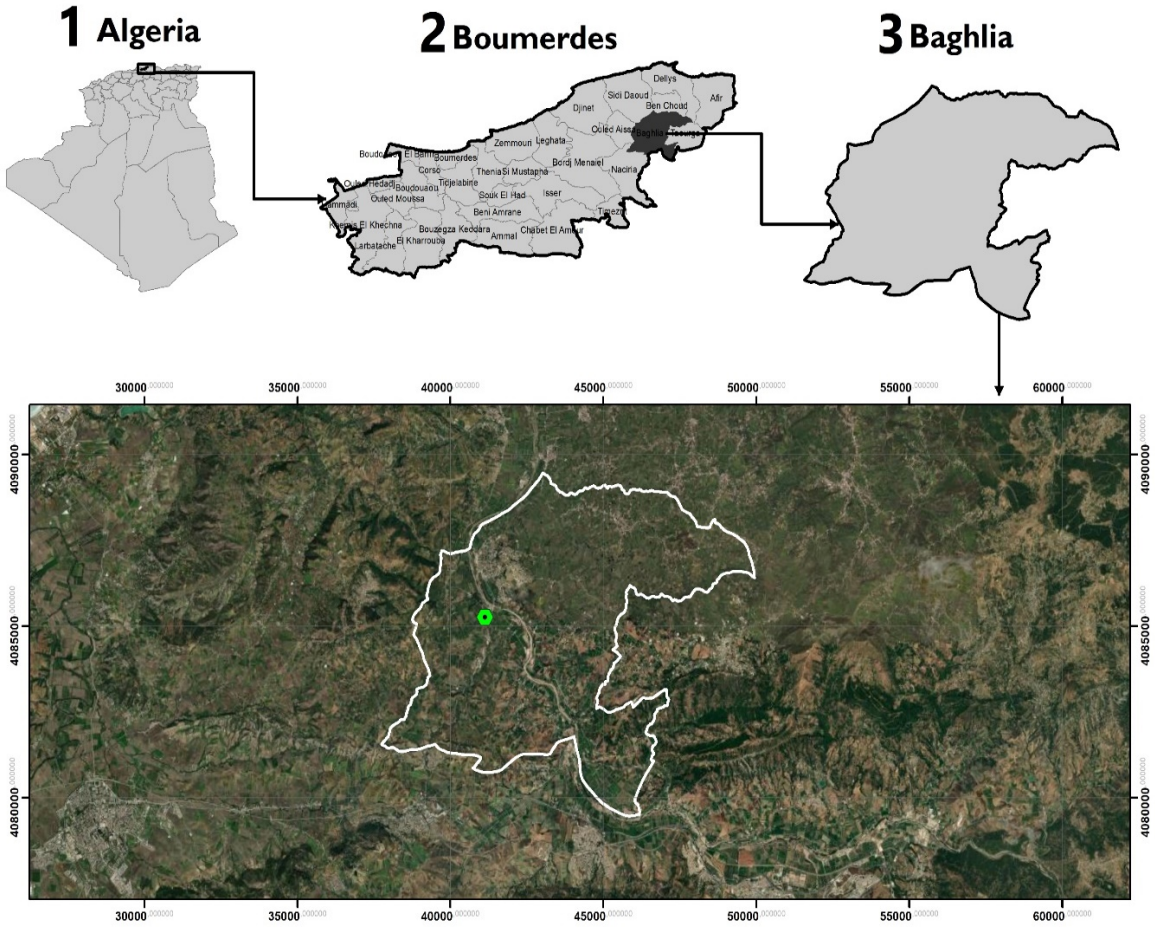


Figure 17: Grape site collection (original photo).

After collection, grape canes were cut into small pieces and air dried until the stabilization of their weight. After that, they were ground using coffee grinder, for extraction of phenolic compounds from the different cane extracts, maceration technique was applied using 100 mg of ground canes macerated in 1000 mL of 60% ethanol solution during 24h in the dark then filtered. Supernatants were evaporated. (Anna Malinowska *et al*., 2020). The resulted extracts were used in the different *in vitro* assays.

In the case of seeds, after collection of grapes, they were washed using distilled water to eliminate any debris then, manually separated from the whole berry, air dried and ground using a coffee grinder, then, kept in the dark until extraction. The ground seeds were macerated in 50% acetone (Yilmaz and Toledo, 2006), filtered, evaporated and thus the dried extracts stored at cold until their use in the different spectrometric and chromatographic analysis.

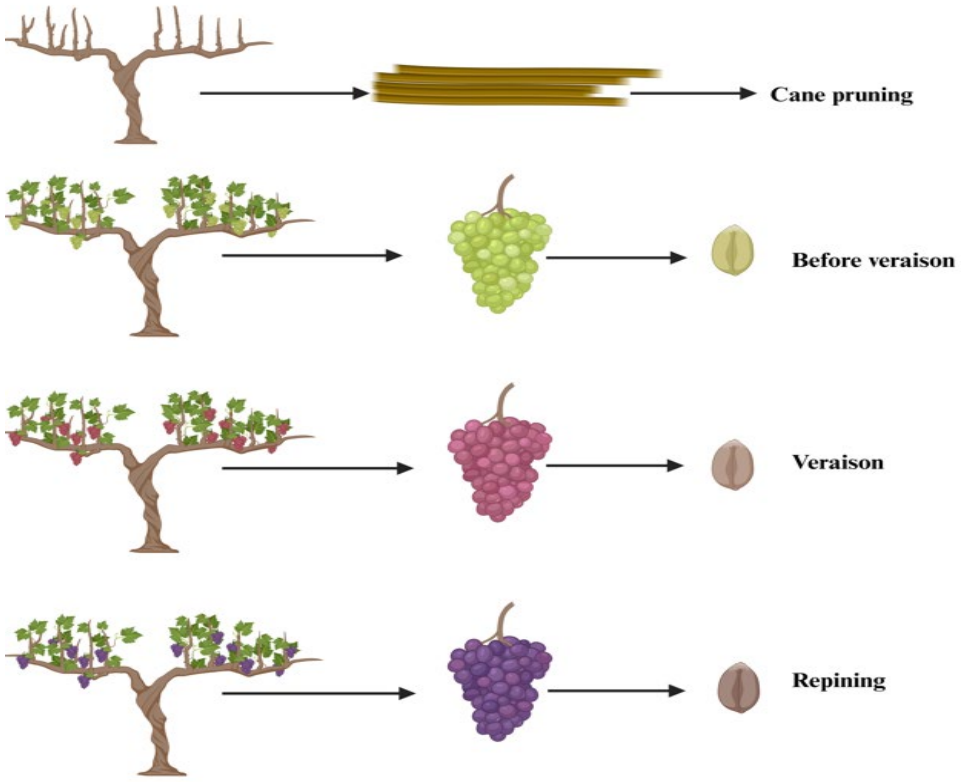


Figure 18: Collection of cane during repining and seeds during different maturity stages (Biorender).

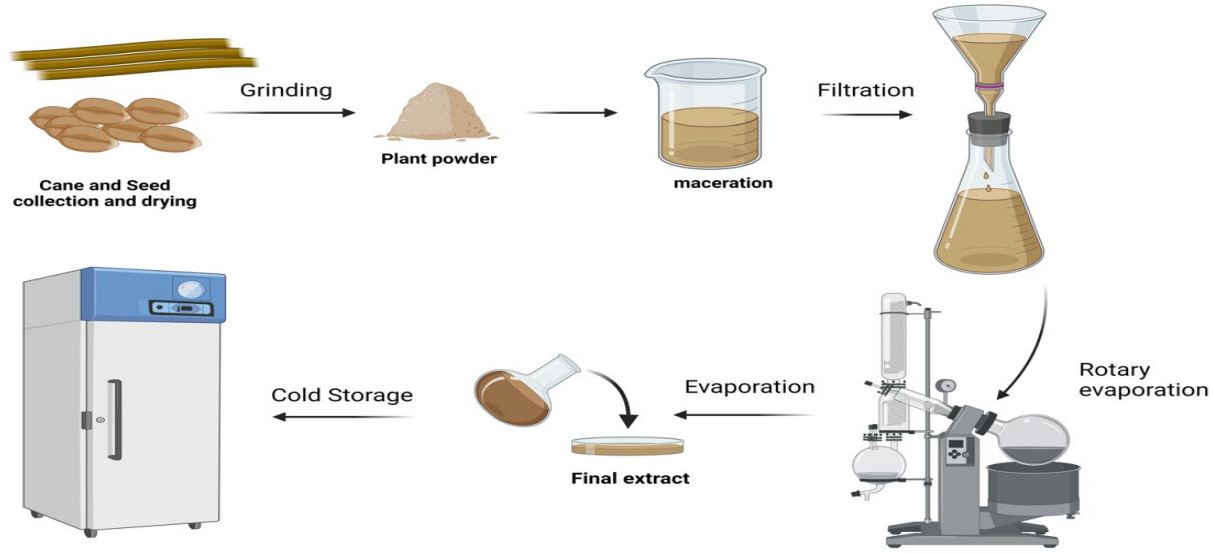


Figure 19: Extraction technique of grape cane and seed polyphenols (Biorender).

2. LCMS/MS analysis

The phenolic compounds were identified following a method similar to that of Erenler et al. (2023) at the Research Laboratory Practice and Research Center, Igdir University. using an LC (Agilent 1260 Infinity) system coupled to a triple quadrupole mass spectrometer (Agilent 6420 Triple Quadrupole LC–MS) and Poroshell 120 SB-C18 reversed-phase column (3.0 × 100 mm, I.D., 2.7 µm) for the separation of different molecules (Figure 20). 50 mg of each extract was dissolved in 1 ml of methanol then 500 µL was added to remove the non-polar fraction. The mixture was centrifugated at 9000 rpm for 10 min. (450 µL) of water and (450 µL) of methanol was then added to 100 µL of the methanolic phase for dilution purpose. Finally, the mixture was filtered (0.22 µm filter) and 5.12 µL of solution was injected for the analyses. The mobile phase consisted of Formic acid (0.1%) and ammonium formate (5.0 mM) in water as eluant A, formic acid (0.1%) and ammonium formate (5.0 mM) in methanol as eluant B. A gradient system was adopted as follow: 25% for 1-3 min, 50% for 4-12 min, 90% for 13-21 min, and 3% for 22-25 min for B mobile phase. nebulizing gas (N2) flow was 11 L/min, and pressure was 15 psi, gas temperature was 300 °C whereas the column temperature was 40°C. MS analysis was performed in both positive and negative ionization modes. the data analyzed using Mass Hunter software by comparing the detected compound retention time with that of the standards whereas the quantification was carried out using the calibration curves of the corresponding standard.

Figure 20: Quantification of polyphenols extracted from the vine wastes using LCMS/MS method (Biorender).

3. Determination of total phenolic contents and biological activities of vine wastes

The determination of the total phenolic content of the different extracts besides to their antioxidant and enzymatic activities were all established through different colorimetric assays where the results were measured 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). Different standards were used in a linear regression analysis to evaluate the results which represented as IC50 and A0.5 values ± SD of three measurement.

3.1. Determination of Total Phenolic, Total Flavonoid, total flavonol, total condensed and hydrolysable tannin Contents

To ascertain the total phenolic content of grape samples, a colorimetric test was conducted using the Folin-Ciocalteu reagent (FCR), in accordance with the methodology outlined by Müller et al. (2010). To obtain the origin solution, 1 mL of methanol was added to 1 milligram of sample extracts. After that, 20 µL of each origin solution was mixed with 75 µL of Na2CO3 (7,5 %) and 100 µl of FCR reagent (1:10). Following that, it was necessary to incubate for two hours in the dark before measuring the absorbance at 765 nm. Using the Gallic acid calibration curve (y = 0.0034x + 0.1044, R2 = 0.9972), the total phenolic content was calculated and reported as milligrams of Gallic acid per gram of dry extract (mg GAE/g).

The technique described by Topçu et al. (2007) was employed with few adjustments to determine the total flavonoid content. 50 µL of samples with a concentration of 1mg/mL were added in triplicate to a mixture of 130 µL MeOH, 10 µL CH3COOK (1M), and 10 µL of Al (NO3)2, 9H2O (10 %). For forty minutes in the dark, the mixture was left at room temperature. The resultant mixes' absorbance was measured at 415 nm, and the TFC was estimated using a quercetin calibration curve (y = 0.0048x, R2 = 0.997).

The determination of the total flavonol was assessed by combining 50 µL of sample solutions with a concentration of 1 mg/mL, 50 µL of 2% aluminum chloride, and 150 µL of 5% sodium acetate according to the method described by Bouzana et al. (2023). The combining mixture was allowed to stand in the dark during 3 hours and the result absorbance was recorded at 440 nm. The calibration curve of quercetin with an equation of y = 0.007X+0.022, (R^2 = 0.998) was used to express the results as mg QE/g.

The method of Broadhurst and Jones. (1978) was used to evaluate the total condensed tannin content. The reaction solution was prepared from 150 µL of 4% vanillin, 25 µL of triplicate extract solution prepared in methanol with a concentration of 1 mg/mL, and of 75 µL of 30% HCl. The combining solution incubated at 30°C during 20 min and the absorbance was recorded at 500 nm. The standard was catechin (0.0012x + 0.1245; R^2 = 0.9156), and the results were expressed as milligram catechin equivalents per gram of extract mg CE/g extract.

The quantification of the hydrolysable tannin content was carried out according to Esma's et al. (2023) method. The reaction mixture contained 50 µL of 1 mg/mL extract with 150 µL of 2.5 % potassium iodate. The absorbance was recorded at 550 nm after 15 min incubation; and the results were expressed as mg tannic acid equivalents (TAE)/ g dry weight using the standard equation (0.0007x + 0.051; R^2 = 0.9975).

3.2. Antioxidant activity

five different *in vitro* widely used assays were employed in the present study: 2,2-diphényl-1-picrylhydrazyl **(**DPPH), 2,2`-azinobis-3-ethylbenzothiazoline-6-sulfonique (ABTS), ferric reducing power (FRAP), phenanthroline, and silver nanoparticles (SNP) to evaluate the antioxidant activity of grape cane and seed 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 at different concentration (800; 400; 200; 100; 50; 25; 12,5 µg/mL) in micro plates of 96-well- using a micro plate reader (Perkin Elmer EnSpire, New York, NY, USA). Both of BHA: butylated

hydroxyanisole and BHT: butylated hydroxytoluene were used in a linear regression analysis as standards to evaluate the results which represented as IC50 and A0.5 values ± SD of three measurements.

3.2.1. Scavenging free radicals' capacity

The capacity of the different extract to scavenge DPPH and ABTS was assessed.

- **2,2-diphényl-1-picrylhydrazyl (DPPH) scavenging activity**

2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable, free radical with a violet color. Its reduction by an antioxidant (proton donor) allows it to become yellow, with the color change proportional to the antioxidant activity. To quantify the reaction, a spectrophotometer at 517 nm detects the solution's absorbance. (Brand-Williams *et al*., 1995).

Seed and canes extracts' capacity to scavenge free radicals was assessed using Blois. (1958) method. 40 µL of samples or synthetic antioxidants (BHT and BHA) at various concentrations were mixed with 160 µL of DPPH diluted solution (0.1 M) with an absorbance of 0.7. The absorbance was recorded at 517 nm after 30 min of incubation at room temperature in the dark. Percentage (%) DPPH free radical scavenging activity was calculated using the Equation:

$$I\%\ (DPPH) = ((Ac-As)/Ac) \times 100$$

Ac is the absorbance of the DPPH solution and As is the absorbance of the sample. The results are expressed as IC50 values.

- **2,2`-azinobis-(3-ethylbenzothiazoline-6-sulfonique) (ABTS) Scavenging activity**

This test is based on an antioxidant's ability to stabilize the blue-green cationic 2,2-azinobis-(3-ethylbenzothiazolin-6-sulfone) radical by converting it into colorless ABTS. The reaction is assessed by using a spectrophotometer to measure the solution's absorbance at 734 nm (Chen et Ho, 1997).

The ABTS assay was assessed using a modified version of Re et al. (1999) method. This involved combining 19.2 mg of ABTS (7mM) with 3.3 mg of potassium persulfate (K2S2O8) to form the ABTS radical cation (ABTS•+). After being stored in the dark for 16 hours, the liquid was diluted to achieve an absorbance of 0.7 at 734 nm. 160 µL of (ABTS•+) solution was mixed with 40 µL of the diluted samples and standards (BHA and BHT), and the mixture was incubated for ten minutes at room temperature. Percentage (%) DPPH free radical scavenging

activity was calculated using the Equation: I% (DPPH) = ((Ac-As)/Ac) ×100. Ac is the absorbance of the DPPH solution and As is the absorbance of the sample. The results are expressed as IC50 values.

3.2.2. Ferric reducing power

This approach relies on an antioxidant's ability to decrease ferric iron (Fe+3) in the ferrous iron (Fe+2) ferricyanide complex (K3Fe (CN)6), resulting in the creation of potassium ferrocyanide (K4[Fe(CN)6]. The yellow shift of Fe+3 to the blue green shift of Fe+2 demonstrates the change in response. The reaction is evaluated by measuring the solution's absorbance at 700 nm with a spectrophotometer (Pellegrini *et al*., 2003).

The ferricyanide reduction method of Oyaizu (1986) with minor modifications was used to validate the extracts' antioxidant activity. 50 µL of 1% potassium ferricyanide solution (K3Fe (CN)6) and 40 µL of phosphate buffer (0.2 M, pH 6.6) were combined with 10 µL of each sample and standards (BHA and BHT) at various concentrations. The reaction was allowed to react for 20 minutes at 50°C. After that, 50 µL of 10% tri-chloroaceticacid (TCA), 40 µL of distilled water, and 10 µL of ferric chloride (FeCl3 0.1%) were added. The absorbance was measured at 700 nm. The A0.5 value was the resultant value (the concentration (µg/mL) at an absorbance of 0.5).

3.2.3. Silver Nanoparticle (SNP) Assay

The approach is based on an antioxidant's ability to convert silver ions (Ag+) to silver atoms (Ag0), which agglomerate in tiny clusters and unite to form colloidal silver particles (SNP). The reaction is measured using a spectrophotometer at 423 nm (Kapoor et *al*., 1994).

the reduction of Ag+ procedure was carried out to assess the antioxidant capacity of the different extracts according to the method described by Özyürek et al. (2012). A combination of 130 µL of SNP solution, 50 µL H2O, and 20 µL of samples or standards (BHA and BHT) contributed to the reaction mixture. The mixture was allowed to stand for 30 minutes at room temperature in the dark, then, the absorbance at 423 nm was determined. The A0.5 value was used to express the results. The A0.5 value was the resultant value (the concentration (µg/mL) at an absorbance of 0.5).

3.2.4. Phenanthroline Assay

The phenanthroline technique employs an antioxidant to reduce ferric ions (Fe3+) to ferrous ions (Fe2+). The generated Fe2+ ion interacts with ortho-phenanthroline to form a red-orange complex (Yefrida et *al*., 2018). A spectrophotometer at 510 nm measures the absorbance of the solution to quantify the reaction.

The method established by Szydłowska-Czerniak et al. (2008) was employed to evaluate the reduction capacity. The mixture composed from 10 µL samples or standards (BHA and BHT), 50 µl FeCl3 (0.2%), 30 µL Phenanthroline (0.5%), and 110 µL Methanol. The final combination was then incubated for 20 minutes at 30°C in the dark before the absorbance at 510 nm was determined. The A0.5 value was used to report the results.

3.3. Enzymatic activity

The capacity of grape cane and seed extracts to inhibit five different enzymes (tyrosinase, α-amylase, butyrylcholinesterase, lipase, and urease) implicated in the development of different diseases were studied *in vitro*. The inhibition of enzymes was determined spectrophotometrically by measuring the absorbance of samples at different concentration in micro plates of 96-well- using a micro plate reader (Perkin Elmer EnSpire, New York, NY, USA). All reactions were considered as colometric assays, different standards were used in a linear regression analysis to evaluate the results which represented as IC50 values ± SD of three measurements.

3.3.1. Anti-tyrosinase activity

The preparation of tyrosinase followed the procedure outlined by Gouzi and Benmansour (2007). 100 g of mushrooms (*Agaricus bisporus*) were crushed in a blender with 120 mL of pH 7 phosphate buffer that had been iced for 30 seconds. Following 30 minutes of mixing and filtering, the filtrate is centrifuged at 18,000 g for 30 minutes at 4°C. The crude tyrosinase enzyme extract is made up of the supernatant.

The preparation of the reaction involved combining 150 µL of buffer solution at pH 6.8, 10 µL of cane and seed extracts at different concentrations (3,125; 6,25; 12.5; 25; 50; 100; 200 mg/mL), and 20 µL of tyrosinase enzyme extracted from mushroom (Figure 21). The mixture then incubated for 10 minutes at 37 °C. Subsequently, 20 µl of L-DOPA was added, the incubation continued for an additional 10 minutes at 37 °C, concluding with a measurement at 475 nm. The method was reported by Deveci et al. (2018). Inhibition percentage calculated

using the following formula: I% = [(Ac - As)/As] * 100, where I%: the inhibition percentage, AC: absorbance of the control, and AS: absorbance of the tested sample.

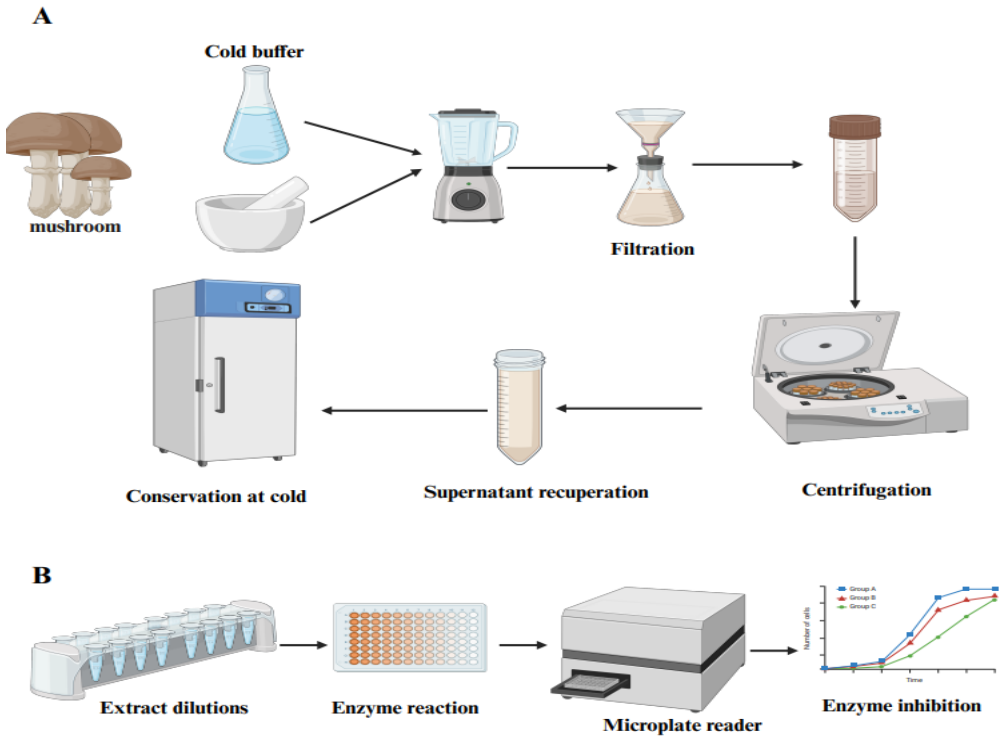


Figure 21: Extraction of mushroom tyrosinase (A) and anti-tyrosinase activity (B) (Biorender).

3.3.2. α-amylase inhibitory Activity

The capacity of the different extracts to inhibit α-amylase was performed according to the method prescribed by Zengin et al. (2014). In a 96 well microplate, a preincubation during 10 min at 37°C of a mixture containing 25 µL of extracts (6,25; 12.5; 25; 50; 100; 200; 400 mg/mL), and 50 µL enzyme prepared in sodium phosphate buffer solution (pH = 6.9) was assessed. Thereafter, 50 µL of 1% starch was added, and a further 20 minutes incubation at 37°C was followed. The addition of 25 µL HCl (1 M) stopped the reaction whereas the addition of 100 µL of iodine reagent noted the color change. The absorbance was recorded at 630 nm. The % inhibition of α-amylase was estimated as follows: I% = 1 - (Absc - Abse) - (Abss - Absb)/(Absc - Abse). Abss = absorbance (extract, starch, enzyme, IKI, HCl); Absb = absorbance (extract, sodium phosphate buffer, IKI); Abse = absorbance (solvent vol extract, enzyme, starch, HCl, IKI); Absc = absorbance (solvent vol extract, sodium phosphate buffer, starch, HCl, IKI).

3.3.3. Anticholinesterase activity

A combination of 150 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of extracts at different concentrations (3,125; 6,25; 12.5; 25; 50; 100; 200 mg/mL), and 20 µL Butyryl choline esterase (6.85-10-3 U) solution was incubated at 25^0C during15 min. An addition of 10 µL of DTNB (0.5 mM), 10 µL of butyrylthiocholine chloride (0.2 mM) was followed. Finally, a lecture at 412 nm, for 0 min and 15 min was assessed to obtain the results (Ellman *et al*., 1961). Inhibition percentage calculated using the following formula: I% = [(Ac - As)/As] * 100, where I%: the inhibition percentage, AC: absorbance of the control, and AS: absorbance of the tested sample.

3.3.4. Lipase Inhibition

Lipase inhibition was determined using the spectrophotometric method described by McDougall et al. (2009). Each well consisted of 20 µL of sample (3,125; 6,25; 12.5; 25; 50; 100; 200 mg/mL), 200 µL of Tris-HCl buffer (100 mM, pH = 8.2), 20 µL of lipase (1 mg/mL), and 20 µL of *p*-nitrophenyl octanoate (5.1 mM). Samples were incubated at 37°C for 30 minutes before absorbance measurement at 410 nm. Inhibition percentage calculated using the following formula: I% = [(Ac - As)/As] * 100, where I%: the inhibition percentage, AC: absorbance of the control, and AS: absorbance of the tested sample.

3.3.5. Urease inhibitory Activity

3.3.5.1. *In vitro* anti-urease activity

The assessment of anti-urease activity was assessed by measuring the ammonia generated during the reaction carried out by Taha et al. (2018). In summary, a 96-well micro plate was filled with 10 µL of extracts at various concentrations (6,25; 12.5; 25; 50; 100; 200; 400 mg/mL), 20 µL of urease solution (5 U/milliliter of urease from jack bean *Canavalia ensiformis*, type IX), 20 µL of urea substrate, 45 µL of phenol reagent, and 70 µL of alkaline reagent. After two hours of incubation at 30°C, the mixture's absorbance at 630 nm was determined. Inhibition percentage calculated using the following formula: I% = [(Ac - As)/As] * 100, where I%: the inhibition percentage, AC: absorbance of the control, and AS: absorbance of the tested sample.

3.3.5.2. Molecular docking study

To study *in silico* the binding mechanisms and the interaction modes of the nine products identified in the cane extracts, a molecular docking study was conducted targeting the active site of urease. The three-dimensional structure of urease was acquired from the Protein

Data Bank (https://www.rcsb.org, accessed on 4 Mai 2024) under the code 4H9M, which has one chain. In the enzyme structure preparation, the co-crystallized inhibitor HAE was used for active region determination. The residues His407, His409, Arg439, Ala440, Thr441, Thr442, Lys490, His492, Glu493, Asp494, His519, Tyr544, His545, Gly550, Gly551, Thr571, His593, Arg609, Asp633, Ala636, Met637, and two metal ions were found to be present in the binding pocket of the proposed target protein. The three-dimensional structure of each tested product was downloaded in the SDF format from the Pubchem database (4 March 2024). The molecular docking experiment was undertaken using FlexX software 2.3.3 (https://www.biosolveit.com/) and an incremental ligand construction method (Rarey *et al*., 1996). Polyphenols products in the extract were ranked using the modified Bohm's scoring function (ΔG, in kJ/mol) (Merzoug *et al*., 2018). The compound thiourea was taken as reference inhibitor.

3.3.5.3. *In silico* Absorption, Distribution, Metabolism, Excretion (ADME), and Toxicity (Tox) Investigation of phenolic compounds

To predict the physicochemical and pharmacokinetic properties of the detected polyphenols, based on their chemical structures, a computational ADME/Tox study was performed using Swiss ADME server at http://www.swissadme.ch/ for Lipinski's rule, gastrointestinal absorption (GI), blood-brain barrier (BBB) penetration, inhibitory properties of the cytochrome P450 (CYP) isoforms (Al Azzam, 2023), and ProTox-II at https://www.tox.charite.de/protox3/ for toxicity. The same proprieties of thiourea were also studied for comparison.

3.4. Sun Protection Factor

The sun protection factor (SPF) of various extracts prepared at a concentration of 2mg/mL was determined by the method described by Mansur et al. (1986). The absorbance was measured at different wavelengths, from 290 nm to 320 nm (UV-B), each 5 nm. The SPF factor was determined by applying Mansur's mathematical formula:

$$SPF_{\text{spectrophotometric}} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs$$

EE: erythemal effect spectrum, I: solar intensity spectrum, Abs: solar product absorbance, CF: correction factor (= 10), EE * I: constant values determined by Sayre et al. (1979).

3.5. *In vitro* anti-inflammatory activity:

To determine the anti-inflammatory properties of the plant extracts, a slightly modified version of the BSA test established by Benmohamed et al. (2023) was employed. The procedure involved filling a 96-well microplate with 100 µL of each quantity of extract or standard (diclofenac sodium) and 100 µL of a 0.2% BSA solution prepared with tris-HCl (pH = 6.6). The combination was allowed to incubate for 15 minutes at 37 °C and 5 minutes at 72 °C within an oven. After cooling, the turbidity was measured at 660 nm using a micro plate reader. The protective effect of samples against the denaturation of BSA is presented as an inhibition percentage calculated using the following formula: I% = [(Ac - As)/As] * 100, where I%: the inhibition percentage, AC: absorbance of the control, and AS: absorbance of the tested sample.

3.6. Antimicrobial activity

The antimicrobial activity of the different extracts was also established against microbial agents.

3.6.1. Bacterial and Fungal Strains

The antibacterial and antifungal activities of the cane and seed extracts were tested against four extended-spectrum β-lactamase (ESBL)-producing *Escherichia. coli* strains provided by Bougouizi et al. (2024), two fungal strains (*Candida albicans* and *Aspergillus niger*) provided by Bouzana (2024) and two reference strains; (*Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231) obtained from the Pasteur Institute, Algiers). The bacterial isolates were collected from a private laboratory in central Skikda, Algeria. They were obtained from urine samples from hospitalized elderly patients, including two females and two males, aged between 80 and 96 years. The four ESBL strains were multidrug-resistant, demonstrating resistance to at least three different classes of antibiotics. Specifically, they were resistant to ampicillin, first and third-generation cephalosporins (cefazolin, cefotaxime, and ceftazidime), ciprofloxacin, and cotrimoxazole. Additionally, two of the strains were also resistant to gentamicin and amoxicillin/clavulanic acid. The strains of *Candida albicans* and *Aspergillus niger* were collected from Dr. Chaib's bacteriology laboratory in Azzaba. They were primarily isolated from pathological samples such as the tongue, the interdigital spaces, nails, and vaginal swabs.

3.6.2. Antibacterial and antifungal activity

- **Determination of Inhibition Zones Diameters**

The antimicrobial activity against *E. coli* producing BLSE and *C. albicans* was evaluated using the Kirby Bauer disk diffusion method on Mueller-Hinton agar plates (CLSI, 2023). Bacterial and yeast inoculant were prepared in physiologically sterile water, with an OD of 0.08 at 625 nm and an OD of 0.12 at 530 nm, respectively and were used to inoculate the plates. Sterilized 6 mm disks were impregnated with 20 µL of varying extract concentrations (20; 10; 5; 2.5; and 1.25 mg/mL). After incubation at 37°C for 24 h, inhibition zones around the disks were measured in millimeters. Dimethyl sulfoxide (DMSO) served as the negative control, while a disk of Gentamycin (10 ug/mL), a broad-spectrum antibiotic, is used as a positive control against bacteria. All measurements were performed in triplicate. The results were interpreted according to the inhibition zone (IZ) intervals determined according to the scale of Ponce et al. (2003):

IZ< 9: resistant microbial agent.

09 < IZ< 14 mm: sensitive microbial agent.

15 < IZ < 19 mm: very sensitive microbial agent.

- **Minimal Inhibitory Concentration (MIC)**

The MIC of various extracts against *E. coli* producing ESBL and *C. albicans* was determined using the broth microdilution method following CASFM protocol (CLSI, 2023). 50 µL of extracts at various concentrations (0.625; 0.312; 0.156; and 0.078 mg/mL), A mixture of 50 µL of bacterial or yeast suspension, and 100 µL of MH broth was added to each microplate well. Controls including wells for bacterial or yeast growth and MH broth sterility were used. After 24 h incubation at 37°C, growth was assessed visually.

- **Determination of the percentage inhibition of *A. niger* growth**

The antifungal activity against *A. niger* was assessed using the disk diffusion method on Sabouraud agar supplemented with chloramphenicol (Hajji *et al*., 2016). Mycelial fragments from 72 h old cultures were placed at the center of Petri dishes. Sterile 6 mm diameter disks impregnated with 20 µL of varying extract concentrations (20; 10; 5 mg/mL) were placed on the plates. A negative control dish with a mycelial disk treated with DMSO was included.

Incubation occurred at 27 ± 2°C for 72 h. Fungal growth was evaluated by measuring the average of two perpendicular colony diameters. The percentage inhibition of growth (%) was calculated using the formula: Percentage inhibition = [(dc - dt) / dc] × 100. where dc is the diameter of the colony in the control dish, and dt is the diameter of the colony in the treated dish. The results were interpreted according to the interpretation scale established by Abd-Ellatif et al. (2011), "From 30% to 40% represents low activity, from 50% to 60% represents moderate activity, from 60% to 70% represents good activity, and >70% represents excellent activity."

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 ($p \leq 0.05$). Pearson's correlation coefficients (r) were used to determine the correlation between variables. Additionally, Principal Component Analysis and hierarchical clusters were performed using Origine Pro2024.

Results and discussion

1. Cane wastes

1.1. Total Phenolic, Flavonoid, and flavonol contents

In the present study, four vine Algerian grape cultivars (FR: Fragola Nera, CR: Cardinale, RG: Red glob, and GR: Gros Noir) were selected for the evaluation of their total phenolic (TPC), total flavonoid (TFC), and total flavonol contents (TF-OL). The highest TPC was detected in the grape cultivar FR from the hybrid (*Vitis vinifera*/*Vitis Labrusca*) with an amount of 309,8 ± 11.5 mg gallic acid equivalent/g extract, followed by RG from *Vitis vinifera* (205,07 ±16,4 mg GAE/g extract), while the lowest concentrations were found in the CR (198,3 ± 5.4 mg GAE/g extract) and GR (123 ± 11,7 mg GAE/g extract) extracts. The FR variety also demonstrated the highest TFC value (55,6 ± 2,06 mg Quercetin equivalent/g extract) followed by CR, RG and GR with an average TFC of 48,5 ± 7,9, 40,5 ± 7,3, and 40,5 ± 0,2 mg QCE/g extract, respectively. The highest TF-OL content was recorded in the RG extract with an amount of 15.98 ±1.20 mg QCE/g extract, whereas non-significant lower amounts were recorded in the other varieties (Table 1). The results of our study suggested that the significant differences ($p < 0.05$) in the TPC, TFC, and TF-OL values of the four Algerian vine-cane types produced under the same conditions may be caused by the vine-cane variety.

Table 1: Total phenolic, flavonoid, and flavonol contents of cane extracts.

Extracts	TPC (mg GAE/g)	TFC (mg QE/g)	TF-OL (mg QE/g)
GR	123c ± 11,7	40,5c ± 0,2	13.49c±0.45
FR	309,8a ± 11.5	55,6a ± 2,06	14.06b±0.40
RG	205,07b ±16,4	40,5c ± 7,3	15.98a±1.20
CR	198,3b ± 5.4	48,5b ± 7,9	13.30c±0.85

GR: Gros Noir, FR: Fregola Nera, RG: Red Glob, CR: Cardinale. total phenolics: TPC (mg gallic acid/g extract), total flavonoids: TFC (mg quercetin/g extract), total flavonol: TF-OL (mg quercetin/g extract). The values in identical columns with various superscripts (a, b, c,) differ significantly ($p < 0.05$).

Ju 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 equivalent 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 influence of the variety, 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. The results showed significant differences with 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 evaluation 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 acidified methanol solution as solvent. The results of this study found to be in some cases lower and in others higher than our results and showed significant differences between the species and between the varieties of the same species represented by different values, which varied from 76.4 to 224.5 mg (GAE)/g extract and 33.1 to 146.6 mg (QCE)/g for TPC and TFC, respectively (Zhang et al., 2011). The results obtained by these studies are in accordance with our suggestion regarding the influence of variety.

The study conducted by Esparza et al. (2021), found that climatic conditions and the variety influenced the TPC and TFC of *Vitis vinifera* stems harvested in two different years, 2016 and 2018. Additionally, 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 time of storage (2, 4, and 6 months) and demonstrated a decrease trend in the TPC content especially after six months of storage.

In another hand, the statistical analysis represented high correlation (Figure 26) between TPC and TFC ($r = 0.86$, $p < 0.01$) which revealed that flavonoids are the major compound contributing to total phenolics in grape cane extracts, Zhang et al. (2011) also mentioned this relation.

1.2. Identification of phenolic compounds by LCMS/MS:

The LCMS/MS results revealed the presence of thirteen phenolic compounds with different concentrations, as represented in Table 2 and Figure 22. Flavonoids, phenolic acids, and stilbenes dominated the phenolic profile of the cane extracts. Flavonoids are the highest class detected in all grape cane varieties, represented by the flavanol catechin and epigallocatechin with a range amount of 571.25-1440.45 mg/kg D.W. and 23.10-116.67 mg/kg D.W., respectively, with a maximum detected catechin concentration in the FR variety (1440.45 mg/kg D.W). In the same case, luteolin, the only representative of the flavone class, was found to vary between 89.79 and 175.52 mg/kg D.W. Regarding the case of flavonol, kaempferol-3-glucoside was only detected in the FR variety, whereas Isoquercitrin (quercetin-3-glucoside) was found in all cultivars except for GR, with an amount of 9.28, 15,96, and 17.49 mg/kg D.W for the FR, RG, and CR cultivars, respectively. Regarding flavanone, hesperetin was detected in the RG variety with a concentration of 26.28 mg/kg DW.

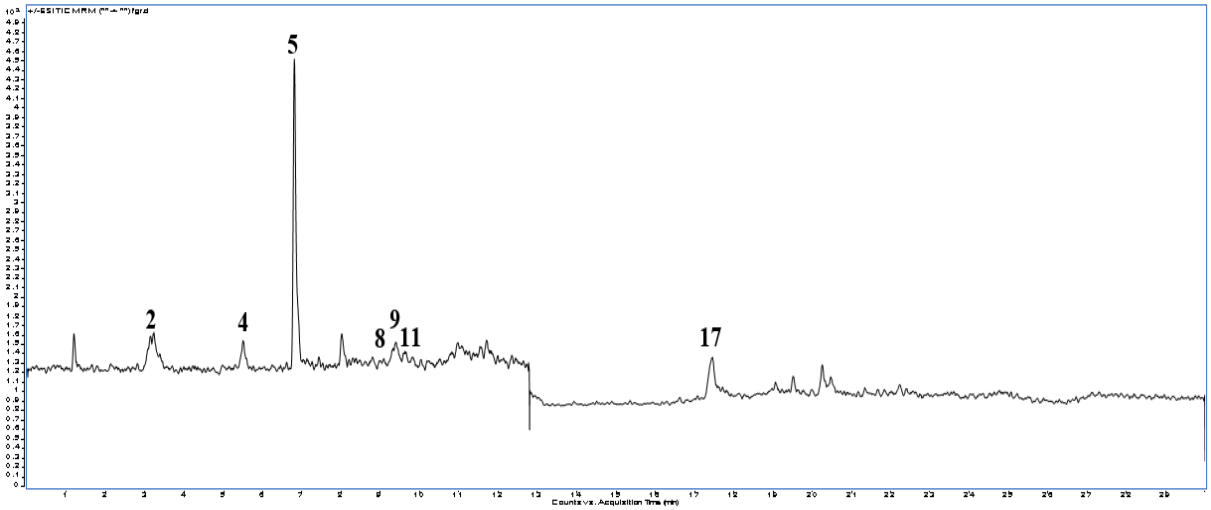
Phenolic acids are the main second class identified in the different extracts represented by hydroxybenzoic acids, which average between 91.69 and 132.43 mg/kg D.W. for gallic acid and from not detected to 105.18 mg/kg D.W. for salicylic acid. Protocatechuic acid was only identified in the FR variety with a concentration of 15.22 mg/kg D.W. However, in the case of hydroxycinnamic acids, o-coumaric acid was identified in a considerably lower amount in three extracts. It should be pointed out that the specific qualities of each variety, the climate and biotic factors, the viticultural methods, as well as the growing conditions can all be used to explain the variations in phenolic content observed in this work and previous research published in the literature (Leal *et al*. 2020). Regarding the influence of variety, our results support earlier findings conducted by Ang Zhang et al. (2011), Leal et al. (2020), and Moreira et al. (2020) whom assumed that grape cultivars have different effects on the phenolic content of grape shoot and stem.

Comparing the amounts of phenolic compounds with data from the literature is difficult since various writers present results in different ways and the different techniques and solvents used for extraction. Although, our results are in agreement with those reported by Esparza et al. (2021) and Quero et al. (2021) whom found that catechin was identified to be the most abundant phenolic compound in the stem extracts with a range concentration of 0.95-3.50 and 0.98 mg/g respectively. Moreira et al. (2018) also confirmed that the most important phenolic acid found in vine shoots is gallic acid with levels ranging from 527 to 1014 mg/100 g D.W. Kaempferol and Quercetin and their derivatives was identified in many studies as a part from flavonol class detected in grape shoots (Moreira *et al*., 2020; Rätsep *et al*., 2021). however, only Kaempferol-3-glucoside and Isoquercitrin were detected in our study.

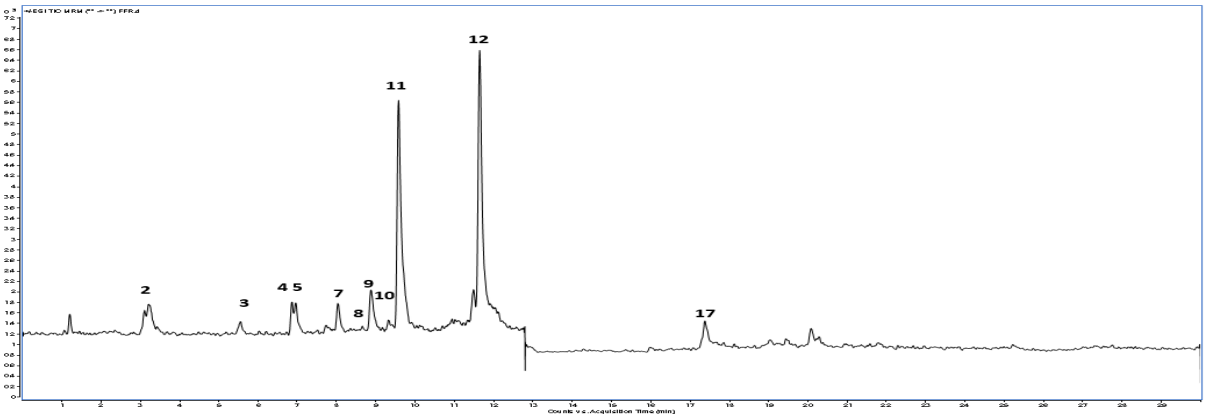
Table 2: Phenolic compounds identified in the grape cane extracts. RT: retention time, GR: Gros Noir, FR: Fregola Nera, RG: Red Glob, CR: Cardinal.

No	Phenolic compounds	RT	GR	FR	RG	CR
		(min)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
1	Shikimic acid	1.414	ND	ND	ND	ND
2	Gallic acid	3,218	91,77c	132,43a	118,65b	91,69c
3	Protocatechuic acid	5,449	ND	15,22	ND	ND
4	Epigallocatechin	6,796	115,48a	23,10c	40,40b	116,67a
5	Catechin	6,904	571,25d	1449,45a	1130,81b	753,53c
6	Chlorogenic acid	7,378	ND	ND	ND	ND
7	Hydroxy-benzaldehyde	7,679	ND	15,97a	14,02a	ND
8	o-coumaric acid	9,441	8,29c	10,81b	ND	11,37a
9	Salicylic acid	9,539	77,33c	105,18a	ND	83,10b
10	Resveratrol	9,791	ND	44,28a	1,38b	1,53b
11	Polydatin	9,807	0.30d	44.21a	9.02c	11.61b
12	Isoquercitrin	11,867	ND	9,28c	15,96b	17,4941a
13	Kaempferol-3-glucoside	13,287	ND	ND	4,14a	ND
14	Quercetin	14,821	ND	ND	ND	ND
15	Hesperetin	15,815	ND	ND	26,28a	ND
16	Kaempferol	16,431	ND	ND	ND	ND
17	Luteolin	17,909	158,68b	174,00a	175,52a	89,782c

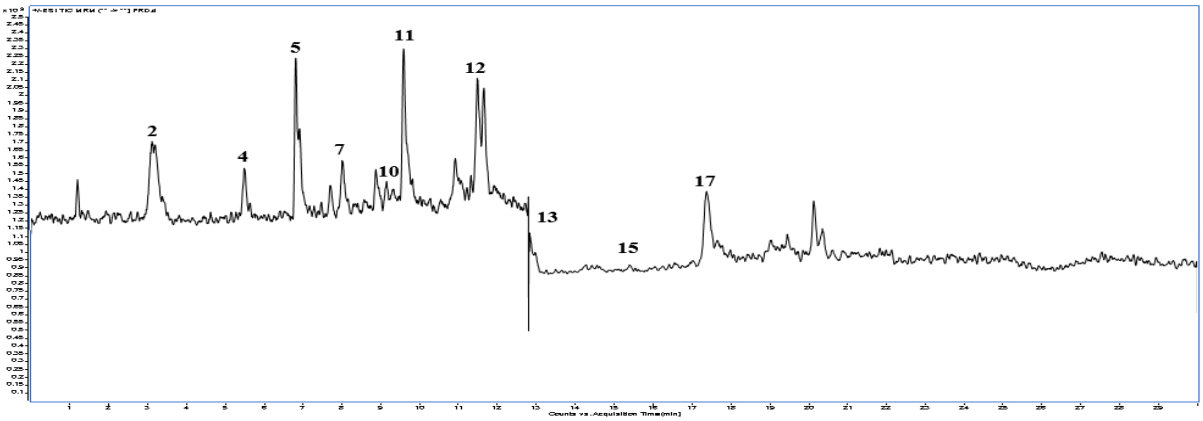
GR



FR



RG



CR

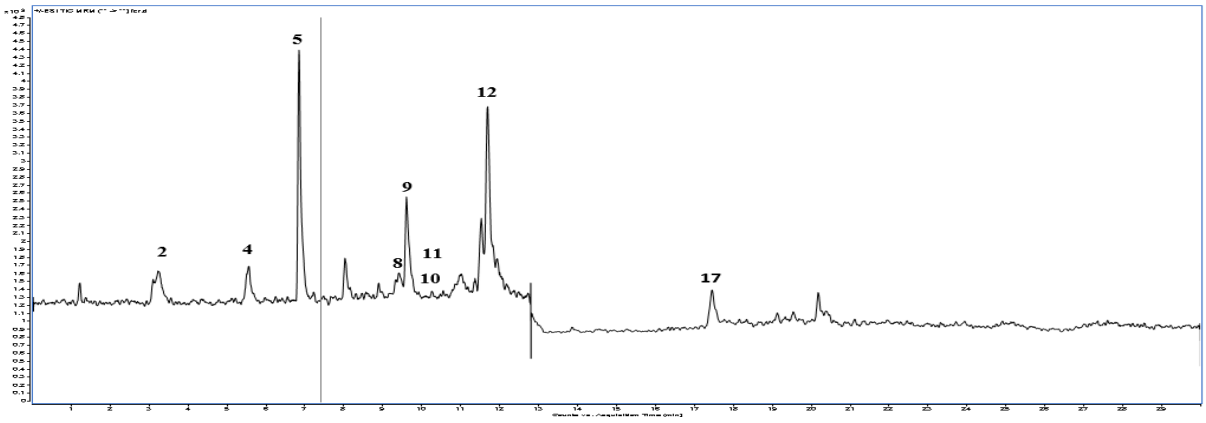


Figure 22: LCMS/MS chromatograms of the studied extracts. 2: Gallic acid, 3: Protocatechuic acid, 4: Epigallocatechin, 5: Catechin, 7: Hydroxy-benzaldehyde, 8: O-coumaric acid, 9: Salicylic Acid, 10: Resveratrol, 11: Polydatin, 12: Isoquercetin, 13: Kaempferol-3-glucoside, 15: Hesperetin, 17: Luteolin.

Regarding stilbenes, both resveratrol and polydatin were detected in considerably lower concentrations in the most extracts in comparison with flavonoids and phenolic acids, ranging from "not detected" to 44.28 mg/kg for resveratrol and from 0.30 to 44.21 mg/kg for polydatin. It is well known that grapevine canes, belonging to both *vinifera* and non-*vinifera* species, are abundant in various stilbene monomers, glycosides, and oligomers. A study conducted by Gharwalova et al. (2018) revealed the presence of resveratrol and its analogue polydatin in 44 analyzed cane extracts with varying concentrations between 4.47-252.79 mg/kg of dry matter (DM) and 4.24–48.73 mg/kg DM, respectively, using 40% ethanol as solvent for extraction. The same stilbene was found to be in the range of 12.65–19.00 mg/g in the methanolic extracts from three different cane varieties studied by Ju et al. (2016). The variation in the concentration of the different studied stilbenes can be due to the various *Vitis* species and cultivars, in addition to the cultivation conditions (plant management, climate, etc.), Additionally, Stilbenes function as phytoalexins, protecting plants from the aggression of pathogenic microorganisms, as a result, plants may exhibit increased resveratrol concentrations in response to infections or additional environmental factors (Chong *et al*., 2009; Guerrero *et al*., 2020).

1.3. Principal component analysis

A principal component analysis (PCA) was performed to investigate the similarities and differences between the cane polyphenol contents of the four studied varieties. 50.48% of the variation could be clarified by the first principal component (PC1), while only 38.55% could be clarified by the second main component (PC2) (Figure 23). Both GR and CR varieties were

grouped together on the negative side of PC1 and characterized by higher amounts of EGC, O-CA, and SA, whereas FR and RG were grouped similarly on the positive side and grouped the rest of the compounds. A hierarchical clustering on the main components (HCPC; Figure 24) was carried out on the first two PCs in order to visualize the proximity of the four cultivars under study. The results confirmed the first suggestion and clustered FR and RG together with the same cluster distance and grouped GR and CR together, giving the possibility that they have the same ancestor.

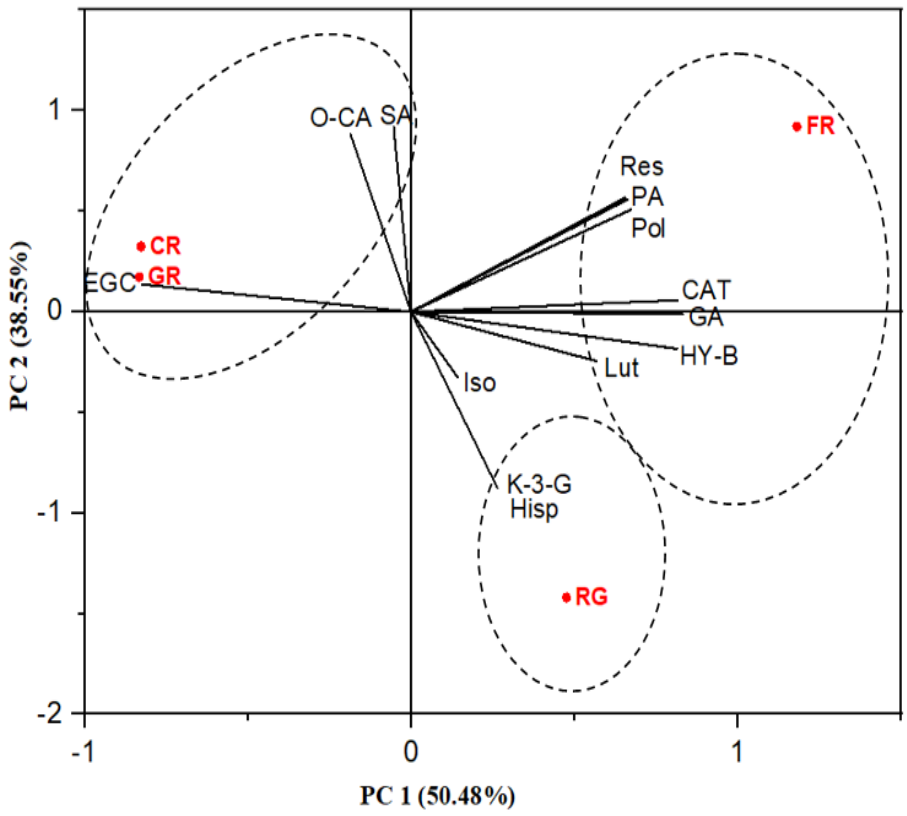


Figure 23: Principal component analysis of the phenolic compounds detected in vine canes. O-CA: O-coumaric acid, GA: gallic acid, SA: salicylic acid, PA: protocatechuic acid, Res: resveratrol, Pol: polydatin, CAT: catechin, EGC: epigallocatechin, K-3-G: kamperol-3-glucoside, Hesp: hesperetin, HY-B: hydroxybenzaldehyde, Iso: Isoquercetin. GR: Gros Noir, FR: Fregola Nera, RG: Red Glob, CR: Cardinale.

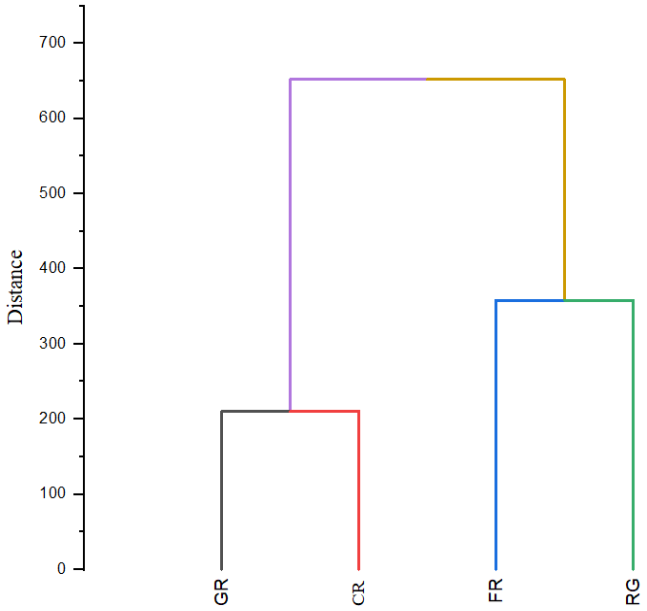


Figure 24: Hierarchical clustering on the main component (HCPC). GR: Gros Noir, FR: Fregola Nera, RG: Red Glob, CR: Cardinale.

1.4. Antioxidant activity

In the present study we used a total of five *in vitro* assays (DPPH, ABT, reducing power (FRAP), phenanthroline, and silver nanoparticles (SNP)) 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 revealed that most extracts demonstrate moderate antioxidant abilities by all of the investigated methods.

1.4.1. 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 its color from purple to pale yellow. It's classified as both electron or hydrogen atom transfer mechanism (Barchan *et al*., 2014; Christodoulou *et al*., 2022). The results of the inhibition assay represented in figure 25 showed that the FR variety attained a maximum percentage of inhibition (89.67%) at a concentration of 25 µg/mL; both

CR and GR were reached it at 50 µg/mL (88.85%, 88.80%, respectively), whereas RG extract (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: CR<GR<RG 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, Zhang et al. (2011) predicted the capacity of different cane extracts to scavenge DPPH and demonstrated values 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, the results represented different capacities to scavenge DPPH.

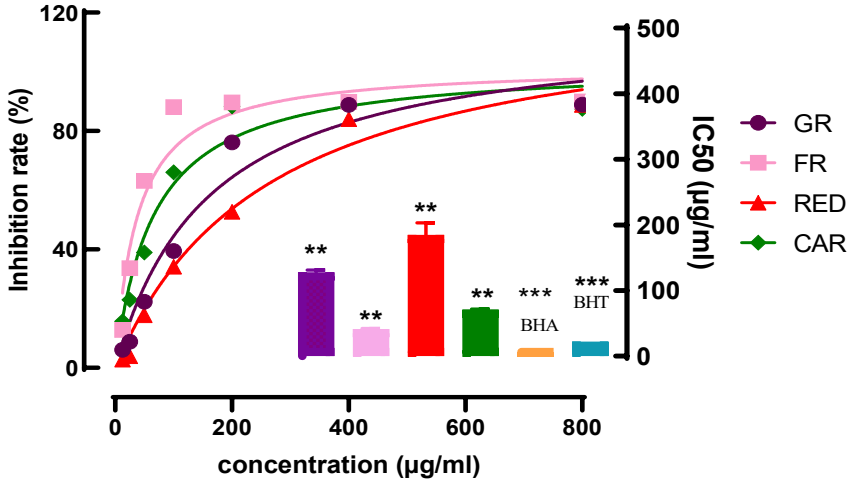


Figure 25: Inhibitory activity and IC50 values of the studied extracts and standard determined using DPPH assay. (**) for comparison inter-compounds and (***) with standard. FR: Fragola Nera, RED: Red Glob, CAR: Cardinale, GR: Gros Noir. IC50: the concentration at the fifty of inhibition. BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene. values represent the means ± SEM of three measures.

In the same context, the correlation coefficients (Figure 26) were found to be negative when comparing the phenolic, flavonoid, and flavonol contents with the IC50 values of the DPPH scavenging assay (r = −0.57, −0.91, and −0.5 for TPC, TFC, and TF-OL, 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 (Balík *et al*., 2008; Zhang *et al*., 2011; Gharwalova *et al*., 2018). Additionally, a high correlation with o-coumaric acid, salicylic acid, kompferol-3-glucoside, hisperitin, and polydatin was detected, giving the suggestion that these molecules are the main phenolic compounds responsible for the antioxidant activity against DPPH (Figure 28).

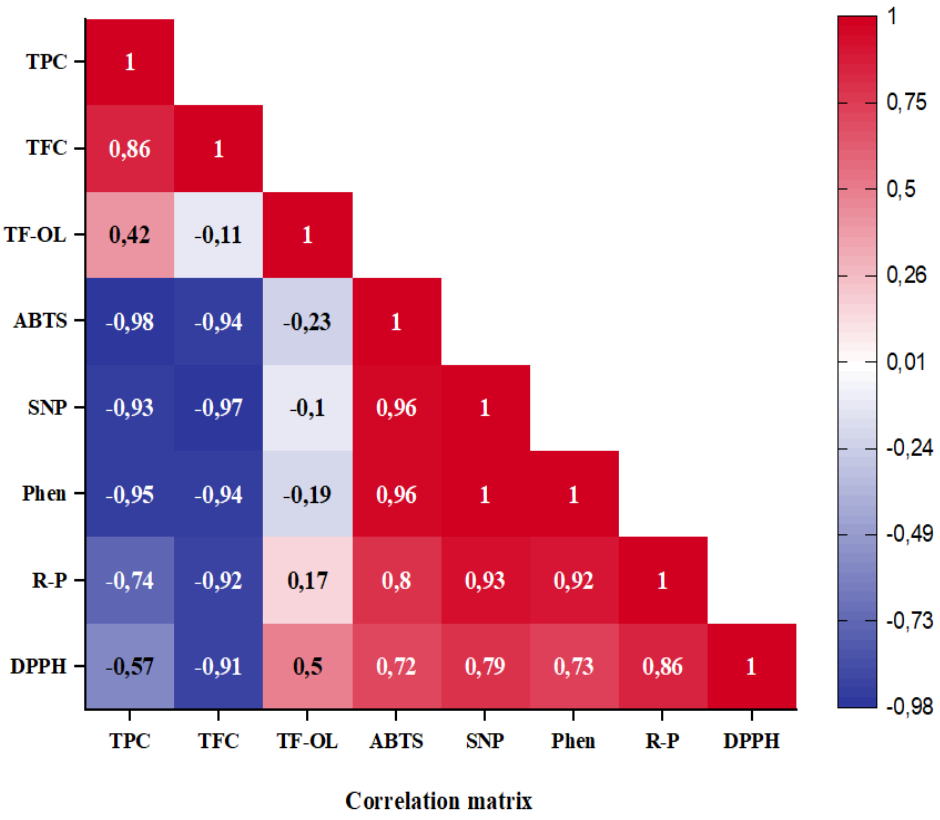


Figure 26: Correlation between TPC, TFC, TF-OL, and the antioxidant activities of the cane extracts. TPC: total phenolic content, TFC: total flavonoid content, TF-OL: total flavonol. RP: Reducing power, Phen: Phenanthroline, SNP: Silver nanoparticles.

1.4.2. 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 make the ABTS assay more adaptable due to its ability to detect the scavenging activity of both polar and non-polar compounds (Zhang *et al*., 2011; Srief *et al*., 2022).

The results of the antioxidant power of the tested extracts presented in figure 27 showed that the inhibition percentage of all extracts reached its highest value (93%) at a concentration of 25µg/mL. According to the recorded results, all the extracts demonstrated strong antioxidant activity with higher IC50 values than the standards 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 was 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 RG variety (9,18 ± 0,2 µg/mL), and an even higher value in the case of the GR (11,61 ± 0,3 µg/mL).

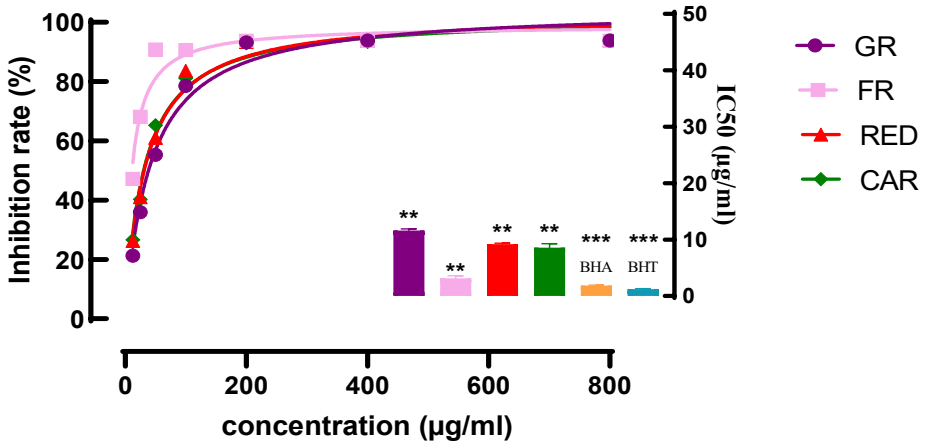


Figure 27: Inhibitory activity and IC50 values of the studied extracts and standard determined using ABTS inhibitory assay. (**) for comparison inter-compounds and (***) with standard. FR: Fragola Nera, RED: Red Glob, CAR: Cardinale, GR: Gros Noir. IC50: the concentration at the fifty of inhibition. BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene. values represent the means ± SEM of three measures.

In the same 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 a higher value 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.

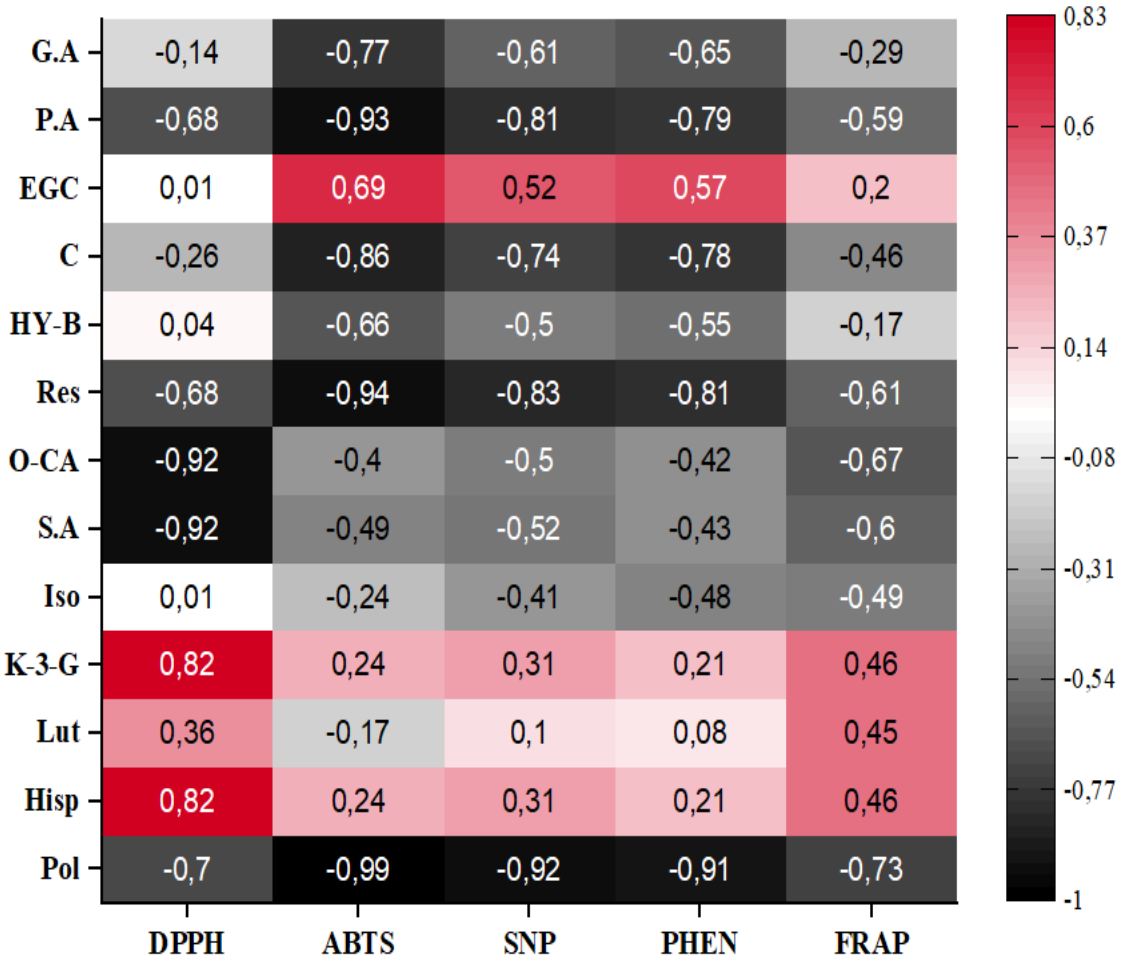


Figure 28: Correlation between the detected phenolic compounds and the antioxidant capacity of the extracts. O-CA: O-coumaric acid, GA: gallic acid, SA: salicylic acid, PA: protocatechuic acid, Res:resveratrol, Pol: polydatin, CAT: catechin, EGC: epigallocatechin, K-3-G: kamperol-3-glucoside, Hesp: hesperetin, HY-B: hydroxybenzaldehyde, Iso: Isoquercetin.

Correlation analysis between phenolic, flavonoid, and flavonol content and IC50 values was conducted (Figure 26). The IC50 values strongly and negatively correlated with TPC ($r = -0.98$, $p < 0.01$) and TFC ($r = -0.94$, $p < 0.01$), and weakly with TF-OL ($r = -0.23$, $p < 0.01$), which was confirmed by Zhang et al. (2011) and Ferreyra et al. (2019). Stilbenes: resveratrol and polydatin were found to demonstrate the highest correlation coefficient (Figure

28), protocatechuic acid, catechin, and gallic acid also exhibited high correlation, revealing that the main cause of ABTS scavenging is these chemicals. Yadav et al. (2009) al also mentioned the antioxidant capacity of grape gallic acid, catechin, resveratrol, and epicatechin.

1.4.3. 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 (Derradji-Benmeziane *et al*., 2014; Christodoulou *et al*., 2022). The study of the reduction capacity of vine cane extracts revealed a similar pattern, with FR having the highest activity when compared to 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, RG, and GR, while at 200 µg/mL The PIs of the extracts are ranked as 74%, 61%, 65%, and 54%, respectively (Figure 29). With regard to A0.5, those of the extracts and standards BHA, FR, CR, BHT, RG, and GR correspond to 8.41 ± 0.67 µg/mL, 40.12 ± 3.41 µg/mL, 41.33 ± 0.77 µg/mL, 50.1 ± 1.53 µg/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 A0.5 values.

The results of the reducing power assay 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. Additionally, grape cane extracts from Southern Italy's typical cultivars were extracted at various pH values. The IC50 ranged from 3.649 to 39.482 µg (AAE)/mg DE, with the best reduction of ferric ions at pH 13.00 (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 (Figure 26) in the current analysis ($r = -0.707$ and -0.914, respectively, $p < 0.01$). in addition, the highest correlation coefficient was detected with polydatin and resveratrol (Figure 28). Other studies have also demonstrated that the reducing

power of grape cane extracts is well correlated with their phenolic contents (Balík *et al*., 2008; Zhang *et al*., 2011). The high correlation represented between the TPC, TFC and the antioxidant tests confirmed that these molecules are the main contribute 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 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).

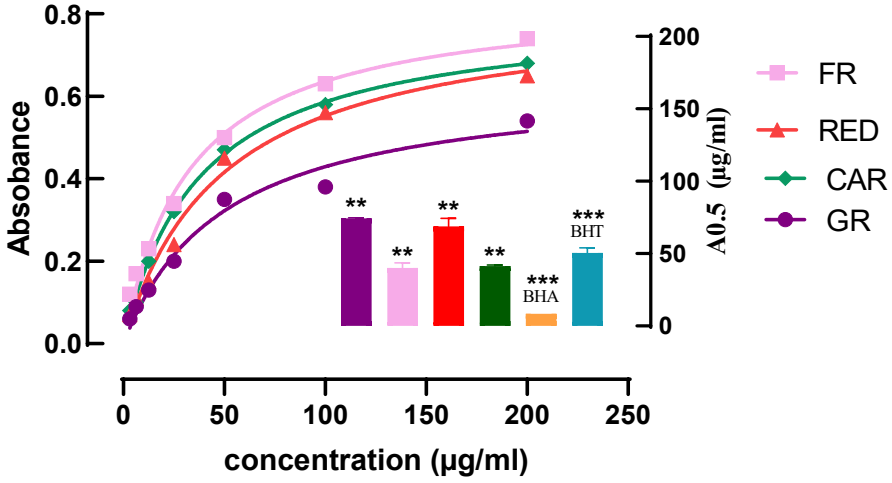


Figure 29: Inhibitory activity and A0.5 values of the studied extracts and standard determined using reducing power inhibitory assay. (**) for comparison inter-compounds and (***) with standard. FR: Fragola Nera, RED: Red Glob, CAR: Cardinale, GR: Gros Noir. A0.5: the concentration at the 0.50 absorption. FR: BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene. values represent the means ± SEM of three measures.

1.4.4. SNP activity

SNP is another accurate colorimetric method to evaluate the antioxidant capacity of different extracts based on Ag+ reduction to highly colored spherical nanoparticles SNPs by phenolic compounds with maximum absorbance at 423 nm (Özyürek *et al*., 2012). The antioxidant activity and the results of the SNP assay revealed a similar trend with ABTS and phenanthroline essays. All samples showed reducing power capacity in a concentration-dependent manner; both of FR and CR appeared to have the highest efficiency with a percentage

inhibition of 100% and 97%, respectively, followed by 93% for RG and 92% for GR at 400 µg/mL. The values of the A0.5 of both standards and extracts increased in the order of FR< CR< BHA< RG< GR <BHT and was 45.02±1.33, 66.94±0.19, 73.47±0.88, 85.33±7.51, 100±9.92 and >200 µg/mL (Figure 30). In the same context, both FR and CR were found to be more potent than BHA and BHT, whereas RG and GR were found to be better only than BHT. A high correlation was observed between SNP and total phenolics and flavonoid content with correlation coefficients of $r = -0.714$ for polyphenols and $r = -0.907$ for flavonoids ($p < 0.01$) (Figure 26). Polydatin and resveratrol showed the highest correlation coefficients among the identified polyphenols, followed by protocatechuic acid and catechin (Figure 28).

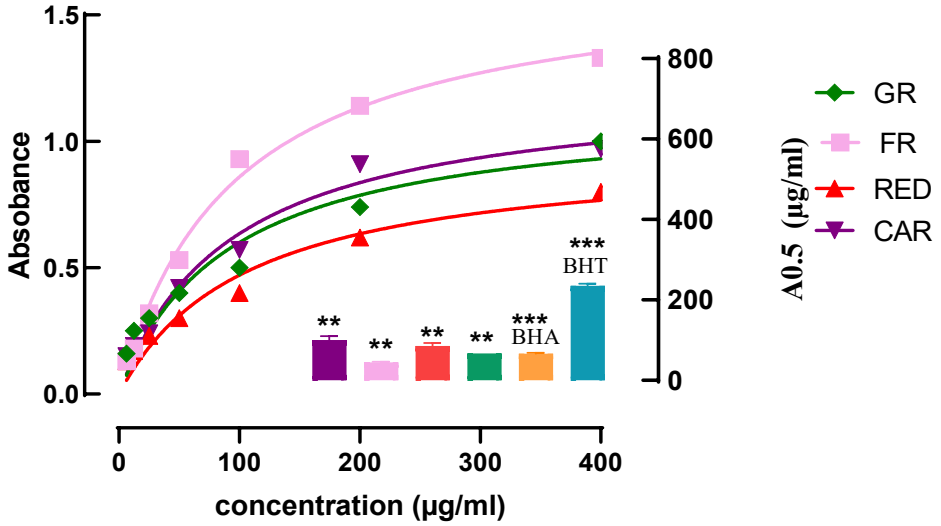


Figure 30: Inhibitory activity and A0.5 values of the different extracts and standards determined using SNP assay. (**) for comparison inter-compounds and (***) with standard. FR: Fragola Nera, RED: Red Glob, CAR: Cardinale, GR: Gros Noir. A0.5: the concentration at the 0.50 absorption. BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene. values represent the means ± SEM of three measures.

1.4.5. Phenanthroline Activity

Regarding the phenanthroline assay it is knowing that this method specifically based on the reaction between ferrous iron (Fe+2) from FeSO4 and 1,10-phenanthroline, ferrous iron produces a reddish-orange tri-phenanthroline complex which can be quantified spectrophotometrically at 510 nm. 1,10-Phenanthroline competes with plant extracts and standards for Fe2+ in aqueous solution (Debanjan *et al.*, 2016). The different extracts showed a strong ability to reduce iron with an inhibition percentage of 100 % at a concentration of 100 µg/mL for CR, RG, and GR and at 50 µg/mL for FR. The ferrous iron chelating activity showed

a same tendency in IC50 values which ranked as follow: FR 10.21±0.53, CA 14.78±0.33, RG 17,31±0,15, and GR 2 1.41±1,16 µg/mL. All the extracts were found to be less potent than BHA and BHT (1.49 ± 0.08 µg/mL, 2.20± 0.04 µg/mL) (Figure 31). The antioxidant activity in phenanthroline assay is well correlated with phenolic and flavonoid content (r = −0.944 and −0.895, respectively, p < 0.01) (Figure 26). Additionally, high correlation with polydatin, resveratrol, protocatechuic acid, and catechin was also detected (Figure 28).

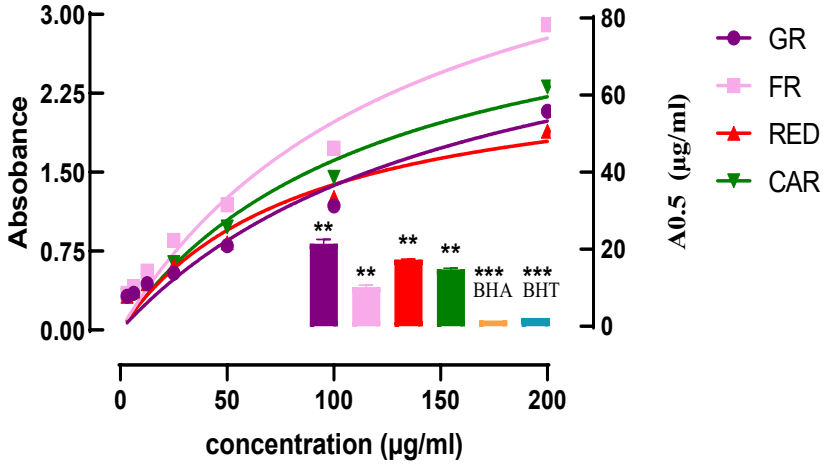


Figure 31: Inhibitory activity and A0.5 values of the studied extracts and standards determined using phenanthroline inhibitory assay. (**) for comparison inter-compounds and (***) with standard. FR: Fragola Nera, RED: Red Glob, CAR: Cardinale, GR: Gros Noir. A0.5: the concentration at the 0.50 absorption. BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene. values represent the means ± SEM of three measures.

In terms of similarity in the mode of action of the two last methods the results of phenanthroline assay appeared to be lower in comparison with the results of the silver nanoparticle assay. However, no reports were found in the literature on the use of these two methods to evaluate the antioxidant activity of grape cane extracts. It is observed that, extracts with an abundance of polyphenols and flavonoids exert the significant antioxidant activity. Therefore, it can be assumed that the plant's phenolic content may be the 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, some detected phenolic compounds, and the AC activity of the different extracts with the different methods. Polydatin and resveratrol showed the highest correlation coefficients among the identified polyphenols, followed by

catechin, protocatechuic acid, and gallic acid. all these molecules were identified in other studies as potent antioxidants.

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 *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).

1.5. Enzymatic Activity

The inhibition of different enzymes was studied and the results were

1.5.1. Anti-tyrosinase Activity

Tyrosinase is an enzyme that plays a crucial role in the biosynthesis of melanin, the pigment responsible for the color of skin, eyes and hair and the protection of skin from UV radiation. While tyrosinase is essential for normal pigmentation, its dysregulation or overactivity can lead to skin disorders, such as hyperpigmentation, wrinkles and even skin cancer (Zolghadri *et al*., 2019; Khodja *et al*., 2023). As such, studying the inhibition of tyrosinase is important in the cosmetic field, as it can be targeted for therapeutic purposes to maintain skin whiteness especially for inhibitors extracted from natural sources due to their availability and low toxicity (Muddathir *et al*., 2016). Using Cojik Acid as the standard reference, the four grape cane extracts' inhibitory potential against mushroom tyrosinase was assessed and represented as inhibition percentages (Figure 32).

GR and CR showed comparable inhibitory effects (36%), the RG variant showed noticeably higher activity (39.11%), while the FR variant displayed the highest inhibition of 46.44%, which seemed to be almost close to the standard (49.46%). The correlation coefficient between the detected phenolics and the inhibitory effect of the different extracts was analyzed (Figure 35) and revealed a high positive correlation with Resveratrol, Catechin, Galic acid, Protocatechuic acid, and Hydroxybenzaldehyde and a lower positive correlation with Luteolin. Another high correlation was also detected, but on the negative side with Epigallocatechin, suggesting that these molecules are responsible of enzyme inhibition.

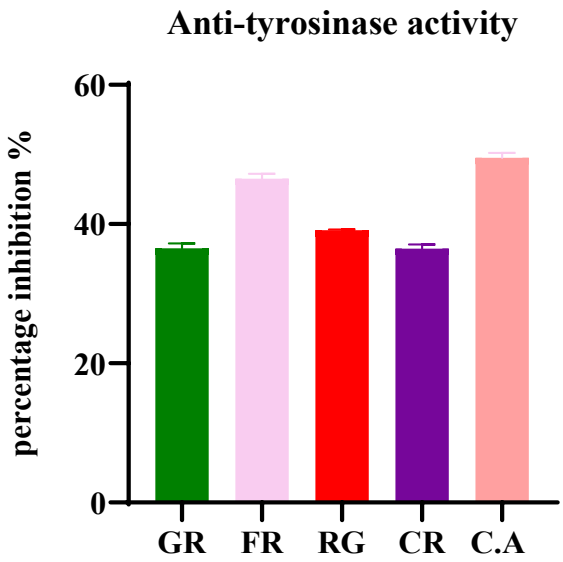


Figure 32: Inhibitory activity of turosinase by the studied extracts and standard. GR: Gros Noir, FR: Fragola Nera, RG: Red Glob, CR, Cardinale, C.A: Cojik Acid. IC50: IC50: the concentration at the fifty of inhibition. values represent the means ± SEM of three measures.

Anna Malinowska et al. (2020) also studied the effect of five grape cane extracts and two pure stilbenes, E-resveratrol and E-ε-viniferin, on inhibition of tyrosinase and found that the latest ones exhibited high inhibition percentages of 75% and 76% respectively, whereas the cane extracts showed different capacities to inhibit this enzyme with different percentages that varied from low effect (30,4%) to high effect (62,5%). Additionally, according to Serra et al. (2023) elastase and tyrosinase enzyme overexpression is linked to skin aging, the discovery of anti-elastase and anti-tyrosinase properties in grape stem extracts enhance the possibility of their use as anti-aging substances. The study of Costa-Pérez et al. (2023) revealed that Syrah stem extracts were the most effective at inhibiting tyrosinase and elastase activity among the studied extracts, suggesting their potential use in the cosmetic sector. Additionally, the potential of the substance to prevent damage to skin cells caused by free radicals, minimize hyperpigmentation, and prevent wrinkles is indicated by the association between its tyrosinase inhibitory action and radical scavenging activity (Rauniyar *et al*., 2007).

1.5.2. 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 (Oyedemi *et al*., 2017; Oluwagunwa *et al*., 2021).

This study investigated the effects of the cane ethanolic extracts on α-Amylase inhibition. As demonstrated by the results of different extracts (Figure 33), there is a dose-dependent effect, increasing inhibitory efficacy with the increase of concentration. CR, FR, GR, RG, and acarbose demonstrated an inhibition of 82.84, 75.49, 71.10, 66.10, and 20,05%, respectively at a concentration of 200 µg/mL. 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 CR, RG, GR, FR, and Acarbose, respectively.

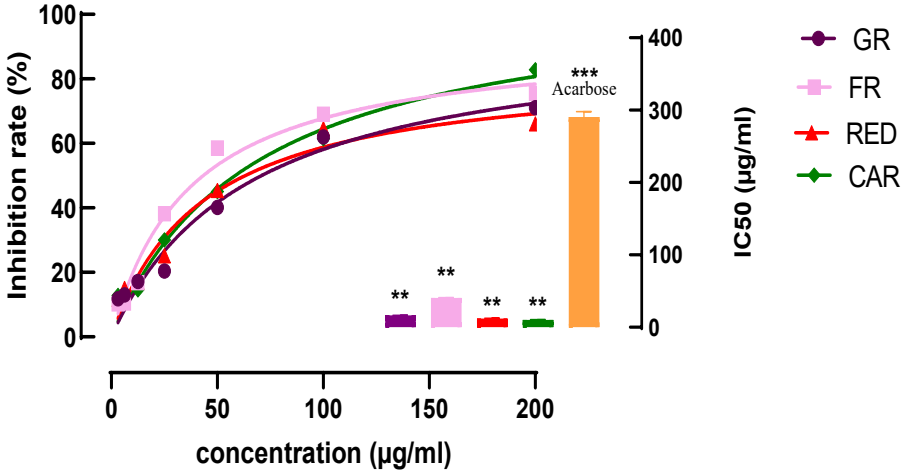


Figure 33: Inhibitory activity and IC50 values of the studied extracts and standard determined using amylase inhibitory assay. (**) for comparison inter-compounds and (***) with standard. GR: Gros Noir, FR: Fragola Nera, RED: Red Glob, CAR, Cardinale, and the standard acarbose. IC50: IC50: the concentration at the fifty of inhibition. values represent the means ± SEM of three measures.

The present results are consistent with those of Ben Khadher et al. (2022), who also observed the inhibitory effects of a macerated ethanolic extract from grape stem plants. This extract demonstrated a high level of inhibition activity against amylase, as indicated by a low IC50 value of 13.4 µg/mL. Additionally, Moreira et al. (2018) evaluated the inhibition of α-amylase by natural stem extracts from two distinct varieties; their results are greater than ours, with an IC50 of 60.37 ± 5.55 and 73.28 ± 6.77 µg/mL for the MAE method. In addition, some *in vivo* and *in vitro* investigations have suggested that phenolic compounds from grape stems

may also have antidiabetic effect 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).

High correlation was detected with polydatin, resveratrol, protocatechuic acid, gallic acid, and catechin (Figure 35). In the same case, Cisneros-Yupanqui 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.

1.5.3. Lipase inhibitory activity

The ability of the cane extracts to inhibit lipase was established. The results revealed that the CR extract was the most effective, with an IC50 value of 12.52±0.07 µg/mL. A non-significant lower inhibition was observed with the RG extract (15.03±3.60 µg/mL), whereas significant lower activity was recorded by the FR and GR extracts with an IC50 values of 46.60±0.55 µg/mL and 54.37±7.03 µg/mL, respectively (Figure 34). All the extracts were found to be less effective than the standard Orlistat (4.98±0.70 µg/mL). high correlation coefficient was detected between the inhibitory activity and Isoquercetin (Figure 35).

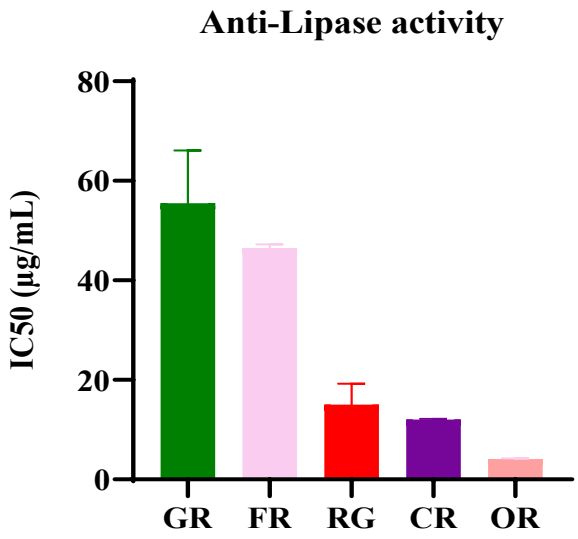


Figure 34: IC50 values of the lipase inhibitory activity by the cane extracts. GR: Gros Noir, FR: Fragola Nera, RG: Red Glob, CR, Cardinale, and standard orlistat (OR). IC50: the concentration at the fifty of inhibition. values represent the means ± SEM of three measures.

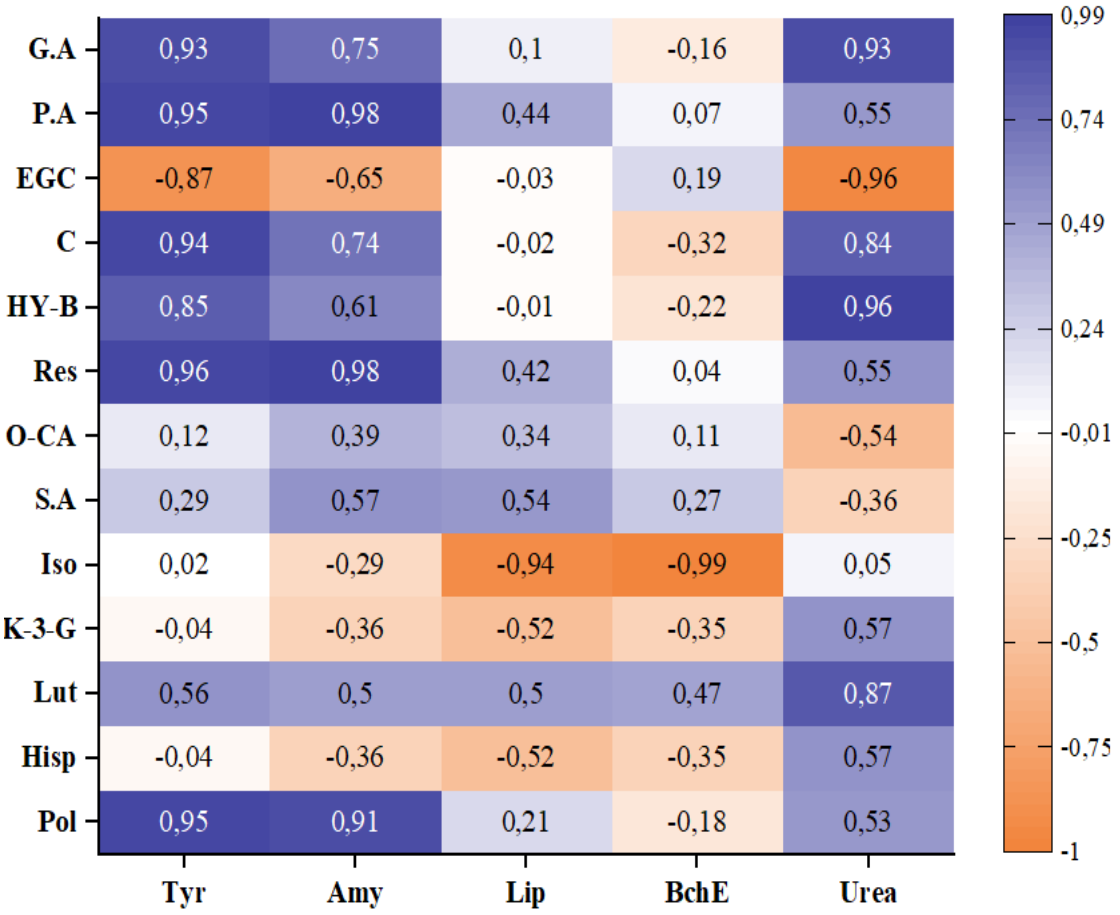


Figure 35: Correlation between enzymatic activities and the detected phenolic compounds. O-CA: O-coumaric acid, GA: gallic acid, SA: salicylic acid, PA: protocatechuic acid, Res: resveratrol, Pol: polydatin, CAT: catechin, EGC: epigallocatechin, K-3-G: kamperol-3-glucoside, Hesp: hesperetin, HY-B: hydroxybenzaldehyde, Iso: Isoquercetin. Tyr: tyrosinase, Amy: amylase, Lip: Lipase, BchE: butyrylcholinesterase, Urea: urease.

Obesity is a lipid metabolic disorders that remains a global problem, accounting for the fifth leading cause of mortality worldwide. It also causes diabetes, cardiovascular disease, musculoskeletal issues, and certain types of cancer. Pancreatic lipase's digestion and absorption of dietary fats, a key source of excessive calorie intake, can be targeted for development of anti-obesity drugs (Lunagariya *et al*., 2014). Natural lipase inhibitors contained in our food products are consumed over extended periods of time as a part of our normal diet, and they may play a significant role in reducing dietary lipid bio-accessibility and, therefore, reducing obesity (Tormási *et al*., 2023). The inhibition of lipase by grape cane is not mentioned in the literature. However, the use of grape extract and other fruits to inhibit lipase in the study of Sudha et al. (2023) was mentioned. Additionally, GSE was found to significantly inhibit pancreatic lipase, lipoprotein lipase and hormone sensitive lipase, key responsible for the fat digestion and

metabolism in human body (Yu and Ahmedna, 2013). This inhibitor activity suggests that GSE might be useful as a treatment to limit dietary fat absorption and the accumulation of fat in adipose tissue.

1.5.4. Butyrylcholinesterase inhibitory activity

Alzheimer's disease (AD) is caused by a lack of the neurotransmitter chemical acetylcholine in the synaptic clefts. This damage affects the functioning of the cholinergic system, which is essential for memory development, learning, and other cognitive processes broadly. Acetylcholine deficiency is caused by diminished production in the presynaptic knobs, increased cholinesterase breakdown at the gaps, or nerve receptor insensitivity to the neurotransmitter. Consequently, one of the approaches used to ameliorate AD involves administration of cholinesterase inhibitors (AChE) such as donepezil and galantamine, among others (Ngai *et al*., 2022).

The capacity of the studied extracts to inhibit butyryl enzyme was also established (Figure 36). The highest activity was recorded in the CR extract with an IC50 value of 16±1.03 µg/mL. the other extracts were showed lower efficacity and ordered as follow: RG 20.13±1.44 µg/mL, FR 26.78±0.67 µg/mL, GR 38.59±2.97 µg/mL. the standard Galantamine had the lowest IC50 (6.27±1.15 µg/mL). high correlation coefficient with isoquercitin was recorded.

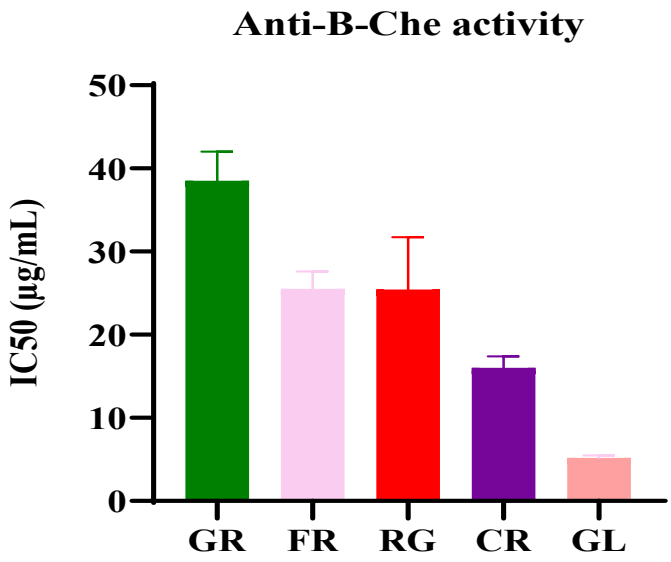


Figure 36: IC50 values of the butyrylcholinesterase inhibition activity. GR: Gros Noir, FR: Fragola Nera, RG: Red Glob, CR: Cardinale, GL: galantamine, B-Che: butyrylcholinesterase. IC50: the concentration at the fifty of inhibition. values represent the means ± SEM of three measures.

Moreira et al. (2018) studied the capacity of the Portugal vine shoot to inhibit acetylcholinesterase and found that all the extracts demonstrated an inhibitory activity with priority to the MAE extracts. Additionally, the ethyl acetate extract of grape stem obtained by maceration demonstrated a high potential regarding several biological activities, among them the AChE activity with an IC50 value of 14.1 µg/mL in the study of Ben Khadher et al. (2022).

1.5.5. Anti Urease Activity

1.5.5.1. *In vitro*

The multi-subunit nickel-containing enzyme urease (also known as urea amidohydrolase; EC 3.5.1.5) is found in plants, fungi, and bacteria, where and is catalysis the hydrolysis of urea to ammonia, it is also playing 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 (Rauf *et al*., 2020; Al-Rooqi *et al*., 2023; Kamah *et al*., 2024). 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 in except of RG 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, RG, CR, and thiourea, respectively (Figure 36). The standard inhibitors 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 CR variety exhibited the best urease inhibitory activity regarding the other extracts with an IC50 value of 25.88 ± 2.26 µg/mL. Hydroxybenzaldehyde, epigallocatechin, gallic acid, luteolin, catechin, were found to highly correlated with the anti-urease activity (Figure 37).

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

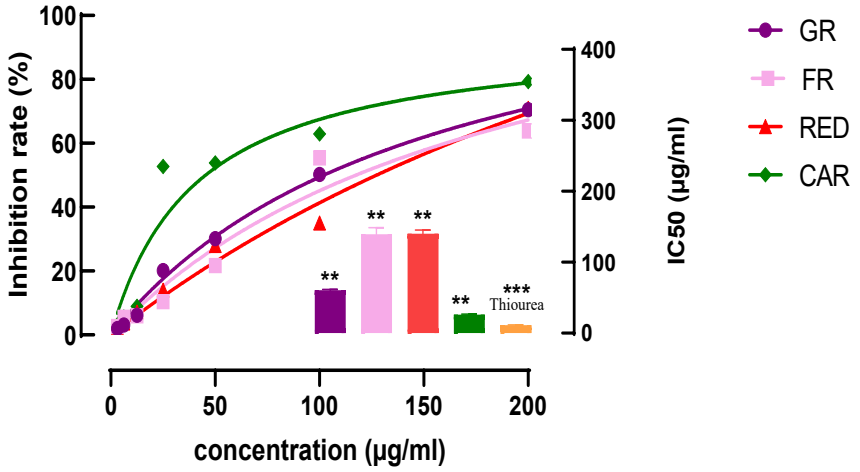


Figure 37: Inhibitory activity and IC50 values of the studied extracts and standard determined using urease inhibitory assay. (**) for comparison inter-compounds and (***) with standard. GR: Gros Noir, FR: Fragola Nera, RED: Red Glob, CAR: Cardinale. IC50: the concentration at the fifty of inhibition. values represent the means ± SEM of three measures

1.5.5.2. Molecular docking results

Molecular docking approach is one of the commonly used computational strategies in drug identification and development. Therefore, in order to support the results obtained in this study experimentally, molecular docking analysis were performed. It was used to search among the nine major polyphenols identified those that bind more strongly than the reference inhibitor, thiourea, to our target urease and to elucidate the mechanism of interaction of these products. As can be seen from the docking results, it was observed that all the docked products showed lower interaction energies than the standard. The docking scores are provided in Table 3.

The comparison of docking scores, interaction energy, revealed that among the studied compounds, gallic acid was the most potent inhibitor and was found to confine in a favorable position within binding site of target enzyme (Figure 38). The gallic acid demonstrated the highest binding affinity followed by o-coumaric acid and salicylic acid, it was attached to the enzyme with a binding energy value of -28.8802 kJ/mol. Docking results show that those three

identified products have similar orientations in the binding site of urease enzyme because of their similarity on atomic composition and chemical property.

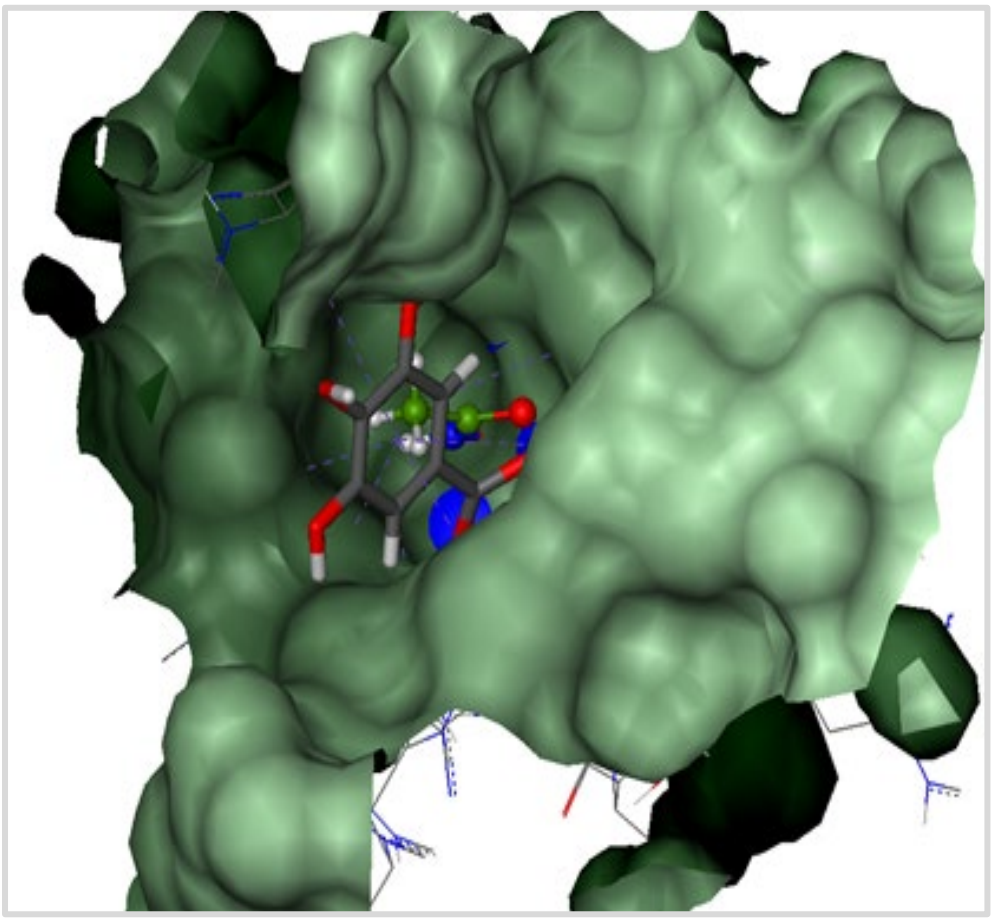


Figure 38: Positioning of gallic acid into the whole urease active pocket.

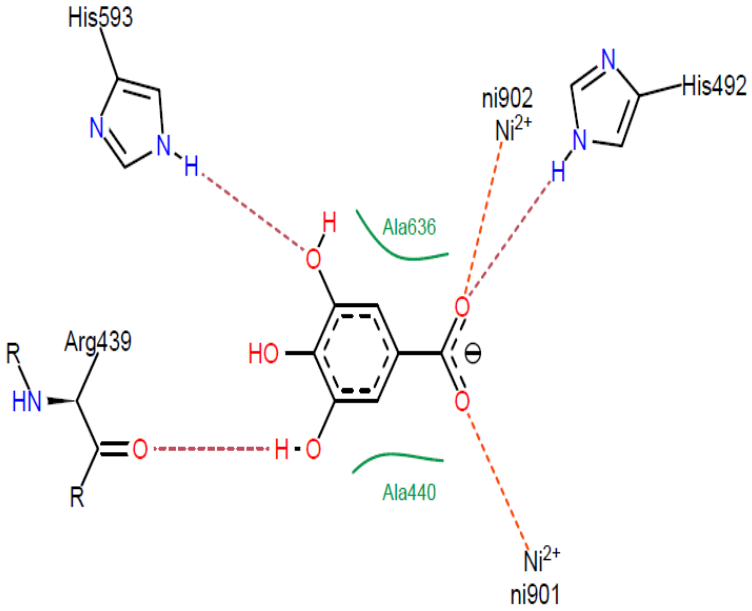
The two-dimensional graphical representations for molecular docking obtained from the enzyme-inhibitor interactions of most three potent inhibitors are given in Figure 39. Compounds gallic acid, o-coumaric acid and salicylic acid chelate the binuclear nickel metallo -center that catalyzes the decomposition of urea to produce ammonia through the carboxylic acid group (Carter *et al*., 2009). They form several hydrogen bridges with the key residues of the binding site, such as His492, Asp494, His593 and Asp633 by their hydroxyl groups (Balasubramanian and Ponnuraj, 2008). Besides, their phenolic rings insert into a hydrophobic pocket composed Ala440, His492, His519, His593, Ala636 and Met637 to stabilize the compounds in the active site of urease.

Interestingly, the present study revealed that gallic acid, o-coumaric acid and salicylic acid act by direct binding to Ni^{2+} in the active site of urease which is similar to that of the binding of phosphodiamidates, fuoride, and hydroxyurea, category of urease inhibitors (Svane

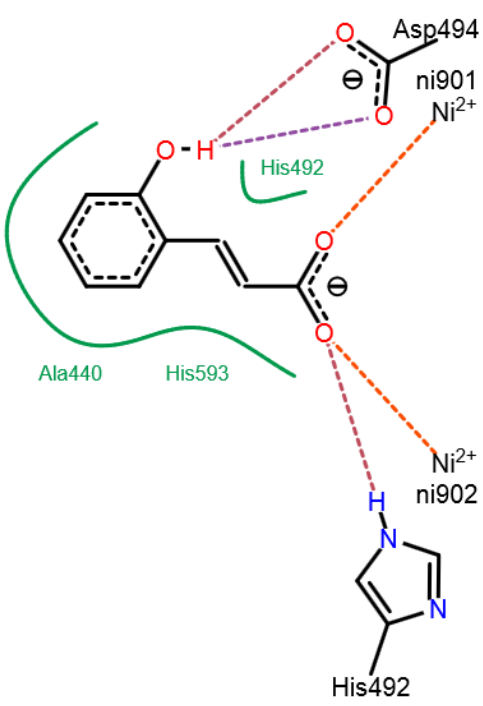
et al., 2020). This effect suggests that these phytochemicals can have a similar anti-urease propriety.

1.5.5.3. ADME/Tox analysis

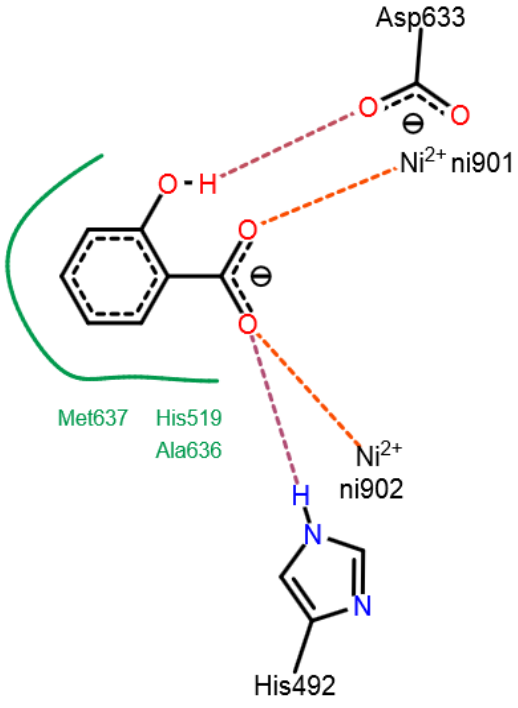
To further evaluate *in silico* the ADME profile of the nine major products identified in the extract of the *Vitis vinifera* s, Swiss ADME server was used. Brain blood barrier (BBB) permeation was predicted for o-coumaric acid, salicylic acid and resveratrol, but not for thiourea and the others. As indicated in Table 4, all the products were predicted to have substantial gastrointestinal absorption and complied with Lipinski's rule of five, indicating their druglike characteristics **(**Lipinski *et al*., 2001; Lagorce *et al*., 2017**)**, only isoquercetin had low gastrointestinal absorption. Swiss ADME also predicted that catechin, epigallocatechin, o-coumaric acid, salicylic acid, isoquercetin and polydatin should not inhibit cytochrome P450 isoforms like that of thiourea. They displayed interesting properties for effective medication development, underscoring their potential as promising drug candidates that deserve further exploration and experimental validation. Furthermore, ProTox-II prediction revealed low toxicity for all identified phytochemicals, especially catechin, epigallocatechin, luteolin, o-coumaric acid and Isoquercetin, compared to the reference inhibitor (Figure 40), which guarantees their use *in vivo.*



(a)



b)



(c)

Figure 39: Prediction interacting mode of the effective compounds in urease active site after enzyme-ligand docking using FlexX. gallic acid (a), o-coumaric acid (b) and salicylic acid (c) Dotted lines indicate hydrogen binding, green lines show hydrophobic interactions.

Table 3: Docking scores of the tested products.

Product with PubChem CID	Total score	Contribution of the matched interacting groups	Lipophilic contact energy	Energy of lipo-hydrophilic contacts	Energy of steric obstruction	Immobilization energy of the rotatable bonds	Number of matches
Thiourea (CID_2723790)	**-12.4830**	**-15.4073**	**-1.6775**	**-2.0523**	**1.2540**	**0.0000**	**4**
Catechin (CID_9064)	-18.9867	-22.8457	-6.2871	-7.1246	3.4707	8.4000	11
Epigallocatechin (CID_72277)	-22.7214	-29.9542	-6.7403	-8.2296	7.0026	9.8000	15
Gallic acid (CID_370)	**-28.8802**	**-34.1941**	**-3.4883**	**-5.4050**	**4.6074**	**4.2000**	**12**
Luteoline (CID_5280445)	-18.5595	-21.1333	-7.9120	-6.1595	5.6453	5.6000	11
O-coumaric acid (CID_637540)	-26.5100	-29.3227	-4.3390	-4.2330	4.5847	1.4000	8
Resveratrol (CID_445154)	-17.1953	-19.9601	-5.1667	-4.9565	3.2881	4.2000	14
salicylic acid (CID_338)	-24.6899	-27.8086	-4.1012	-4.9234	5.3435	1.4000	11
Isoquercetin (CID_5280804)	-22.7213	-34.6594	-8.7234	-10.5519	10.4134	15.4000	16
Polydatin (CID_5281718)	-17.2791	-26.8194	-6.1772	-7.5508	5.2684	12.6000	12

Table 4: In silico ADME profiles of the identified products.

Compounds	BBB penetration	GI absorption	CYP inhibitor	Lipinski's rule of 5
Thiourea	No	High	None	Yes
Catechin	No	High	None	Yes
Epigallocatechin	No	High	None	Yes; 1 violation: NHorOH>5
Gallic acid	No	High	CYP3A4 inhibitor	Yes
Luteolin	No	High	CYP1A2 inhibitor CYP2D6 inhibitor CYP3A4 inhibitor	Yes
O-coumaric acid	Yes	High	None	Yes
Resveratrol	Yes	High	CYP1A2 inhibitor CYP2C9 inhibitor CYP3A4 inhibitor	Yes
Salicylic acid	Yes	High	None	Yes
Isoquercetin	No	Low	None	No; 2 violations: NorO>10, NHorOH>5
Polydatin	No	High	None	Yes; 1 violation: NHorOH>5

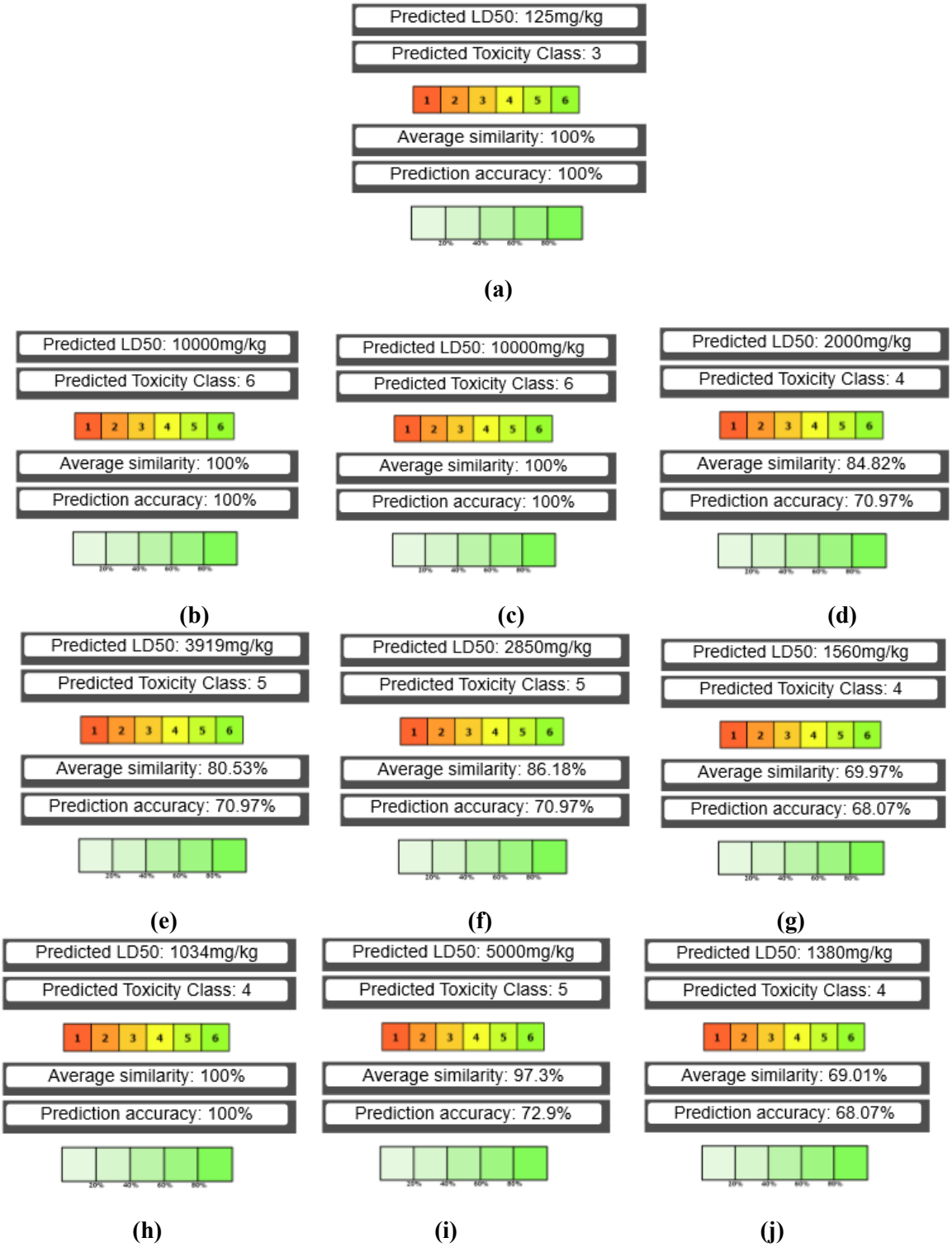


Figure 40: Toxicity, predicted by Protox-II, of identified polyphenols in the cane extracts. The products include thiourea (a), catechin (b), epigallocatechin (c), gallic acid (d), luteolin (e), o-coumaric acid (f), resveratrol (g), salicylic acid (h), isoquercetin (i) and polydatin (j).

1.6. 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 CR, GR, and RG were given the highest SPF index with no significant differences between the samples whereas FR showed the lowest SPF value (Figure 41). It can be suggested that all extracts have a photoprotective effect with high SPF value according to the Recommendation of the Commission of the European Communities (2006) (Table 5). However, the use of grapevine stem extracts as a raw material in cosmetics in order to fight skin damages 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) 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 as collagen breakdown and pigmentation induced by UVB radiation. From that, Grapes and their derivatives provide potential photoprotective properties against UV radiation, making them appropriate for herbal cosmetic compositions (Soto *et al*., 2015).

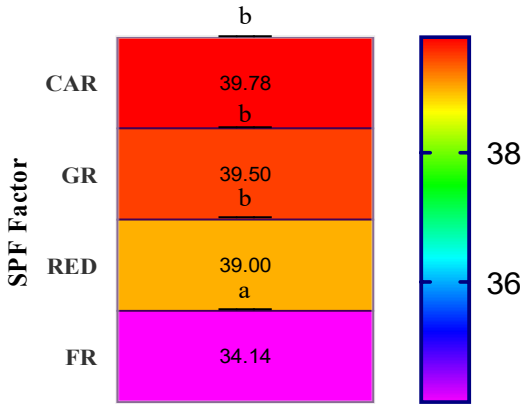


Figure 41: SPF values of cane extracts. the values in identical columns with various superscripts (a, b) differ significantly ($p < 0.05$). FR: fregola Nera, RED: Red Glob, GR: Gros Noir, CAR: Cardinale. SPF: Sun protection factor.

Table 5: 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 ≤

1.7. 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 into loss of both configuration and functionality (Dharmadeva *et al*., 2018). In diseases like rheumatoid arthritis, cancer, and diabetes, which are inflammatory disorders, denaturation of protein induces the generation of autoantigens. Therefore, potential anti-inflammatory medication prospects may come from medicinal plant extracts that prevent protein denaturation and maintain cell membrane 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 of the concentration (Table 6). The four extracts exhibited more than 40% inhibition of BSA denaturation at 1000 µg/ml whereas both of RG 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 diclofenac 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 dose of lipopolysaccharides. using 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 64.5% and the lowest IC50 = 26.6 µg/mL (Ben Khadher *et al*., 2022).

Table 6: Percentage inhibition of BSA by both cane extracts and standard.

C (µg/ml)	GR (%)	FR (%)	RG (%)	CR (%)	D.S (%)
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

C: concentration, GR: Gros Noir, FR: Fragola Nera, RG: Red glob, CR: Cardinale, D.S: diclofenac Sodium. The values represent the means ± SEM of three measures.

1.8. Antimicrobial activity

Recently, the wide use of antibiotics leads to an increase in the drug-resistance bacteria which is considered a global health problem (Leal *et al*., 2020; Jesus *et al*., 2022). One of these antibiotics are beta lactams which have been widely used to treat infections caused by gram negative bacteria since 1980 (Saeide *et al*., 2014). β-lactam resistance generated through the production of bacterial β-lactamase which inhibit the amide bond of their β-lactam ring (Al-Hayanni and El-Shora, 2020). It is well noticed that *Esherichia coli* are important causes of infections, especially urinary tract infections and considered as the main bacteria produced extended spectrum beta-lactamase (ESBL) (Saeide *et al*., 2014). from that, the discovery of new natural antimicrobial compounds derivates from plants is becoming crucial to limit and diminish the spread of drug resistance.

In the same context, the studied extracts were tested for their capacity to inhibit microbial agents. The results were represented in Table 7. The majority of the extracts were found to have good inhibition activity. The FR extract was found to be more effective against *E. coli* ATCC 25922 (15 ± 0.14 mm), *E. coli* 04 (11 ± 0.76 mm) and *C. albicans* ATCC (1023120 ± 1.23 mm), the RG inhibited *E. coli* 01 (15 ± 0.09 mm) and *E. coli* 03 (11 ± 0.65 mm) with the highest inhibition diameter whereas the GR extract trended in inhibition of *E. coli* 02 and *C. albicans* 01 (16±2.3mm and 15±0.9 mm , respectively). Furthermore, the CR extracts also showed the highest inhibition of *C. albicans* ATCC 10231 (20 ± 2 mm) and *C. albicans* 01 (15 ± 1 mm). The minimal inhibition concentration (MIC) was found to be 0.6 mg/mL for

all extracts against all the bacterial and fungal strains. The capacity of grape canes to inhibit microbial strains was poorly studied in the literature, however, Pop et al. (2022) demonstrated the antimicrobial activity of grape canes, the red and wight grape pomace and their combination and found good inhibition diameter against all the studied strains with highest inhibition in the combination. Moreira et al. (2018) also tested the efficacity of two Portugal vine shoot against microbial agents and found good inhibition of different bacteria and yeasts. In addition, according to Bogdan et al. (2020) the phenolic composition of wine and *V. vinifera* extracts such as: flavanols, gallic acid, hydroxycinnamic acid, trans-resveratrol, and epicatechin is the main contribute to the antimicrobial activity exactly through the inhibition of extracellular enzymes or the complexation of metal ions from bacterial media by phenolic acids. To the best of our knowledge, no study was reported the inhibition of bacterial strains producing beta lactamase by grape cane extracts.

Table 7: Antimicrobial activity of the cane extracts.

Extracts	**FR**		**RG**		**GR**		**CR**	
Strains	**IZ (mm)**	**MIC (mg/mL)**	**IZ (mm)**	**MIC (mg/mL)**	**IZ (mm)**	**MIC (mg/mL)**	**IZ (mm)**	**MIC (mg/mL)**
***E. coli* ATCC25922**	15±0.14**	0.6	10±0.29*	0.6	12±2.2*	0.6	10±0.5*	0.6
***E. coli* 01**	10±0.4*	0.6	15±0.09**	0.6	10±0.6*	0.6	/	/
***E. coli* 02**	/	/	10±0.26*	0.6	16±2.3**	0.6	11±0.6*	0.6
***E. coli* 03**	/	/	11±0.65*	0.6	10±1.2*	0.6	/	/
***E. coli* 04**	11±0.76*	0.6	10±1.76*	0.6	9±0.16*	0.6	10±1.9*	0.6
***C. albicans* ATCC 10231**	20±1.23**	0.6	11±0.96*	0.6	14±0.8*	0.6	20±2**	0.6
***C. albicans* 01**	12±1.26*	0.6	11±0.43*	0.6	15±0.9**	0.6	15±1**	0.6
Percentage inhibition (%) of growth								
	C (mg/mL)	**IP (%)**	**C (mg/mL)**	**IP (%)**	**C (mg/mL)**	**IP (%)**	**C (mg/mL)**	**IP (%)**
***A. niger* 01**	10	20±1.79	-	-	5	5±0.09	5	10±1.9

IZ: inhibition zone, MIC: minimal inhibition concentration, GR: Gros Noir, FR: Fregola Nera, RG: Red Glob, CR: Cardinale, C: concentration, IP: inhibition percentage. /: resistant, *: sensitive (09 < Ø < 14 mm), **: very sensitive (15 < Ø < 19 mm), -: no activity.

2. Seed wastes

2.1. Changes in the total polyphenol contents and their sub-groups during grape ripening

The content of total polyphenols (TP); and their four sub-groups; total flavonoid (TF), total flavonol (TF-Ol), total condensed tannins (TCT), and total hydrolysable tannin (TYT) in the seed extracts of three different varieties (Sabel: SB, Cardinale: CR, and GR: Gros Noir) at the three repining stages (Before veraison, V: veraison, and Repining: R), were determined by spectrophotometric assays as represented in Figure 42. Significant variation ($p < 0.05$) in the total phenolic compound content was observed between grape seeds (527.4 to 716.1 mg/g) across different ripening stages. All the extracts showed high concentrations of TPC, and each extract trended differently. SB trended at before veraison, RG at veraison and CR at repining stage. For the CR and SB varieties, the highest concentrations were detected before veraison (BV), decreasing then from 650,7 and 669,5 mg GAE/g to attain a concentration of 595.1 and 5527.4 mg GAE/g respectively, at veraison. From veraison to repining, a further increase was observed. In contrast, for the RG variety, the highest amount was detected around veraison (716,1 mg GAE/g) and lower concentrations were observed in both the BV and R stages (554.80 and 574,2 mg GAE/g) putting into consideration that the R stage remains more elevated concentration than at BV.

Variety is one of the main factors influencing grapes' phenolic content. Furthermore, factors such as maturity, environmental stress, agronomic techniques, geographic regions, irrigation, the presence of plant pathogens, extraction techniques, and solvents have an impact on grapes' phenolic content (Dudoit *et al*. 2020; Du *et al*. 2021). This difference based on variety is consistent with previous studies. Labri *et al*. (2020) used 80% methanol as an extraction solvent to find out the TPC content in Algerian grape seeds at ripening. The study revealed that the Red Globe variety had 398.01 mg GAE/g and the Valenci variety had 335.11 mg GAE/g. Elagamey et al. (2013) used the same extraction method to look at the total phenolic compounds in six Egyptian seeded grapes. The results demonstrated that the TPC levels were different between the varieties, with Black Rose, Red Globe, and Roumi Ahmer having the highest levels.

Regarding maturity, our results align with those of Dudoit et al. (2020), who found higher seed TPC values at the veraison stage. Ivanova et al. (2011) reported the highest TPC for some varieties at veraison and for others at physiological ripening. Benbouguerra et al.

(2021) also noted that the highest TPC content in three grape skin varieties occurred at the first stage, with subsequent stages showing a decrease. Studies by Kurt-Celebi et al. (2020) and Prakash et al. (2020) demonstrated an increasing trend in TPC from the unripe to the ripe stages of grape berries. These results confirm that grape maturity influences the TPC content of different varieties. The partial oxidation of phenolic compounds during the ripening phases may be responsible for the decrease in TPC content (Dudoit *et al*., 2020). Additionally, the continual transformation of phenolic compounds into other substances, the increase in fruit weight, and the reduction in bitterness by polyphenols, which serves to protect young fruits from animal consumption, can explain this decrease (Zhang *et al*., 2022).

The TFC content ranged from 24.5 to 40.7 mg QE/g, with the highest concentrations detected in the RG and CR varieties. The flavonoid content in the CR and SB varieties evolved similarly to the total polyphenols, showing the highest concentrations in the unripe stage, followed by a decrease at veraison, and then another increase until the ripe stage. In the RG variety, the flavonoid content followed the same pattern as the TPC, with the highest concentration detected at the veraison stage. Total flavonols also followed this trend, except in the CR variety, which showed a decrease from the unripe to the ripe stage.

Our results showed higher TFC values compared to those reported by Derradji-Benmeziane et al. (2014), who estimated the TFC content in five Algerian grape varieties. Kurt-Celebi et al. (2020) also observed a similar trend in TPC and TFC during maturity, noting an increase in concentrations from unripe to overly ripened berries.

Regarding Total flavonols content, they followed the same trend except of the CR variety, which showed a decrease from the unripe to the ripe stage. Fang et al. (2013) studied the evolution of total flavonol during grape berry development and observed two accumulation peaks; the first one appeared at about 20 days after full bloom, followed by a remarkable decrease until veraison where another accumulation peak was detected.

In the case of total condensed tannin (TCT), each variety behaved differently. RG showed an increase in TCT from unripe to veraison, followed by an obvious decrease until the ripe stage. Regarding the CR variety, the TCT demonstrated a decreasing trend from the first to the last stage, whereas SB variety followed the same trend as the TPC and TFC. In contrast, the highest concentration of the TYT was detected at the veraison stage in all cultivars. Benmeziane and Cadot (2019) analyzed the quantity of TCT in the seed and the skin of

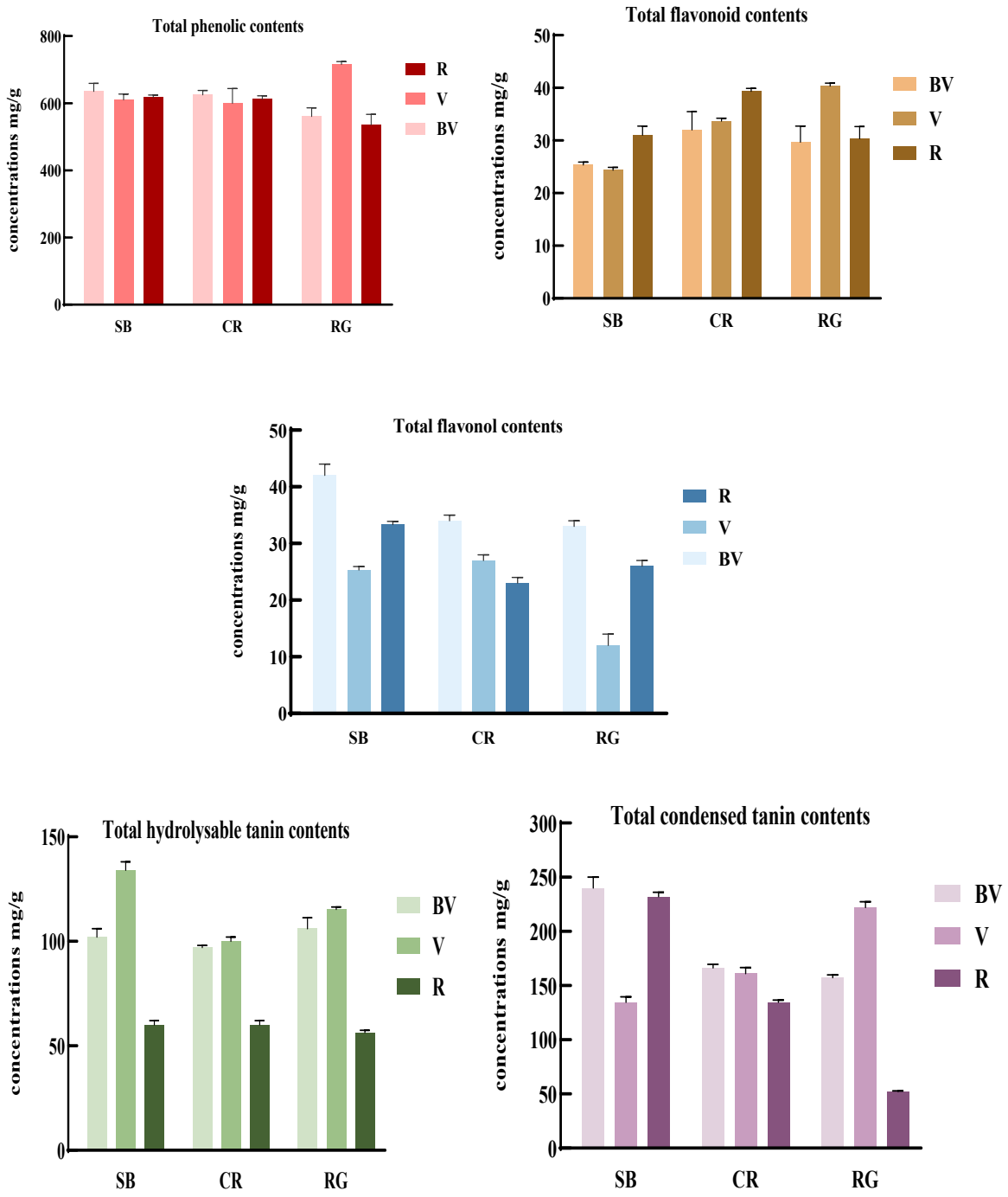


Figure 42: Total phenolic, flavonoid, flavonol, hydrolysable and condensed tannin contents of grape seed extracts at different repining stages. SB: Sabel, CR: Cardinal, RG: Red glob. BV: before veraison, V: veraison, R: repining. values represent the means ± SEM of three measures.

Algerian grape variety Cardinale at the repining phase, and found an amount ranging from 537.25 mg/g to 1332.90 mg/g of berries with higher contents in the seed.

Many factors can affect the quantity of the TCT in grapes and explain the difference between the results like: variety, climate conditions which differs from year to the other, number of seeds, berry size, in addition to the year of harvest (Benmeziane and Cadot, 2019). Regarding the changes of TCT during repining, the decrease of the tannin content can be explained by: vineyard practices include irrigation, vigor, vintage, altitude, and shading, climate conditions, continuous growth of the tannin polymers to produce longer chains, in addition to the association of tannin with other compounds in the berry during fruit ripening (Kennedy *et al*., 2007). Erna et al. 2019 confirmed the suggestion of the effect of climatic and maturity conditions by studying the development of seed tannin in two different years during maturation. The same results were found by Wang et al. (2023) where the amount of CT differed in the two studied vineyards and the seeds' soluble CT content revealed an overall decreasing pattern during repining.

2.2. Evolution of the individual phenolics detected by LCMS/MS

Flavan-3-ols are considered as the most reduced form of the flavonoids (Padilla-González *et al*. 2022), they are the most predominant class of phenolic compounds in grape berries, and generally distributed in skins and seeds with the predominant monomer being catechin and its derivatives, gallocatechin, epigallocatechin, epicatechin gallate and epigallocatechin gallate (Liu *et al*., 2010; Garrido and Borges, 2013; Pérez-Navarro *et al*., 2019).

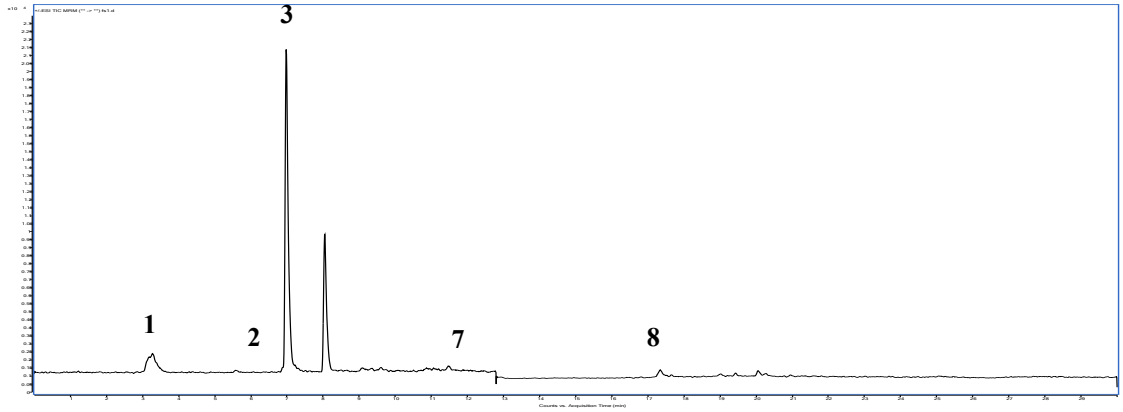
The results of the LCMS/MS revealed the presence of two flavanols: catechin and epigallocatechin. Catechin was the most abundant flavanol in all cultivars at different ripening stages. epigallocatechin was found in lower concentrations than catechin. The BV stage contained the highest concentration of catechin in all cultivars. The evolution of catechin in the SB and RG varieties showed a similar trend, shifting from the BV to the R stage. In the CR variety, catechin decreased from BV to V, then increased again to a value lower than BV. Epigallocatechin evolved differently in each cultivar: the SB cultivar showed a decreasing pattern from the first to the last stage, the RG variety had the same amounts in the BV and R stages with none at veraison, and the CR variety detected epigallocatechin only in the R phase (Table 8, Figure 42).

Table 8: Phenolic constituents (mg/kg) in the seed extracts during different repining stages as estimated by LC-MS/MS.

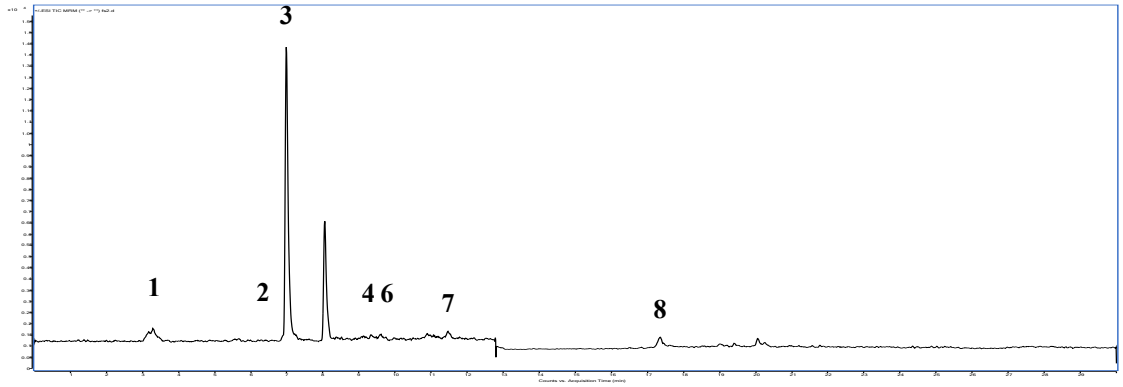
Variety	Maturation stage	GA	O-CA	C	EGC	Iso	Lut	Dios	Pol	Res
Sabelle (SB)	BV	275.16^{a}	Nd	72645.40^{a}	12.00^{a}	7.23^{b}	124.12^{b}	Nd	Nd	Nd
	V	124.25^{b}	12^{b}	46576.47^{b}	8.44^{b}	8.81^{a}	103.97^{c}	Nd	1.62^{a}	Nd
	R	271.70^{a}	13.89^{a}	18245.33^{c}	4.99^{c}	6.022^{c}	162.74^{a}	Nd	1.52^{a}	Nd
Red Globe (RG)	BV	1130.75^{a}	12.08^{a}	24586.40^{a}	4.58^{a}	6.35^{b}	110.17^{c}	Nd	1.67^{a}	Nd
	V	365.95^{b}	11.86^{a}	23109.15^{b}	Nd	7.70^{a}	146.03^{a}	Nd	1.77^{a}	Nd
	R	119.13^{c}	10.95^{b}	4537.68^{c}	3.97^{b}	6.62^{b}	133.30^{b}	Nd	0.88^{b}	Nd
Cardinal (CR)	BV	387.54^{b}	Nd	31148.85^{a}	Nd	Nd	164.44^{a}	Nd	Nd	Nd
	V	119.13^{c}	Nd	13700.78^{c}	3.97^{b}	Nd	85.06^{c}	6.81^{a}	0.9^{b}	Nd
	R	698.61^{a}	9.80^{a}	20337.11^{b}	5.26^{a}	5.25^{a}	115.0^{b}	Nd	3.00^{a}	2.00^{a}

GA: Gallic acid, **O-CA**: O-coumaric acid, **C**: Catechin, **EGC**, Epigallocatechin, **Iso**: Isoquercitrin, **Lut**: Luteolin, **D**: Diosgenin, **Pol**: Polydatin, **Res**: Resveratrol, **BV**: Before veraison, **V**: Veraison, **R**: Repining, **Nd**: not detected. The values in identical columns with various superscripts (a,b,c) differ significantly ($p < 0.05$). **BV:** before veraison, **V:** veraison, **R:** repining. values represent the means ± SEM of three measures.

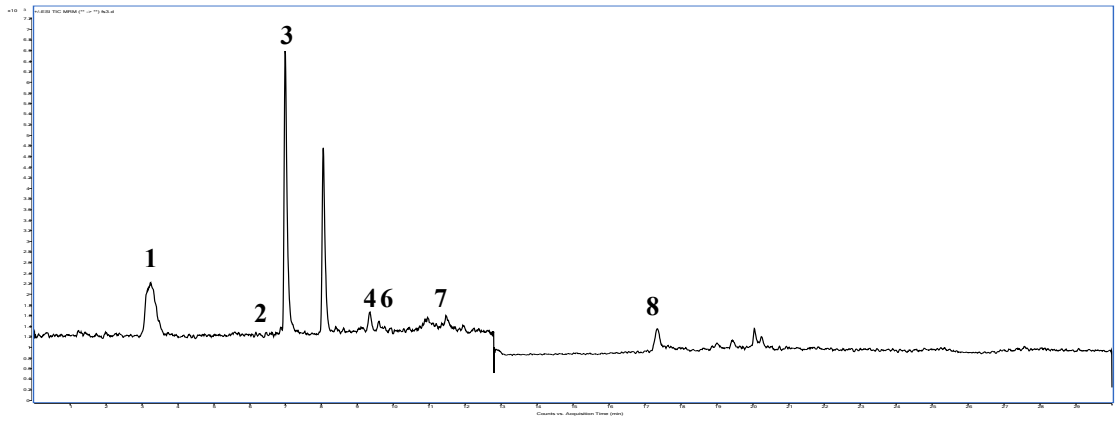
Sabel: befor veraison



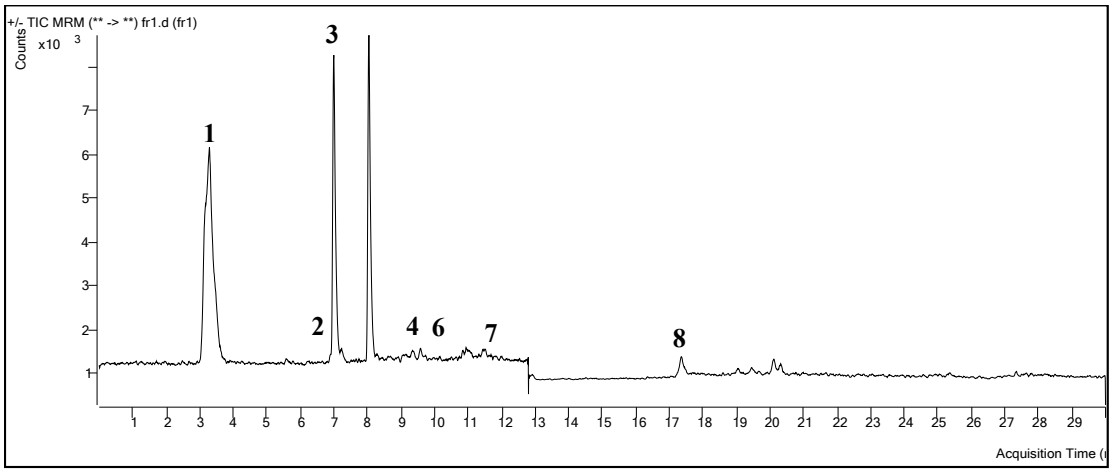
Veraison



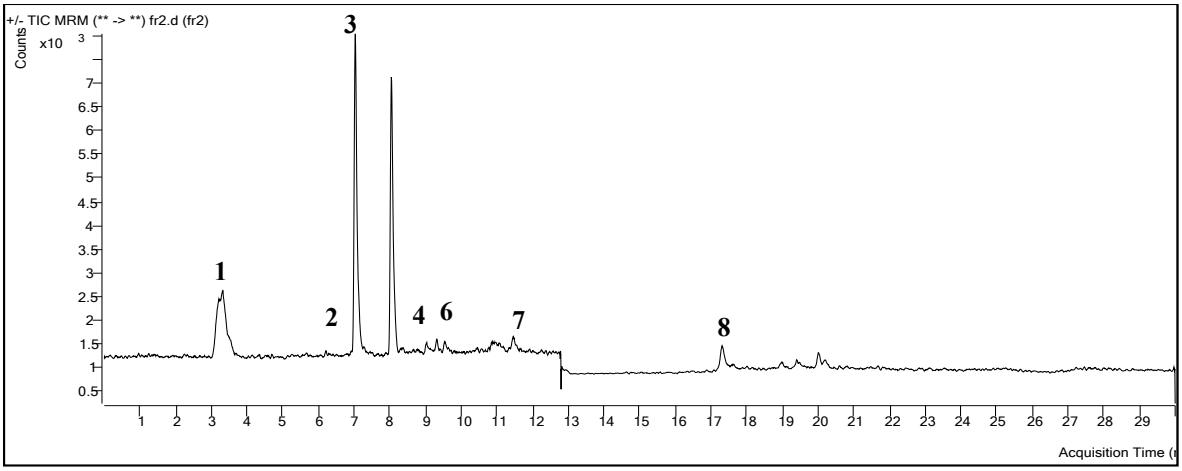
Repining



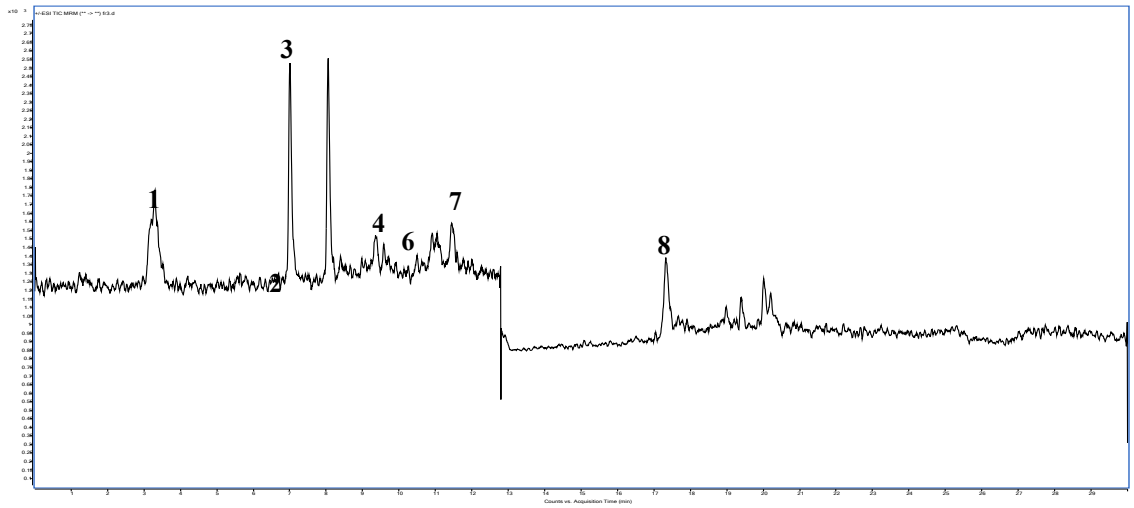
Red glob: before veraison



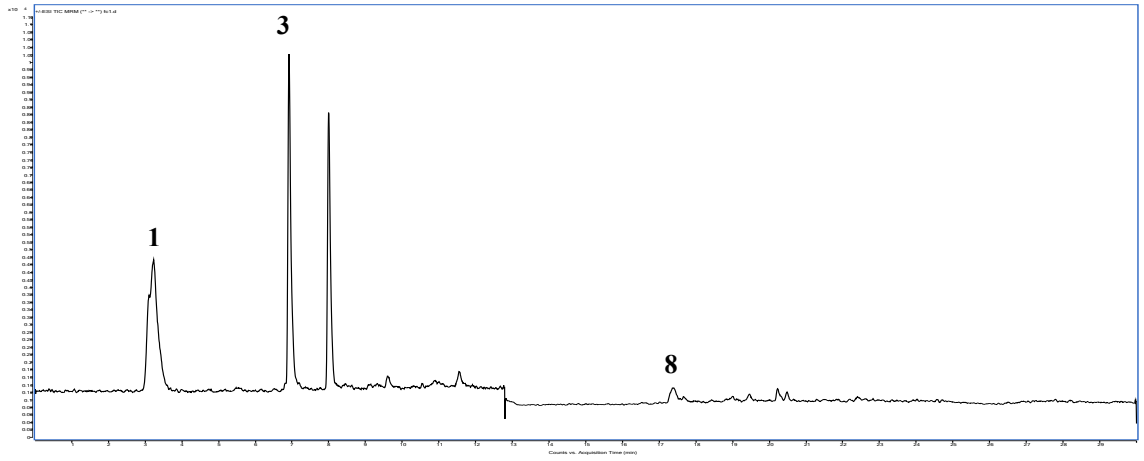
Veraison



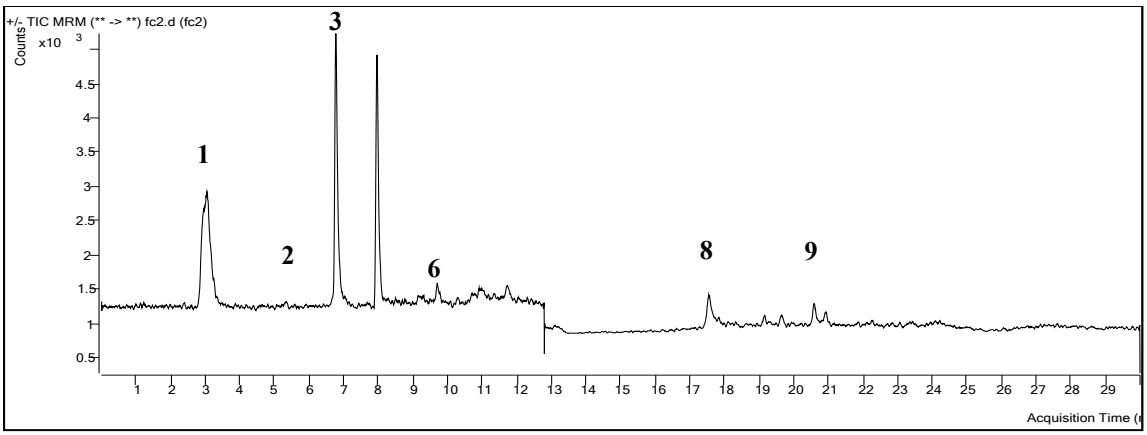
Repininng



Cardinal: Before veraison



Veraison



Repining

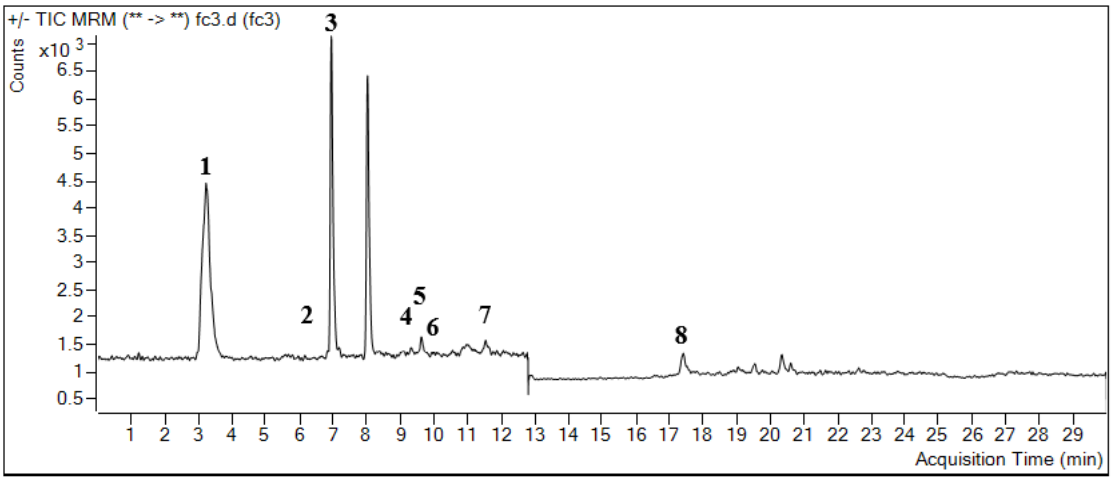


Figure 43: LCMS/MS chromatograms of the different seed extracts. 1: gallic acid, 2: epigallocatechin, 3: catechin, 4:o-coumaric acid, 5: resveratrol, 6: polydatin, 7: isoquercetin, 8: luteolin, 9: diosgenin.

The detection of catechin as the most abundant flavanol aligns with findings by other authors. Obreque-Slier et al. (2010) and Du et al. (2021) found that catechin was the predominant flavanol detected in grape skin and seed, respectively. A decrease in the concentration of the three monomers C, EC, and ECG was detected in the study of Obreque-Slier et al. (2010) and Liu et al. (2010). Additionally, the amount of grape seed flavan-3-ol monomers demonstrated two different changes in the study of Kyraleou et al. (2017); an increase during the first stage, followed by a decrease until harvest. The reduction of the monomeric flavan-3-ol in the grape seeds is a result of phenolic chemical oxidation and their fixation on the seed coat., it also possible due to their polymerization and interactions with other substances, like proteins and polysaccharides, which make them less extractable (Liu *et al*., 2010; Andjelkovic *et al*., 2013).

All cultivars contained gallic acid and o-coumaric acid, with gallic acid being present in higher quantities than o-coumaric acid. The highest amount of gallic acid was detected in the RG variety during the green stage, and the highest amount of o-coumaric acid in the SB cultivar during the ripening phase. The amount of gallic acid evolved similarly in the SB and CR varieties, with the highest amount occurring at the first phase, decreasing at veraison, then increasing until maturation, while the RG variety showed a shifting trend. No specific trend was observed in the case of o-coumaric acid.

The significant differences in gallic acid content between varieties and the impact of ripening stages support findings by those who observed variations in phenolic acids during grape ripening and maturation (Topalovic and Mikulic-Petkovsek, 2010; Liang *et al*., 2011).

Regarding the class of stilbene, for polydatin, the SB variety represented low concentrations at veraison and repining phases with no detected amount in the green stage. Low concentrations were also found in the RG variety, with no significant differences between the first and second phases, while a decrease pattern at the repining phase was observed. Regarding the CR variety, the polydatin was only detected during the repining stage with highest concentration in comparison to the two other varieties. In the case of resveratrol, it was only found in the CR cultivar at the repining stage in low quantity.

It's well known that stilbenes are present in grape berry with very low concentrations, and they are mainly concentrated in the skin Benbouguerra et al. (2020). No concentration of stilbenes was also detected in the grape seed during the repining stages in the study of Benbouguerra et al. (2020) and Dudoit et al. (2021). Moreno et al. (2008) also found that the concentration of trans-resveratrol in the seed was found to be much lower in comparison to that of the skins, and that cis-resveratrol has not been detected in any of the seed samples.

Regarding the flavone luteolin, the concentration of this secondary metabolite ranged from 85.06 to 164.44 mg/kg in all cultivars during the different repining phases. In the case of SB and CR, a decrease in the concentration of luteolin from BV to V was detected, in contrast of RG cultivar which showed an increase trend. During the second period from veraison to repining, the concentration of luteolin increased in SB and CR and decreased in the RG variety. Fang et al. (2013) also observed slight modification in the luteolin content during grape berry development characterized by a rapid increase around veraison to kept high in ripe fruit.

Isoquercitrin was the only flavonol detected in grape seeds of different varieties, and found in low concentrations across different ripening stages. The SB variety had the highest amount of Isoquercitrin. no significant differences between ripening phases were noted, except for the CR variety, where Isoquercetin was only detected at the ripening phase.

It is well noticed that quercetin is the primary component of the flavonoid class which is generated during the first period of veraison in the study of Ivanova et al. (2011). Topalovic et al. (2010) also remarqued an increase in the content of quercetin-glucoside in the grape skin

during maturity. In the case of diosgenin, it was only found in CR variety at veraison stage with low concentration.

2.3. Antioxidant activity

Several biological or dietary systems have investigated the antioxidant properties of grape extracts and related compounds. It is well known that's Grape extracts and related products have the potential to reduce oxidative stress in biological systems and prevent degradation of food. Therefore, the extracts from grape seeds are a promising antioxidant for dietary supplement. they are considered as the grape part which displayed the highest antioxidant activity followed by skin, and the flesh (Xia *et al*., 2010).

Grape seed extracts demonstrated potent antioxidant activity across all ripening stages, with higher activity noted at BV and R stages (Table 9). The extracts showed effective scavenging activity against ABTS free radicals and strong iron reduction in the phenanthroline test, surpassing their efficacy in the DPPH assays. CR and SB extracts from the BV stage exhibited higher antioxidant activity than the standard BHT in the DPPH and phenanthroline assays, respectively. In the SNP test, all samples showed higher potency than the standards BHA and BHT.

The seed extracts' antioxidant activity varied with the ripening stage and changes in phenolic content. IC50 values exhibited a trend similar to that of TPC, TFC and some detected phenolic compounds. In the DPPH assay, the scavenging ability was most potent at the BV stage, decreased at veraison, and increased again at ripening in all cultivars. The ABTS test showed that the CR and SB cultivars had similar patterns. However, the RG variety had lower IC50 values and stronger activity at the BV and V stages compared to the R stage. The reducing power test showed that the SB variety was most active at veraison, while the RG and CR varieties were more active at the BV and R stages. For all cultivars, the phenanthroline assays showed close IC50 values across different ripening stages. The SNP test revealed high activity at BV and V for RG and CR, as well as at BV and R for SB.

In the same context, the phenolic profile is in accordance with the antioxidant activity, the higher polyphenol concentration corresponded to the higher antioxidant activity. Additionally, the variation in the concentration of some antioxidant molecules like condensed tannin, catechin, epigallocatechin, phenolic acids (gallic acid and o-coumaric acid) and the stilbene resveratrol (Sarkhosh-Khorasani *et al*., 2021) can explain the changes in the antioxidant

capacity of the different extracts. Grape seed extracts are thought to have higher antioxidant potential than vitamins C and E (Ananga *et al*., 2017). Additionally, the decrease in the antioxidant activity of grape skins with the decrease of TPC content during the repining stages was also reported by Benbouguerra et al. (2021). Furthermore, significant differences in the antioxidant capacity of grape skins observed during ripening was also noted by Du et al. (2021) who reported that the tendency of the scavenging activities of DPPH, ABTS and the reducing power is similar to that of TPC and TFC.

Table 9: Antioxidant activity of the different seed extracts during repining. values represent the means ± SEM of three measures.

Variety	Maturation stage	DPPH IC50 (µg/mL)	ABTS IC50 (µg/mL)	Phenanthroline A0.5 (µg/mL)	Reducing power A0.5 (µg/mL)	SNP A0.5(µg/mL)
Sabelle (SB)	BV	24.14±2.01^{c}	4.68 ±0.06^{c}	2.14 ±1.81^{c}	9.40±0.80^{c}	10.45± 0.91^{c}
	V	36.46±1.92^{a}	9.51±1.47^{a}	6.31± 0.34^{a}	13.13± 0.33^{a}	14.67±1.34^{a}
	R	29.67±8.0^{b}	6.09± 0.29^{b}	4.77 ± 0.63^{b}	10.29±0.23^{b}	13.23±0.86^{b}
Red Globe (RG)	BV	16.14±1.90^{c}	2.88± 0.08^{b}	3.85 ± 0.82^{c}	12.30±2.08^{b}	14.82± 3.17^{b}
	V	56,76± 4.16^{a}	12.94±1.45^{a}	5.34 ± 0.60^{a}	11.39± 0.4^{c}	13.16±1.43^{c}
	R	26,52± 0.9^{b}	2.77 ±0.80^{b}	4.62 ± 0.74^{b}	13.50± 1.64^{a}	23.27±0.29^{a}
Cardinal (CR)	BV	29,42±5,31^{b}	5.58± 0.32^{b}	4.00±0.30^{a}	10.55±1.49^{c}	12.67±2.9^{c}
	V	55,50±10,54^{a}	6.30± 0.49^{c}	4.63±0.33^{a}	14.04±1.09^{a}	14.06±1.57^{b}
	R	29,68±1,25b	14.45±2^{a}	4.06±0.04^{a}	11±1.38^{b}	18.54±1.24^{a}
BHA	NT	5.73 ±0.4	1.8±0.1	1.49±0.08	8.41±0.67	73.47±0.88
BHT	NT	22.32±1.2	1.29d±0.1	2.20±0.04	50.1±1.53	>200

BHA: butylated hydroxyanisole, BHT: butylated hydroxytoluene, NT: not treated. The values in identical columns with various superscripts (a, b, c) differ significantly ($p < 0.05$). A0.5: the concentration at the 0.50 absorption and IC50: the concentration at the fifty of inhibition. BV: before veraison, V: veraison, R: repining.

2.4. Principal Component Analysis

A Principal Component Analysis (PCA) was used to establish and clarify the possible associations and correlations between seed development, phenolic profiles, and the antioxidant activities of the studied varieties (Figure 44), a total of 100% variance was detected in all cultivars. For SB variety, PC1 represented a percentage variance of 55.06%, whereas PC2

represented 44.94%. the majority of the variables found to have positive correlation with PC1 while catechin (C), epigallocatechin (EGC), gallic acid (GA), luteolin (Lut), total condensed tannin (TCT), and total flavonoid (TFC) seemed to have negative correlation. C, EGC, TPC, TF-OL, TCT, and GA were the key markers of the BV stage. the veraison stage was characterized by higher amount of Iso, Pol and TYT. At least, TFC Lut, and O-CA were the main markers of the repining phase. Regarding the antioxidant activity, it was appeared that all the tests were positively correlated with PC1, located at the veraison stage area, where they represented the lower antioxidant capacity represented by the highest IC50 values.

In the case of CR variety, PC1 and PC2 exhibited 55.97% and 44.03% of the total variance respectively. The area of the BV stage represented higher concentrations of C, GA, Lut, TPC, TCT, and TF-OL where the two last variables were found to be negatively associated with PC1. No specific markers in except of Dios and TYT were detected in the area of the veraison stage whereas a total of six markers were found in the area of repining stage. Regarding the antioxidant activity, the lowest efficacity of the Phen, ABTS, and DPPH were found to be located at the veraison area, and the two other tests were disposed at the area of the repining phase.

Regarding the RG variety, PC1 explained 58.40% of the total variance and has a positive correlation with the majority of the variables. PC2 explained 41.60% of the total variance. C, EGC, GA, O-CA, TF-OL, TCT and TYT are the main markers of the first stage with high concentrations, the second stage characterized by higher amount of Pol, Lut, Iso, TPC, TFC, TYT and TCT. Regarding the last stage no specific markers was observed. The distribution of the antioxidant tests according to their higher IC50 values and lower effect was found to be divided between the repining stage (SNP, ABTS, Phen) and the veraison stage (DPPH, RP), in contrast of the before veraison stage which exhibited potent antioxidant activity which may be due to the quality of the phenolic compounds and their concentrations in this stage.

The PCAs results characterized by higher concentrations of TPC, TCT, C, and GA During the first stage (BV), these molecules act as potent antioxidants, and represented high correlation with the different tests, that is what explain the highest antioxidant capacity of the different extracts during this stage.

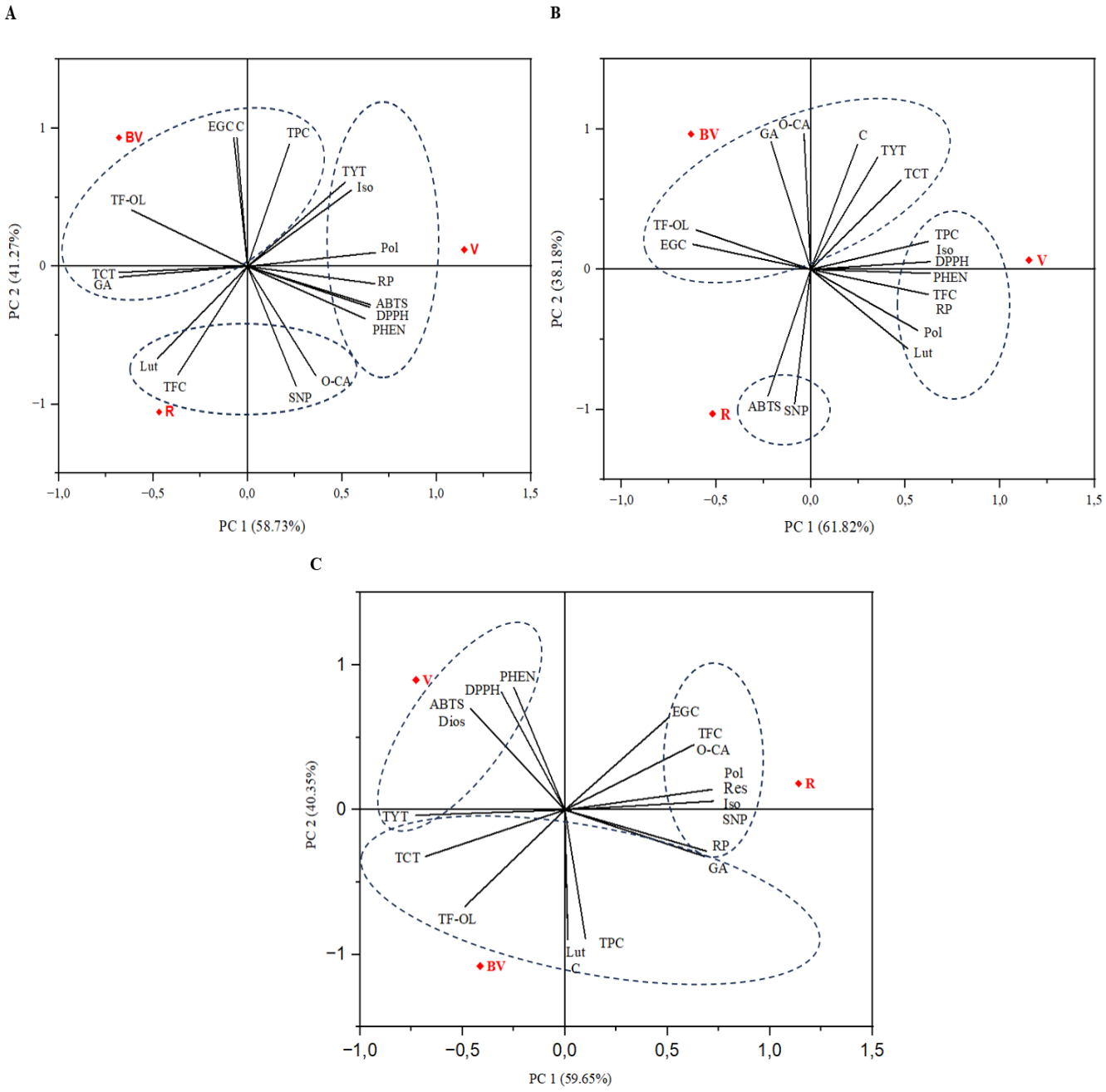


Figure 44: Principal component analysis of the different seed extracts. **TPC:** Total phenolic, **TFC:** flavonoid, **TF-OL:** flavonol, **TCT:** condensed tannin, **TYT:** hydrolysable tannin, **O-CA:** O-coumaric acid, **GA:** gallic acid, Res: resveratrol, **Pol:** polydatin, **CAT**: catechin, **EGC**: epigallocatechin, **Iso**: Isoquercetin. **A**: Sabel, **B**: Red Glob, **C** Cardinal. **BV:** before veraison, **V:** veraison, **R:** repining. **A:** Sabel, **B:** Cardinale, **C:** Red glob.

2.5. Enzymatic Activity

2.5.1. Anti-tyrosinase Activity

Besides of their antioxidant characteristics, polyphenols have the ability to block the functions of numerous enzymes responsible of skin aging like tyrosinase, collagenase, and elastase (Baroi *et al*., 2022). Tyrosinase is the enzyme responsible of melanin production due to skin hyperpigmentation, because of that, blocking the synthesis of melanin through the inhibition of tyrosinase activity is considered as therapeutic approaches for treating hyperpigmentation (Dwibedi *et al*., 2022). The anti-tyrosinase activity of the three extracts during the different repining phases was assessed and the results showed that the last phase represented the highest inhibition percentage in all cultivars (Table 13). CR variety demonstrated the most potent inhibition percentage (45%), followed by SB which exhibited a non-significant lower activity (44%), while RG showed the lowest activity with a percentage of 38%. The standard Cojik Acid was found to have an inhibition percentage of 36,29 %.

The UAE extracts of the seed extracts also demonstrated a high inhibition activity of tyrosinase in the study of Michailidis et al. (2021) represented by high inhibition percentage (75%). In contrast, a study conducted by Rauniyar et al. (2014) revealed that the inhibition of mushroom tyrosinase by grape seed extract showed lower effect than our results (7%). Furthermore, grape seeds considered as an important source of antioxidants which protect skin from UV damage and give them the potential to enhance the value of cosmetic formulations such as sunscreens (Baroi *et al*., 2022). In addition, Inhibitors of tyrosinase are not important only for the skin protection, they are also can be used to enhance the quality of fruits and vegetables by inhibiting their browning caused by tyrosinase, that what making plants as natural, potent, and safe source of these molecules. (Rauniyar *et al*., 2014; Michailidis *et al*., 2021). It is well noticed that one of the most effective phenolic classes exhibited the inhibition of tyrosinase activity are flavonoids represented essentially by: kaempferol, quercetin, morin, catechin and rhamnetin, while resveratrol with other stilbenes like piceatannol, coumarin, oxyresveratrol, chlorophorin and andalasin have been also reported to have the inhibitory activity (Dwibedi *et al*., 2022).

2.5.2 Anti α-amylase activity

As the data represented in Table 10, the extracts showed moderate activity against α-amylase and represented a dose dependent effect with a more effective inhibitory activity compared to the acarbose. At a concentration of 7.81µg the inhibitory power of the different extracts was found to be low, increased then with the increase of concentration to attain their maximum at 250 µg. no significant differences was detected between the majority of the extracts. RG and CR varieties trended similarly. Same inhibitory effects were demonstrated at the first and second phases, whereas a lower effect at the last stage was reported. SB variety inhibited α-amylase in lower efficacy in the first stage, whereas more potent effects were found at veraison and repining stages with no significant differences between the two phases.

Table 10: Inhibition percentage and IC50 values of the different seed extracts at different repining stages against α-amylase.

Extracts		Inhibition percentage %							
		7.81 µg	15.62 µg	31.25 µg	62.5 µg	125 µg	250 µg	500 µg	IC50 µg/mL
SB	BV	5,72±0.83	20,84±4.21	34,43±2.32	73,25±3.94	73.30±4.76	73.40±2.32	73.40±2.19	**53.30±3.44b**
	V	9,35±0.33	63,81±5.95	75,97±6.44	83,86±5.52	83.88±2.45	83.90±3.94	83.90±3.43	**47.09±2.55a**
	R	20.65±1.69	60,54±3.21	79,13±7.39	86,06±3.43	86.20±6.34	86.20±.87	86.20±2 ?21	**47.01±3.23a**
RG	BV	4.96±0,30	12.83±1.06	36,15±2.74	60,81±2.45	62,19±3.42	62.20±4.32	62.20±3.02	**48,90±3.42a**
	V	5,39±0.21	10,01±1.12	42,78±1.86	61,41±6.09	61,90±2.67	61.95±3.25	61.95±2.93	**48,34±5.04a**
	R	5,08±0.34	9,33±0.76	57,75±3.94	62,13±4.02	72,53±4.34	72.60±5.38	72.60±1.55	**31,94±4.22b**
CR	BV	6,63±0.40	17,15±0.34	48,31±3.21	81,26±3.44	83,11±5.21	83.14±4.87	83.14±2.97	**48.90±3.65a**
	V	7,20±0.55	13,38±1.05	57,17±2.32	82,07±7.29	82,72±1.43	82.74±6.45	82.74±6.43	**48.34±5.34a**
	R	6,78±0.13	57,74±3.34	77,18±5.23	83,03±5.48	96,93±6.65	96.95±9.04	96.95±3.41	**51.79±4.39b**
Acarbose		7,76±0,17	8,08±0,30	9,46±0,11	10,70±0,96	31,81±2,89	37,21±3,54	53,05±1,59	**3650,93±10,70**

SB: Sabel, RG: Red Glob, CR: Cardinale. BV: Before veraison, V: veraison, R: repining. IC50: the concentration at the fifty of inhibition. values represent the means ± SEM of three measures. The values in identical columns with various superscripts (a, b, c,) differ significantly ($p < 0.05$).

Yilmazer-Musa et al. (2012) studied the capacity of grape seed extract to inhibit both α-amylase and αglucosidase activity, and found higher activity than the standard acarbose. Furthermore, the study conducted by Dudoit et al. (2020) investigated the α-glucosidase inhibitory effect of seed grape extracts at four ripening stages and observed that the activity remains almost constant during the first three stages of ripening then shifted at maturity. The

different extracts were found also to be more effective than the standard acarbose by inhibiting α-glucosidase with more potent effect. According to Dwibedi et al. (2022) flavonoids are the main contribute to the α-amylase inhibition, whereas other phenolic compounds like: hydroxycinnamic acids and tannins was found to have less inhibitory activity than flavonoids. Procyanidins of grape seed were found to have more potent inhibitory effect on α-amylase and α-glucosidase due to the higher number of potential attachment sites compared to monomeric phenolic compounds, their high degree of polymerization and the abundance of hydroxyl groups in their structure (Cisneros-Yupanqui *et al*., 2023). However, monomeric flavanols like catechin, epigallocatechin, tannin, and hydroxycinnamic acids were found also to be effective but in less potent manner (Dwibedi *et al*., 2022).

2.5.3. Inhibition of lipase activity

The capacity of the different extracts to inhibit lipase was assessed. The results presented in Table 13 demonstrated the high efficacity of the SB and RG extract to act as inhibitors of the studied enzyme. The two extracts revealed higher effect than the standard Orlistat. The inhibition capacity changed with the changes of the phenolic profile. Concerning the CR and the SB extracts, an increasing in the inhibition capacity from the green to the mature stage was detected. İn the case of RG, the highest inhibition was recorded during the second phase, whereas the two other phases exhibited lower efficacity. This result implies that GSE, especially those of the SB and RG varieties, could be useful as a treatment to reduce dietary fat absorption and accumulation of fat in adipose tissue. The study conducted by Moreno et al. (2003) indicated that grape seed extract has been reported to inhibit several lipases, including pancreatic and lipoprotein lipases with a percentage of 80% and 30%, respectively. Additionally, several studies have indicated the potential use of condensed tannins (proanthocyanidins) extracted from grape solid parts (seeds and skins) as promising anti-obesity molecules with lack of toxicity effect. Furthermore, the ethanol grape seed extract (EGSE) contained higher concentration of proanthocyanidins, had higher inhibitory effect against pancreatic α-amylase and lipase in comparison with water grape seed extract (WGSE) in the study of Hassan (2014). Compounds in grape seed extract that prevent the gastrointestinal breakdown of fats by blocking lipase enzymes (pancreatic lipase, Lipoprotein lipase and hormone-sensitive lipase *in vitro*) could offer a natural, safe, and affordable weight-loss solution (Yadav *et al*., 2009).

2.5.4. Butyrylcholinesterase inhibition activity*:*

Another inhibition test of the enzymes responsible of diseases was established to confirm the pharmacological activities of grape seed extracts. The increasing prevalence of neurodegenerative diseases has attracted researchers to examine various components that can be used to treat or prevent neurodegeneration (Yadav *et al*., 2009). Most of the extracts were found to inhibit butyryl in higher effective way than the standard Galantamine. There was a dose-dependent effect; the highest inhibition percentages were determined at a concentration of 100 µg. SB and CR varieties trended in inhibition of the enzyme with the lower IC50 values (1.04± 0.62 and 1.41±0.17 µg/mL, respectively). The best activity for RG and SB extracts was recorded during the green stage, whereas lower activity was recorded in the two other stages (Table 11). In contrast, the best activity for the CR extract was shown at the veraison phase.

Table 11: Inhibition percentage and IC50 values of the different seed extracts at different repining stages against Butyrylcholinesterase activity.

Extracts		Inhibition percentage %							
		3.12 µg	6.25 µg	12.5 µg	25 µg	50 µg	100 µg	200 µg	IC50 µg/mL
SB	BV	40.04±2.03	48.96± 0.87	68.18± 0.72	83.58± 0.63	86.03± 0.36	88.12± 1.45	88.36 ±1.53	**3.46±0.67a**
	V	49.37±1.97	65.91±0.45	75.58±0.27	84.6±2.07	89.01±1.66	91.76± 2.3	91.82±1.23	**1.04±0.62b**
	R	27.07± 1.3	55.35 1.72	68.27 0.98	89.69 2.43	93.65 0.64	97.93 1.22	98 0.84	**1.45±0.49b**
RG	BV	31.66± 3.59	33.67± 2.28	48.10± 1.03	71.14± 1.78	94.30± 2.56	95.01± 1.05	95.10± 1.3	**8.85±1.21a**
	V	33.78± 2.05	35.46± 2.04	63.42± 0.65	81.99± 1.27	90.16± 1.03	90.18± 1.5	90.18± 1.6	**8.80±2.15a**
	R	14.32±1.5	27.85±1.6	52.91± 0.19	73.49±1.21	84.34±0.84	94.20±0.50	94.3±0.86	**3.25±0.46b**
CR	BV	34.55± 2.07	49.73± 5.5	54.53± 1.28	75.81± 0.38	88.01± 2.85	88.02± 1.08	88.3± 2.2	**3.90±0.78b**
	V	12.83±1.02	20.39±1.3	32.01±3.5	56.56±3.53	69.20±2.80	88.38±5.02	89.14±2.4	**9.96 ±2.05a**
	R	48.72±2.20	80.46±5.28	88.96±3.76	91.72±1.90	94.12±1.00	97.82±1.78	97.9 ±0.63	**1.41±0.17c**
Galantamine		35,93 ±2,28	43,77 ±0.00	68,50 ±0,31	80,69 ±0,41	85,78 ±1,63	91,80 ±0,20	94,77 ±0,34	**6.27±1.15**

SB: Sabel, RG: Red Glob, CR: Cardinale. BV: Before veraison, V: veraison, R: repining. IC50: the concentration at the fifty of inhibition. values represent the means ± SEM of three measures. The values in identical columns with various superscripts (a, b, c,) differ significantly ($p < 0.05$).

The inhibitory activity of grape leaf, seed, and pulp of six seeded grapes against anticholinesterase activity (acetyl-(AChE) and butyrylcholinesterase (BChE)) enzymes was established in the study of Karatas et al. (2022), revealing higher inhibition activity in the pulp, and lower activity in the leaf extracts. According to Insanu et al. (2021) grape pomace such as: skin, seed, and fruits had the potential to act through numerous mechanisms as potent anti-

Alzheimer agents. Additionally, GSE as dietary supplement improves the brain memory, decreased protein carbonyl level, reduced reactive oxygen species production, reduced hypoxic ischemic brain injury, and increased thiol level in the central nervous systems (Gupta *et al*., 2020).

2.5.6. Anti-urease activity

The results of the anti-urease capacity of grape seed extracts during the different repining stages demonstrated that all the extracts showed anti-urease activity in different potent ways. The best activity was detected in the SB variety at the last stage, with an IC50 value close to that of the standard. There was a dose-dependent activity; the inhibition activity was found to be low in the range of concentrations from 3.125 to 25 µg, increased clearly at a concentration of 50µg in the most extracts, and reached its maximum at 100 µg, where the majority of the extracts attained 80% of enzyme inhibition.

Table 12: Inhibition percentage and IC50 values of the different seed extracts at different repining stages against urease.

Extracts		Inhibition percentage %							
		3.12 µg	6.25 µg	12.5 µg	25 µg	50 µg	100 µg	200 µg	IC50 µg/mL
SB	BV	10.16±0.25	11.45± 0.09	12.52 ±0.12	12.96± 4.06	37.46± 0.20	66.28± 0.06	80.35± 0.15	**53.97± 3.89**
	V	11.52±0.36	13.11± 4.33	14± 0.09	26.61± 0.27	57.72± 6.39	73.13± 0.29	80.35± 0.15	**46.54±3.32**
	R	7.61± 0.09	11.72± 0.06	13.79± 0.1	30.46± 2.02	65.75± 0.1	72.40± 1.33	83.51± 0.32	**34.29±6.75**
RG	BV	5.94± 0.11	5.80± 0.2	9.17± 0.6	12.29± 0.07	14.06± 2.3	53.33± 0.14	66.51± 2.7	**135.53± 3.66**
	V	5.80± 0.1	7.64± 0.04	8.04± 0.48	13.54± 1.7	39.13± 0.08	58.96± 0.09	86.14± 0.14	**79.62±0.26**
	R	8.60± 001	8.84± 0.03	15.05± 1.22	20.39± 0.41	34.04± 1.42	71.21± 0.30	83.08± 0.7	**73.83±2.51**
CR	BV	9.06± 0.12	9.43± 0.06	11.51± 0.08	36.31± 0.9	37.20± 0.1	76 ±0.5	84.70± 0.9	**60.54±0.05**
	V	10.93±0.09	11.33± 0.07	11.85± 0.1	12.79± 0.07	14.46±3.3	33.9±6. 2	62.68± 0.2	**153.14±6.42**
	R	10.16±0.25	11.45± 0.09	12.52± 0.12	12.96± 0.3	37.46± 0.2	66.28± 0.06	88.94± 2.59	**73.32±0.40**
Thiourea		4,49±0,78	19,85±2,74	55,64±4,24	94,17±0,15	98,42±0,19	98,49±0.41	98,90±0.05	**11.57±0.68**

SB: Sabel, RG: Red Glob, CR: Cardinale. BV: Before veraison, V: veraison, R: repining. IC50: the concentration at the fifty of inhibition. values represent the means ± SEM of three measures.

Comparing the activity of the different extracts with their repining phases, it was observed that both RG and SB cultivars inhibit urease with a more potent effect during the V and R phases, in contrast CR extract exhibited the highest activity at the unripe stage with an

IC50 value of 60.54±0.05 µg/mL. The variation of the activity with the variation of the phenolic profile during repining indicates that phenolic compounds are responsible for enzyme inhibition.

The use of phenolic compounds from grape seed extracts provides scientific evidence for the effective use of this plant to treat stomach ulcers caused by urease. Moreover, the ability to reduce ammonia production in the soil from urease activity makes the current extracts useful as an agent for improving the environment. While different authors have described the various biological impacts of grape seeds, no treatment has been performed to evaluate the inhibition of urease activity. luteolin-7-Oglucuronide, rosmanol, and rosmadial and phenolic diterpenes, Quercetin, epicatechins, resveratrol, and its derivatives have been shown in numerous studies to exhibit strong urease inhibitory activity (Dwibedi *et al*., 2022).

2.6. SPF Factor

Due to the fact that heightened UV radiation levels reaching the Earth's surface have contributed to an increase in skin-related disorders, there has been significant interest in the exploration of natural chemicals as photoprotective agents (Kamah *et al*., 2025).In the current study, the photoprotective effect represented by the SPF factor of each extract was established and the results revealed that no significant differences between the varieties and between the same variety at different repining phases, in except of the CR variety which demonstrated the highest SPF factor at the repining stage (Figure 45). The SPF factor of the most extracts found to be around 30, this value was considered as high protection effect according to the Recommendation of the Commission of the European Communities (2006).

Natural cosmetic formulations have been exploring the use of grapes and their derivative products and byproducts, as they are natural sources with possible photoprotective qualities against UV radiation (Nunes *et al*., 2015). The addition of grape pomace to the sunscreen formulation in the study of Hübner et al. (2020) revealed an increase in the SPF factor which confirmed the photoprotective effect of grape pomace. Furthermore, the research of Katiyar *et al*. (2008) has demonstrated that grape seed proanthocyanidins can prevent experimental photo-carcinogenesis in both *in vitro* and *in vivo* experiments with low toxicity effect which makes them suitable as sunscreen formulation constituents.

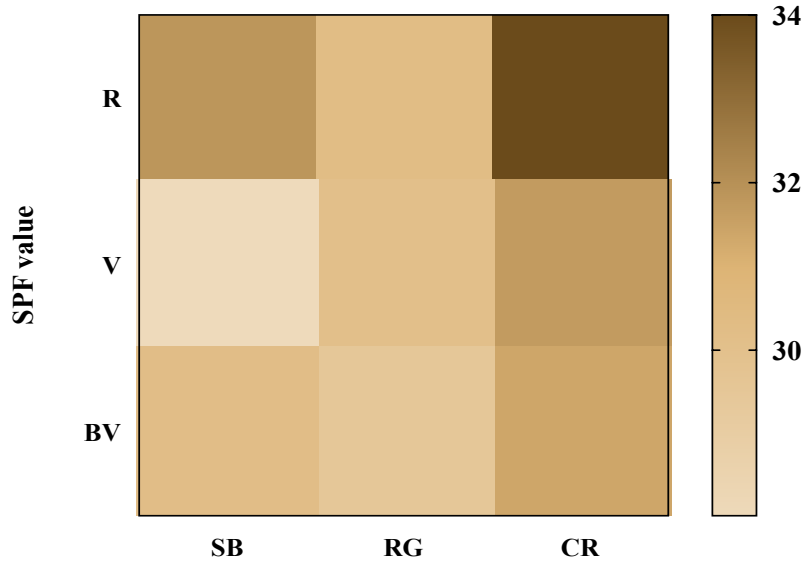


Figure 45: SPF values of seed extracts during different repining stages. GR: Gros Noir, FR: Fregola Nera, RG: Red Glob, CR: Cardinale. BV: Before veraison, V: veraison, R: repining.

2.7. Anti-inflammatory effect

Tissues use inflammation as a defense mechanism against pathogen attacks, cell damage, and irritation. It also acts as an approach for the removal of necrotic and damaged cells. But even so, chronic inflammation is thought to be a key factor in the development of chronic diseases such cancer, Alzheimer's, neurological disorders, cardiovascular diseases, diabetes, arthritis, autoimmune, and pulmonary diseases (Soto *et al*., 2015). One of the metabolic processes that might lead to tissue function loss during an extended inflammatory response is protein denaturation through the disruption of the electrostatic, hydrogen, hydrophobic, and disulphide linkages in the protein structure (Anyasor *et al*., 2019; Bailey-Shaw *et al*., 2017). Lead-based anti-inflammatory medication options may be found in extracts that prevent protein denaturation and maintain cell membrane stability against lysis (Anyasor *et al*., 2019).

The anti-inflammatory effect of the different extracts by inhibiting the denaturation of BSA was investigated. Different inhibition percentages were found during the repining phases. The highest inhibitory effect was detected in SB variety whereas the lowest in CR. For SB variety, before veraison and ripening stages demonstrated the highest activity whereas less potent effect was detected at veraison. Regarding RG cultivar, the denaturation effect was found to be more effective at BV and V while a significant lower effect was reported at repining. In

the case of CR, low inhibitory activity at BV and V was reported whereas an increase in the inhibitory effect was also reported at the last stage.

Table 13: Inhibition percentage of tyrosinase and BSA, and anti-lipase IC50 values.

Variety	Repining stage	I.P (%) of tyrosinase	IC50 of lipase (µg/mL)	I.P (%) of BSA
SB	BV	10±1.3c	22.96±3.10a	41±2.1a
	V	31±1.0b	17.48±3.68b	32±1.4b
	R	44±0.4a	1.20±0.03c	47±1.09a
RG	BV	34±1.5b	15.93±1.75b	30±1.9a
	V	30±2.1b	3.98±1.38c	32±2.02a
	R	38±1.4a	43.53±0.41a	16±0.5b
CR	BV	25±1.3b	31.51±3.85a	15±0.6b
	V	39.7±1.3c	28.02±1.91a	17±0.9b
	R	45±1.6a	14.17±1.00b	36±0.4a
Cojik Acid	/	60±1.6	/	/
Diclofenac sodium	/	/	/	36,29±1.8
Orlistat	/	/	4.98±0.70	/

GR: Gros Noir, FR: Fregola Nera, RG: Red Glob, CR: Cardinale. BV: Before veraison, V: veraison, R: repining. IP: inhibition percentage, IC50: the concentration at the fifty of inhibition. IC50 values represent the means ± SEM of three measures. The values in identical columns with various superscripts (a, b, c) differ significantly ($p < 0.05$)

It was noted that the phenolics extracted from grape seeds have the potential to inhibit enzyme systems linked to inflammatory responses and the generation of free radicals (Xia *et al*., 2010). Gogoi et al. (2015) also studied the capacity of grape seed extract to inhibit the denaturation of BSA and found that the results showed that it has strong anti-inflammatory properties in comparison to the standard diclofenac sodium. procyanidins, flavonols, and flavanols are potential important phenolic components found in grapes, particularly in grape seeds, have demonstrated notable anti-inflammatory effects on rats, mice, and humans (Xia *et al*., 2010). Additionally, Other techniques were used to establish the anti-inflammatory effect of grape seeds. Benbouguerra et al. (2021) also studied the anti-inflammatory effect of Skin extracts from three *Vitis vinifera* varieties during the same repining stages and found that all of the varieties under study had stronger NO and ROS inhibition (>50%) in the before-veraison skin extracts, confirming their anti-inflammatory and antioxidant abilities which changed with the change of the maturity stage.

2.8. Antimicrobial activity

The antibacterial assay against *E. Coli* ATCC 25922 and the ESBL strains (*E. Coli* 01, 02, 03, 04) revealed good activity of the majority of the extracts (Table 14). CR extract trended in inhibition of *E. Coli* ATCC 25922 with RG extract, whereas a non-significant lower inhibition was recorded by the SB extract. CR extract demonstrated also the highest inhibition zone of *E. coli* 01, 02, and 04 (with SB). RG exhibited the most important inhibition diameter of *E. coli* 03. SB extract trended in inhibition of *C. albicans* ATCC 10231 and *C. albicans* 01. Additionally, low activity was recorded against *A. niger* 01 with the highest inhibition percentage being 40%; recorded in the RG extract. The MIC was found to be 0.6 mg/mL for all the cultivars.

It was noticed that GSE have demonstrated good antimicrobial properties especially against Gram negative bacteria like-*Pseudomonas aeruginosa* or *Escherichia coli* compared to Gram positive (Gupta *et al*., 2020). Additionally, according to Georgiev *et al*. (2014), Grape juice, skin, seed, stem and wine were demonstrated good microbial inhibitory activity with catechin, epicatechin, and epicatechin-galate being the main active compounds. The antimicrobial activity of three additives (grapevine seeds, grape and rosehip pressings) was assessed and the results demonstrated reduction in the growth of pathogenic *E. coli* and *Clostridium perfringens* (Jakubcova *et al*., 2015). Resveratrol, flavonoids (flavanols), caffeic acid, quercetin, quercetin-3-O-rutinoside, and polymeric phenolic fractions of GSE were found to be responsible of the antimicrobial activity according to Gupta et al. (2020). This propriety gives GSE the potential to be incorporated into skin care cosmetics besides to an important role in modulation of human gut microflora (Georgiev *et al*., 2014).

Table 14: Antimicrobial activity of seed extracts during the last repining stage.

Extracts	**SB**		**RG**		**CR**	
Strains	**IZ (mm)**	**CMI (mg/mL)**	**IZ (mm)**	**CMI (mg/mL)**	**IZ (mm)**	**CMI (mg/mL)**
***E. coli* ATCC25922**	14±0.41*	0.6	15±0.82**	0.6	15±0.7**	0.6
***E. coli* 01**	10±0.65*	0.6	10±0.42*	0.6	15±0.65**	/
***E. coli* 02**	/	/	13±0.23*	0.6	17±0.78**	0.6
***E. coli* 03**	/	/	10±0.67*	0.6	12±1.25*	/
***E. coli* 04**	10±0.76*	0.6	/	/	10±2.12*	0.6
***C. albicans* ATCC 10231**	10±1.71*	0.6	/	/	/	/
***C. albicans* 01**	16±1.89**	0.6	16±0.76**	0.6	15±0.32**	0.6

Percentage inhibition (%) of growth

	C (mg/mL)	**IP (%)**	**C extract (mg/mL)**	**IP (%)**	**C (mg/mL)**	**IP (%)**
***A. niger* 01**	/	/	10	40±1.65	20	5±0.55

IZ: inhibition zone, MIC: minimal inhibition concentration, SB: Sabel, RG: Red Glob, CR: Cardinale, C: concentration of the extracts, IP: inhibition percentage. /: resistant, *: sensitive (09 < Ø < 14 mm), **: very sensitive (15 < Ø < 19 mm), -: no activity.

Conclusion and perspectives

The total phenolic and flavonoid contents, phenolic profile, and biological activities of four Algerian vine canes and three seed cultivars at three different repining stages were reported for the first time in this work. All the extracts demonstrated a good source of bioactive compounds. For cane cultivars, the FR variety represented the highest content of both phenolic and flavonoid compounds. The LCMS/MS revealed the presence of thirteen phenolic compound in the different extracts with catechin being the highest molecule detected in the FR variety. The same variety exhibited the highest antioxidant activity towards all method tested (DPPH, ABTS, Reducing Power, SNP, and Phenanthroline) which can be explained by the relation between the phenolic composition of the different extracts and their antioxidant activity. This relation was confirmed by a high correlation coefficient. The different extracts demonstrated also a good enzymatic activity towards numerous enzymes (amylase, tyrosinase, urease, lipase, and butyrylcholinesterase), a potent protective agent against denaturation of protein (BSA), a high protective effect against solar radiation represented by high SPF value, and good antimicrobial activity especially against *E. coli* strains and *C. albicans* with inhibition diameter varied from 10 to 20 mm. Regarding seed extracts, phenolic compounds were changed with the evolution of maturity stage. Catechin was the highest molecule detected in all cultivars with Before veraison stage represented the highest concentration detected in all cultivars. The changes in the biological activities with the change of the phenolic compounds was also recorded especially in the antioxidant activity. The seed extracts also exhibited potent antioxidant and enzymatic activities. The antimicrobial activity of the seed extracts at the repining stage revealed also potent activity with the highest inhibition diameter being 17 mm in the CR extract against *E. coli* 2. Grape seeds showed the best antioxidant capacity whereas grape canes demonstrated the highest activity against the studied enzymes and microbial agents. These results encourage the use of grape canes and seeds as a source of bioactive phytochemicals in pharmaceutical, cosmetic, and food industries as antioxidants providing an effective, economical, and eco-friendly solution for generated wastes. From an economic standpoint, it can be suggested that grape canes are more likely to be utilized than grape seeds, because of their greater availability and accessibility.

Perspectives

- Optimization of the cane and seed polyphenol extraction technique.
- Purification of the phenolic compounds and testing their biological activities.
- Identification of other phenolic compounds in the extracts using other standards.

- Study *in vivo* of numerous biological activities of the different extracts to confirm their *in vitro* activity.
- Exploitation of the extracts in the cosmetic industry.
- Synthesis of nanoparticles from the different extracts.
- Studying the evolution of the phenolic compounds in the period of harvest to choose the appropriate time with the highest phenolic contents in the harvested grape berries.

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