

Contribution of the polyphenol study at autochthonous Moroccan grapevine varieties for the discrimination of descriptive similarities

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Abstract- Morocco is endowed with a very diversified viticultural heritage autochthonous and introduced grape varieties but unfortunately, we are witnessing a strong regression of these local vines, confusions in the identification of these varieties as there are similar names that differ from one region to another. This study aims to characterize the vine varieties of the species *Vitis vinifera* of the Moroccan Rif region, namely Taferyalet Bayda (TB), Taferyalet Chahba (TC), Boukhanzir (BK), Bzoult Aouda Kahla (BAK), Bzoult Aouda Hamra (BAH), Dibanni (DB), Lebyed (LB), and Fakass (FA) with a phytochemical study. The descriptive ampelographic studies in physiological stages have shown similarities that make difficult the discrimination between our study varieties, for this purpose a phytochemical study is carried out in order to bring some varietal distinctions. Indeed, some varieties are rich in polyphenols such as Bzoult Aouda Kahla and Bzoult Aouda Hamra, Boukhanzir and Taferyalet Bayda, the high content of flavonoids in Taferyalet Kahla and Taferyalet Bayda and the variety Dibanni is the richest in tannins. From this study it can be affirmed the possible role of phytochemical analysis of the distinction between varieties with morphological similarity.

Index Terms- *Vitis vinifera*, autochthonous, Morocco, phytochemicals, ampelography.

I. INTRODUCTION

The vine is a particular plant that has ancient historical links with the development of human culture [1]. During its long and complex history of domestication, thousands of vine varieties have been developed. The grapevine varieties grown today are thought to have been shaped by the combined action of selection, breeding, blending and migration [2]. The history of the vine in Morocco dates back to the Phoenician and Roman times to which has been added the influence of Arabic since the Middle Ages [3]. However, in recent decades, the modernization of agriculture and the transformation of agricultural systems have placed plant genetic resources in a worrying situation. Indeed, modern vineyards made from European varieties are gaining

space to the detriment of traditional varieties which have declined or even disappeared from the usual growing areas. With the objective of identifying the diversity encountered within the Moroccan autochthonous grape varieties and their possible conservation in the collection, we took in our study to characterize nine (9) individuals of *Vitis vinifera* L. of the Moroccan North-West, according to different phytochemical approaches.

II. MATERIALS AND METHODS

The nine accessions studied in this study are listed in Table 1. The varieties studied are randomly arranged and planted in an experimental greenhouse at the Ibn Tofail University. The growing conditions are identical in terms of soil texture and structure as well as in the irrigation and fertilization programs.

Table 1: Grape varieties studied

Varieties	Abbreviation
Bezoul Alaouda Kehal	BAK
Bezoul Alaouda Hmar	BAH
Boukhanzir	BK
Taferyalet Bayda	TB
Taferyalet Chehaba	TC
Taferyalet Kehala	TK
Dibanni	DB
Fakass	FA
Labyed	LB

1. Preparation of extracts

The leaves of the different varieties studied were air-dried and ground into a fine powder using a mortar and stored in a glass container. The extraction was done by infusion of 5%.

2. Phytochemical screening

The identification of the bioactive chemical compounds existing in the aqueous extract is carried out according to known phytochemical methods [4].

a- Identification of polyphenols

In a test tube we put 1ml of extract and we add 2 to 3 drops of FeCl₃ at 5%. The development of a greenish or blackish blue coloration indicates the presence of polyphenols [5].

b- Identification of tannins

- Research of tannins

To 1 ml of extract, a few drops of a diluted 1% FeCl₃ solution are added. The appearance of a green-blackish color indicates the presence of tannins [6].

- Research of catechic tannins

30 ml of extract, add 15 ml of Stiasny's reagent, then heat in a water bath at 90° for 15 min, the appearance of precipitates shows the presence of catechic tannins.

- Research of gallic tannins

We filtered and saturated the filtrate with sodium acetate, and then a few drops of FeCl₃ solution were added. The blue color indicates the presence of gallic tannins, not precipitated by the Stiasny reagent [7].

c- Identification of flavonoids

- Research of total flavonoids

The presence or absence of flavonoids in an extract can be detected by a simple and fast test. We put some drops of concentrated HCL (2N) and 0.5 g of Mg in 5 ml of the extract. Leave to act for 3 min. The pink-red or yellow coloration, after 3 min of incubation at room temperature, indicates the presence of flavonoids [8].

- Research of anthocyanins

1 ml of extract is added 1 ml of sulfuric acid, then 1 ml of ammonium hydroxide. In the presence of anthocyanins, the color is accentuated by acidification, then turns to purplish blue in basic medium [7].

- Research for leucoanthocyanins

1ml of extract added to 1ml of hydrochloric alcohol and 1ml of isoamyl alcohol were introduced in a tube and then put in a water bath at 90°C for 15min. In the presence of leucoanthocyanes, a cherry red or purplish coloration develops [9].

3. Determination of phenolic compounds by the colorimetric method

a- Determination of total polyphenols

The total phenolic compounds of the extracts were determined using the Folin-Ciocalteu [10] and [11], 20 µl of sample added to 1.58 ml of distilled water, 100 µl of Folin-Ciocalteu reagent and mixed with 300 µl of saturated sodium carbonate (7.5%). The mixture was heated for 30 min at 45°C. The absorbance was measured at 764 nm after cooling to room temperature. Total phenolic content was expressed as mg gallic acid equivalents per g of sample (mg GAE/g).

b- Determination of total flavonoids

The total flavonoid content was performed according to the method reported by [12]. This method consists of mixing 1 ml of the sample with 1 ml of AlCl₃ solution (2%). The samples were incubated for 1 h at room temperature. The absorbance was determined using a spectrophotometer at 415 nm. Total flavonoids were expressed as quercetin equivalents by reference to the standard calibration curve (mg EQ/g).

c- Determination of condensed tannins

Condensed tannins were determined by the vanillin method in an acid medium as a procedure by [13]. Thus, a 200 µL aliquot of each sample was added to the vanillin reagent (8% HCl, 37% methanol, and 4% methanolic vanillin). Then the tubes were placed in a water bath at 30°C for 20 min. This method is based on the ability of vanillin to react with condensed tannins in the presence of acid to produce a colored complex measured at 500 nm. Results were expressed as mg catechin equivalents per 1 g sample (mg CE/g).

III. RESULTS AND DISCUSSION

1. Phytochemical screening

A phytochemical investigation revealed various secondary metabolites. The experimental results of the phytochemical screening carried out on the aqueous extract of *Vitis vinifera* leaves showed the presence of polyphenols, catechic tannins, flavonoids, and leucoanthocyanins and the absence of gallic tannins and anthocyanins.

Table 2: Chemical revelation of aqueous extract of the studied varieties

Variety Active principle	BAK	BAH	BK	TB	TC	TK	DB	FA	LB
Polyphenols	+++	+++	+++	++	++	++	++	++	+++
Free tannins	+++	+++	+++	+	+	+	+	++	+++
Catechic tannins	+++	+++	+++	++	++	++	++	++	+++
Gallic tannins	-	-	-	-	-	-	-	-	-
Flavonoids	++	+	+++	++	++	++	++	+	+
Anthocyanins	-	-	-	-	-	-	-	-	-
Leucoanthocyanins	++	++	+++	++	+	++	++	+	+

+++ : Strongly positive; ++ : Moderately positive ; + : Weakly positive ; - : Negative.

These results are in agreement with those obtained by [14] having worked on the antimicrobial activity of extracts of the leaves of the wild vine (*Vitis vinifera sylvestris*) which revealed the presence of flavonoids and gallic tannins on the other hand the absence of catechic tannins, anthocyanins and leucoanthocyanins. In addition, [15] also found similar results on the red vine variety Ahmar Bouamar. Indeed, these authors report the presence of gallic tannins, flavonoids, anthocyanins and leucoanthocyanins. According to [16] and [17], the exposure of a vine variety to different environments influenced the production of secondary metabolites both in quantity and quality.

2. Results of the dosage of extracts from the leaves of the studied grape varieties

a- Total polyphenols contents

Using the Gallic acid calibration curve below, we deduced the total phenol content.

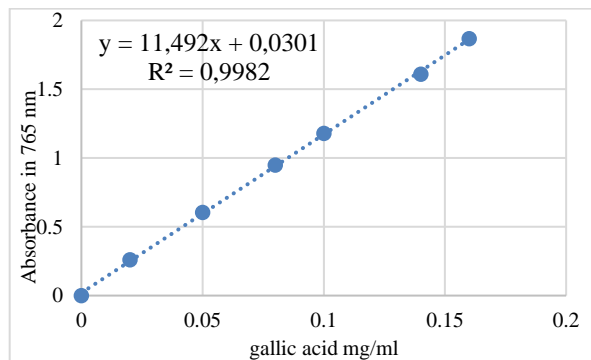


Figure 1: Calibration curve of gallic acid for the determination of total polyphenols

Summarizes the results obtained for the total polyphenol content of the extracts for each variety studied (Figure 2).

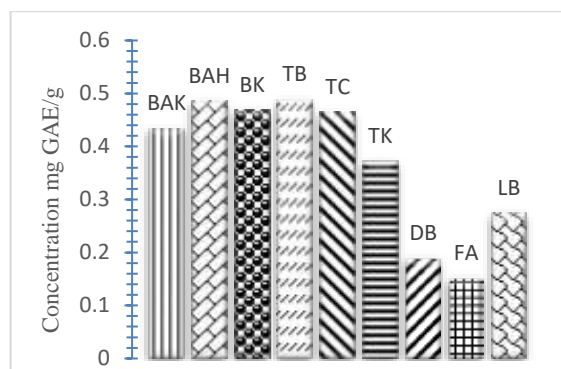


Figure 2: Polyphenol contents of the leaves of the studied varieties of *Vitis vinifera* L.

The highest polyphenol content was found in TB leaves with a value of 0.488 ± 0.032 mg SAG/g followed by the variety BAH with 0.485 ± 0.026 mg SAG/g. Subsequently, a concentration of 0.372 ± 0.035 mg WTG/g is noted in TK, a value of 0.273 ± 0.092 mg WTG/g in LB and a concentration of 0.188 ± 0.141 mg WTG/g in DB. The lowest concentration is marked in the variety FA with 0.151 ± 0.163 mg GAE/g.

b- Flavonoids content

Flavonoid content was determined using the Quercetin calibration curve, shown below.

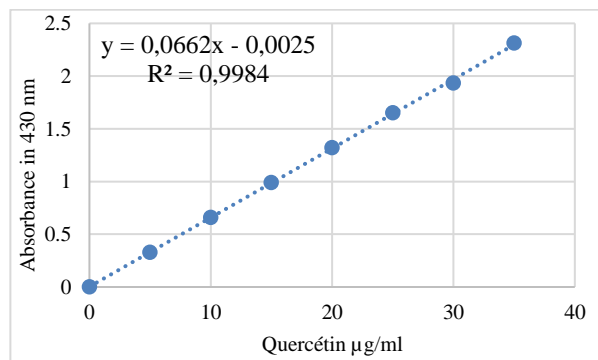


Figure 3: Quercetin Calibration Curve for Total Flavonoids

Summarizes the results obtained for the flavonoid content of the extracts for each variety studied (Figure 4).

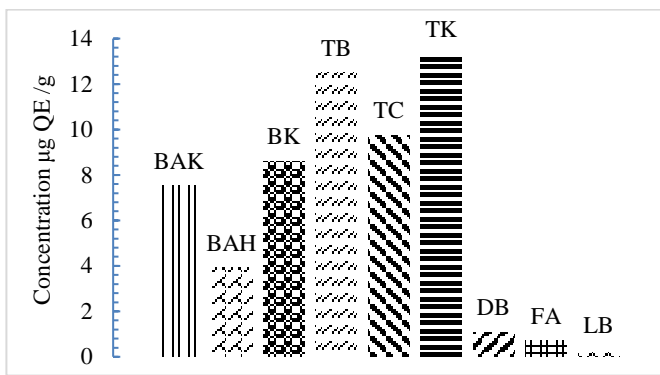


Figure 4: Comparison of flavonoid contents in the leaves of the varieties studied

The leaves of TK contain the highest flavonoid content with the value of 13.16 ± 0.265 mg EQ/g, followed by TB with 12.51 ± 0.064 mg EQ/g. The lowest contents are found in DB, FA and LB leaves with values of 1.08 ± 0.006 mg EQ/g, 0.71 ± 0.009 mg EQ/g and 0.28 ± 0.051 mg EQ/g, respectively.

c- Condensed tannins content

Tannin determination was expressed as mg catechin equivalent per gram of the extract (mg EC/g).

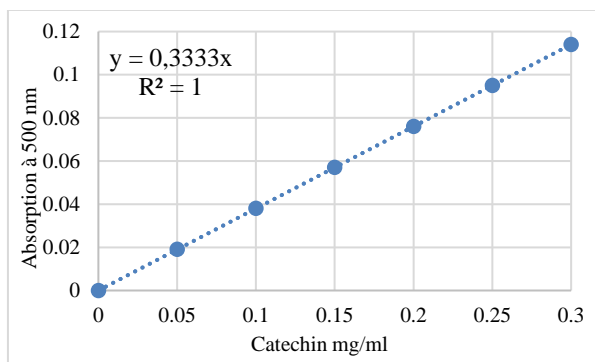


Figure 5: Catechin calibration curve for condensed tannin determination

Summarizes the results obtained for the condensed tannin content of the extracts for each variety studied (Figure 6).

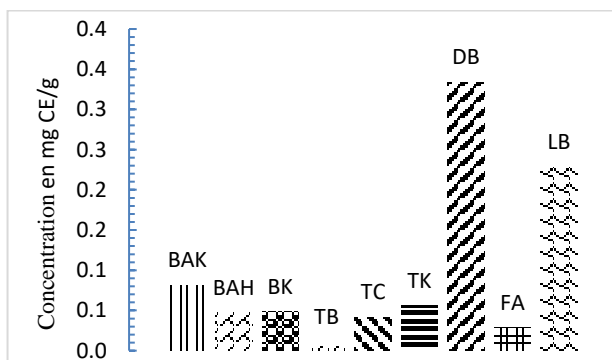


Figure 6: Comparison of condensed tannins content in the leaves of the varieties studied.

The highest condensed tannin content was found in DB leaves with a value of 0.333 ± 0.011 mg EC/g followed by a LB variety with 0.225 ± 0.003 mg EC/g. The lowest amounts were found in the leaves of TK, BAH, BK and FA with values around 0.057 ± 0.005 mg EC/g, 0.048 ± 0.038 mg EC/g, 0.042 ± 0.018 mg EC/g and 0.030 ± 0.008 mg EC/g, respectively. The lowest value of condensed tannins was noted in TB with the value 0.006 ± 0.005 mg EC/g.

Distinction between grape varieties on the basis of their polyphenolic profiles

The ampelographic description has grouped the varieties studied at the same level, which makes it difficult to separate them at the vegetative budding stage, but with this phytochemical study, we have been able to remove the ambiguity between the varieties that remain similar at the descriptive ampelographic.

According to [18] the OIV 003 descriptor which characterizes the intensity of the anthocyanin coloration of the young shoot has grouped our varieties in 4 subgroups, the first group with a null intensity which includes FA, BK and LB, the second group with a weak intensity which includes TB and TC, the third group with a medium intensity which includes BAK and TK and the fourth group with a strong intensity which includes BAH and DB.

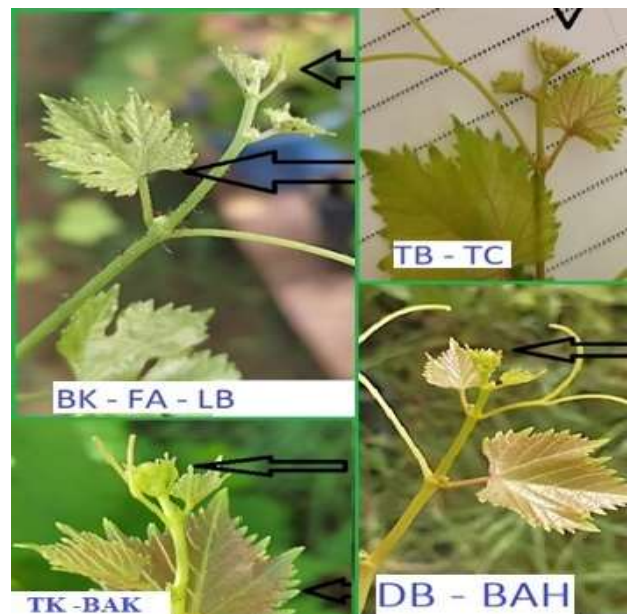


Figure 7: Young branches of the varieties studied [18]

It was possible to discriminate between identical varieties using phytochemistry; indeed, group 1 FA includes a high level of polyphenols, FA is low in tannins and flavonoids, and variety LB has a high tannin content. For group 2 TB is high in flavonoids and low in tannins, and TC high in tannins and low in flavonoids. For group 3, TK being rich in flavonoids and BAK being low. Finally, group 4 BAH is low in tannins and DB high in tannins.

The results are in agreement with [19] which studied the polyphenolic profiles of 50 grape varieties of different genetic-geographical groups, in fact the genetic-geographical groups were clearly separated according to their polyphenolic profiles, with all classes of compounds contributing to discrimination.

IV. CONCLUSION

The objective of this study was to characterize the phytochemistry of aqueous leaf extracts of nine autochthonous Moroccan varieties of *Vitis vinifera* in order to distinguish their descriptive similarities. Phytochemical analysis showed that all cultivars studied contained polyphenols, catechic tannins, flavonoids and leucoanthocyanins, but no gallic tannins or anthocyanins. Total polyphenols, flavonoids and condensed tannins were quantified, and results showed that some varieties, such as Bzoult Aouda Kahla and Hamra, Boukhanzir and Taferyalet Bayda, are rich in polyphenols. Taferyalet Kahla and Taferyalet Bayda also have high flavonoid contents, and the Dibanni variety has the highest tannin content. Descriptive ampelography which is clearly divided into variants can be completed through this phytochemical study. Depending on the polyphenolic profiles, varieties that remain similar in some OIV descriptors have been clearly separated, especially for vegetative organ traits at the early bud break stage.

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