Analytical characterizations of anthocyanins of the hydro-alcoholic extract of fruits of *Grewia coriacea* Mast

Madiélé Mabika A.B.[1,4,*], Ngakegni-Limbili A.C.[1], Agnaniet H.[2], Nyegue M.A.[3], Quio Zao J.M.[4], Thiery V.[4], Ouamba J.M. [1]

[1] Unité de Chimie du Végétal et de la vie (UC2V), Université Marien Ngouabi, Congo.
[2] Laboratoire des Substances Naturelles et de Synthèse Organométallique (LASNSOM), Université des Sciences et Techniques de Masuku, Franceville (Gabon).
[3] Laboratory of Microbiology, Faculty of science, University of Yaounde I (Cameroon).
[4] Littoral Environnement et Sociétés (LIENSs), Université de La Rochelle, France.

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Abstract :
Grewia coriacea Mast (Malvaceae-Grewioideae) is a plant species of the Central African indigenous forest that produces edible fruits. It is the most commercialized fruit in Congo Brazzaville. However, *Grewia coriacea* just like many other fruits of the African indigenous forest is still to be thoroughly studied. In effect, the dietary potential of its fruits has been on the spotlight of some works, though obvious that its coloring property in food remains unknown. The urge to valorize tropical plant resources as well as the diversity of their metabolites for the food and cosmetic industry guided us to undertake the characterization of the anthocyanins found in the fruits of *Grewia coriacea*. The anthocyanin malvidine-3-*O*-glucoside was isolated after analytical characterizations carried out using chromatographic and spectroscopic methods such as the preparative thin layer chromatography (TLC), analytic high pressure liquid chromatography (HPLC), semi-preparative HPLC, visible-ultra violet spectrometry (UV) , nuclear magnetic resonance (NMR), high resolution mass spectrometry (HRMS). This compound (malvidine-3-*O*-glucoside) had not been previously identified in *Grewia coriacea*not even in any of the species of *Grewia*.

Keywords : Characterization, anthocyanins, hydro-ethanolic extract, *Grewia coriacea* Mast.

Caractérisations analytiques des Anthocyanes de l'extrait Hydro-alcoolique des fruits de *Grewia coriacea* Mast

Résumé :
Grewia coriacea Mast (Malvaceae-Grewioïdeae) est une espèce végétale de la forêt spontanée d'Afrique centrale qui produit des fruits comestibles. C'est l'un des fruits de cueillette le plus commercialisé au Congo Brazzaville. Cependant, comme beaucoup d'autres fruits de la flore spontanée Africaine, *Grewia coriacea* reste mal étudié et ses potentialités alimentaires et tinctoriales de ces fruits sont inconnues. La valorisation des ressources végétales tropicales ainsi que la diversité des métabolites pour l'alimentation et l'industrie cosmétique, nous ont conduits à la caractérisation des anthocyanes présents dans les fruits de *Grewia coriacea*. Cette étude nous a permis d'isoler un anthocyane, le malvidine-3-O-glucoside, après une caractérisation analytique menée au travers des méthodes analytiques (CCM-préparative, CLHP-analytique, CLHP semi-préparative, UV, RMN, SMHR). A notre connaissance, ces résultats seraient les premières données publiées concernant la composition chimique de l'extrait hydro-éthanolique de *Grewia coriacea*.

Mots clés : caractérisations, anthocyanes, extrait hydro-éthanolique, *Grewia coriacea* Mast

Introduction

From time immemorial, man in all civilizations has been using dyes from natural origin to color their fabrics and craft works. They are pigments or compounds from plants and animals. The fact that they are visible has triggered their study and facilitating the research techniques. The notion of color, an indispensable factor in the domain of arts, decoration and craftwork could equally become a sign of recognition, hierarchical mark or a means of expressing sentiments could be obtained from natural elements up to the XIXth century. These colorants were pigments of mineral origin: iron oxide for yellow, ocher and red, manganese oxides for brown. From 1500 before our era, Egyptians produced dyes with saffron (yellow), pastel (blue) and madder (red). The latter are either extracts from plants, trees, lichens, or from animal origin, extracts from insects like kermes, mulluscs. In effect, nature offers a wide variety of colors, marvelously rich in dyes and nuances.Elsewhere, a mastery of compounds or materials and techniques used in the making of arts works, has evolved with scientific progress ancient times to the contemporary era. In effect, the identification of natural chemical constituents used on different medicinal, archeological and artistic surfaces is of great interest in valorizing them. The oral transmission of traditional values endowed with ancestral wisdom remains a stumbling block to the handing down of these values and the absence of a data base rooted on scientific studies which is a reference point is a major disadvantage. The development of science and art has gone hand in hand with the perception that our heritage which in a large part is fragile and dispersed should be saved and highlighted. It is in this light that our research team for the past years has initiated the validation of plants used in this domain in collaboration with « ARRDHOR CRITT of Roche Fort sur Mer» experts in the domain of pigments, resins and colorants.

These colorant substances are secondary metabolites of the polyphenol class (figure 1) among which are

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(*) **Correspondance :** Madiélé Mabika Aimé Bertrand ; e-mail: madieleaime@gmail.com ; tél : (+242) 06 620 90 84

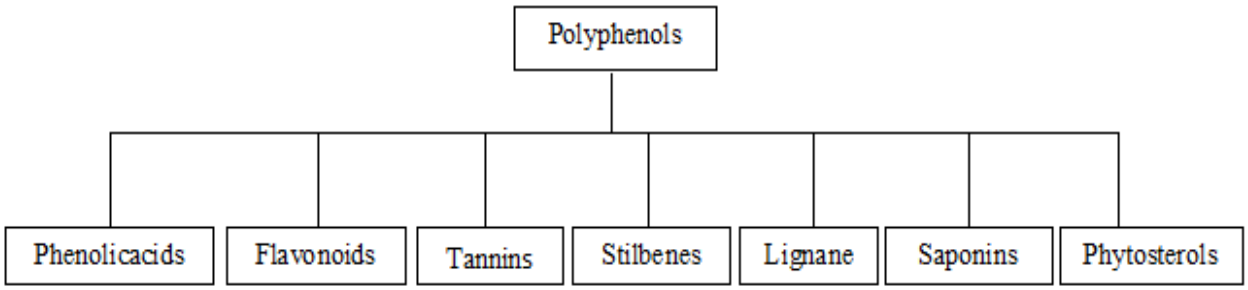

Figure 1: The different classes of phenolic compounds

anthocyanins, molecules of the flavonoids family, are pigments that are responsible for the coloring of some plant parts (flower, fruits leaves, stems roots or grains) into blue, red, mauve, pink or orange (Brouillard, 1993).

In general, literature review shows that anthocyanins have been the subject of many works from the structural (structure and synthesis) and physiological points of view (Blank, 1947, 1958; Sannie and Sauvain, 1952). Chemical studies have been particularly focused on the anthocyanins from red wines (Eder *et al*, 1990, 1994; Mattivi*et al*, 1990; Roggero*et al*, 1998; Arozarena*et al*, 2000, 2002; Garcia-Beneytez, 2002; Revilla *et al*, 2001; Heier*et al*, 2002; Burns *et al*, 2002; Ottenederet *al*, 2002) wherein different analytical methods have been used. Thus, Wulf *et al* (1978) detected and identified 21 anthocyanins meanwhile Heier et al (2002) identified 40 by liquid chromatography coupled to mass spectrometry. In the same way, several secondary metabolites from different species of *Grewia* have been isolated principally triterpenoids, fatty compounds, flavonoids, steroids, saponins and tannins, alkaloids and glycosides (Goyal, 2012). The composition of anthocyanins could be very complex. Generally, these pigments always exist in the form of heterosides (Malien, 2002) and are made up of three parts namely:

- ***The flavylium nucleus (chromophore)*:** It is made up of the flavylium cation base molecule otherwise called 2-phenyl-1-benzopyrilium. The positive charge is usually located on the central oxygen atom. The oxonium notation is just a conventional writing because the positive charge is delocalized on the entire structure (Onyamboko, 2012) (figure 2).
- ***Sugars*:** Anthocyanins are glycosylated usually at position 3 and 5 and the most common sugars (monosaccharides) are glucose, galactose, rhamanose and arabinose but could also be polysaccharides associated in di and/ or tri saccharides formed by the successive combination of monosaccharides.

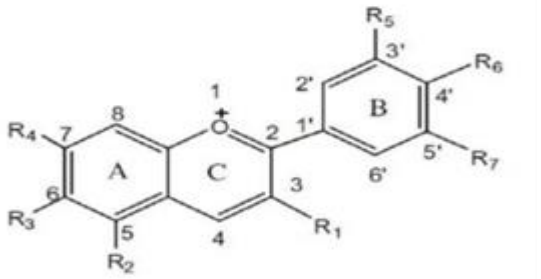

Figure 2: Base skeleton of anthocyanins: structure of flavylium cation (2-phényl-1-benzopyrilium)

In nature, not all the hydroxyl groups of anthocyanins are glycosylated, one free hydroxyl group is necessary to generate all the variety of colors responsible for the flower and fruits pigmentation.

- ***Acylated acids*:** Sugars on their part could be acylated or not by acids: para coumaric acid, cafeic acid, ferulic acid, cinnamic acid or better still malonic acid.

Valorization of tropical plant resources as well as the use of fruits as food or dyeing organ and its abundance in anthocyanins led us to characterize the hydro ethanolic extracts of *Grewia coriacea* whose anthocyanins have never been a subjected to any study.

Grewia species belongs to the Malvaceae family which is made up of shrubs, small trees found in the hot regions (tropical and subtropical) of the world and it is among the genre of this family whose fruits are comestible but some of them are known for their medicinal properties in different parts of the world (Goyal, 2012 ; Dalziel, 1937). There are about 48 genres and 725 species round the world. Close to 600 species of this genre have been identified in

Africa, among which we find 29 species in Congo distributed in 10 genres. *Grewia coriacea* (Malvaceae-Grewioïdeae) is a fruit-tree of the indegenous forest of Central Africa. It is a tree or shrub of 4 to 25 m high measuring 12 to 40 cm in diameter (Annonyme, 1963). Fruits of *Grewia coriacea* commonly called tshui-teke in Congo Brazzaville are categorized as grapes. They are piriform ovoid drupes with a tough exocarp and a grain (Attibayeba et al, 2007, 2010). They are green when immature and become bright reddish-black or purplish when mature. At maturity, the fruits exhale a reddish juice used in the manufacture of many drinks (juice, syrup, liquor) as reported by Nkourissa (2005). Moreover, they are rich in proteins, lipids, ascorbic acid and sugars representing a huge nutritional potential (Attibayéba et al, 2007, 2010). Due to their color depicting that of haemoglobin and drawing inspiration from the theory of signature, *Grewia coriacea* fruits are traditionally used to treat anemia. Dyes extracted from its fruits are used to dye raphia by the indigenous people. The red extract is usually mixed with clay (kaolin) to decorate many ornaments in red during traditional marriage ceremonies (make-up of the bride). The phytochemical screening of the mature fruits of *Grewia coriacea* shows that they are rich in anthocyanins (Ongoka et *al,* 2006, Madiélé et al, 2013; 2015). We too equally analyzed by HPLC-DAD a fraction of concern then isolated and identified by visible UV spectrometry, high resolution mass spectrometry and the 1D and 2D NMR malvidine-3-*O*-glucoside which till date has not been isolated thereby raising our curiosity.

Material and methods

- ***Plant material:*** Mature fruits of *Grewia coriacea* were harvested in July 2012 in a forest near Mbandaka village (Imvouba) at 137 km on the national 2 (North of Brazzaville). They were washed and frozen at a temperature less than or equal to – 5 ^{0}C. A few days later, the fruits were thawed out and dried in an oven at 35 ^{0}C.

- ***Preparation of the hydro ethanolic extract:*** The extracts were prepared using mature fruits in a 1 % acidified hydro ethanolic solvent mixture by maceration. 1 kg of the whole fruits were macerated for 2 hours at 60 ^{0}C under mechanical agitation then pressed in 1000 mL of water/ ethanol mixture (1: 1 v/v).The extract was dried in an oven at 35 ^{o}C after filtration and evaporation at a reduced pressure. The dried extract was ground using a blender to obtain a colored powder (pigment) in the form of oxonium chlorides (red). The pigment is then dissolved in a hexane/ water mixture (1: 1 v/v) to get rid of its lipid content and the aqueous extract lyophilized prior to characterization.

- ***Identification of anthocyanins:*** Rapid identification tests relying basically on the structural transformation of anthocyanins were used. In the presence of 1 N HCl, the extract picks up a bright red coloration. On contact with a solution of NH_4OH, the color changes to green then greenish-yellow to yellow before ultimately decolorizing in the presence of sodium bisulfite solution.

- ***Fractionation and purification of the hydro ethanolic extract:*** The fractionation and purification of the hydro ethanolic extracts were realized using 10 g of the extract according to the experimental procedure here below (figure 3).

- ***Preparative thin layer chromatography*** **:** The compounds were separated by preparative thin layer chromatography. The plates used are silica gel plates of 1 mm thickness on 20 cm x 20 cm glass (Merck 60 F 254). The extracts were dissolved in acidified (1 % HCl) methanol solution and then deposited on the plates. The plates were then eluted in a chromatographic tank saturated with a mixture of solvents CH_3COOCH_2/ CH_3COOH/ HCOOH/ H_2O (80:10:10:25 v/v/v/v). The plates were then revealed under UV lamp at 254 nm. With the aid of a spatula, each plate was then retrieved, absorbed in a small quantity of solvent prior to filtration to recover the compounds.

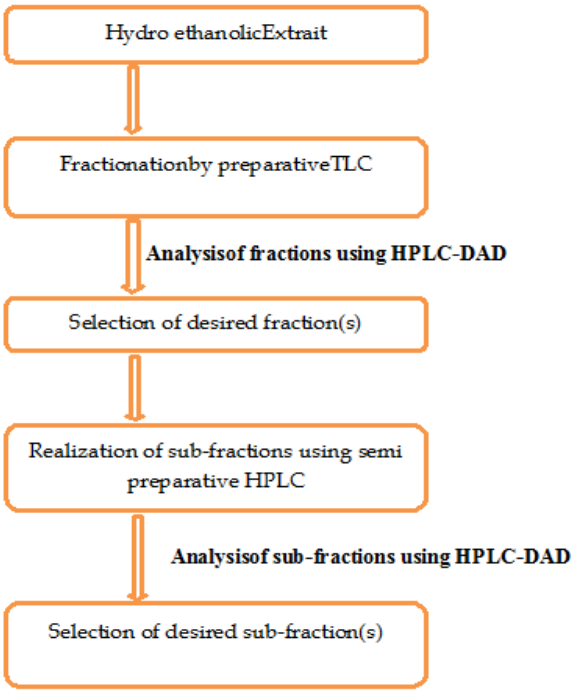

Figure 3 : Experimental procedure of fractionation and purification of the hydro-ethanolic extract.

- Sample preparation for analysis by HPLC-DAD: The dry extracts were dissolved in ketonitrile then filtered using a 0.45 µm membrane. The HPLC-DAD was realized with the help of a set of HPLC Waters 2996 fitted with a W600 pump and a UV detector with a visible range coordinated by a Waters software. The analyses were carried out in reverse phase with a HPLC column Uptisphere 300 °C C18 (5 µm, 250 x 4.6 mm). The actual separation of the anthocyanins was done by an elution gradient using a binary system. All the solvents used were of HPLC grade. They included acidified ultra-pure water (H_2O, solvent A) and an organic modifier (ketonitrile, solvent B). The detection of the anthocyanins was done using a diode electrode that produces different spectra for the anthocyanins eluted. Here below are the experimental conditions.

Injection volume:	30	mL
Flow rate:	1	mL/min
Temperature :	40	°C
Time of travel:	60	min
Time to revert to initial conditions:	5	min
Pressure :	112	bars
Detection :	280nm	

Elution gradient:

Time (min)	0	10	35	45	53	60
% A	70	50	0	0	70	70
% B	30	50	100	100	30	30

- Separation by semi preparative HPLC: The purification was realized using a set of Agilent 1200 semi preparative HPLC equipped with two pumps G136A and an injection inlet (2 mL for semi preparative column) and a UV detector. Separation of the compounds was done on an Uptisphere Strategy 100 A˚ (10µm, 30 x 250 mm) semi preparative column using an Uptisphere RP18 pre-column. The chromatographic conditions are the same to those of the analytical mode but for the following modifications: flow rate (10 mL / min), injection volume (900 mL) and the column. The mobile phase is made up of two solvents: A: water/ AcOH (99: 1 v/v) and B: ketonitrile/ AcOH (99: 1 v/v). The fractions were in bottles after evaporating the organic solvents using a rotavapor R-201 BUCHI at 45 °C and then lyophilized to remove traces of water from them.

- Identification and structure elucidation: The Three characterization techniques were used in order to obtain the necessary information to identify the anthocyanins. They include visible UV spectrometry, high resolution mass spectroscopy and the nuclear magnetic resonance spectroscopy.

- Visible UV spectroscopy: The Anthocyanins present absorption maxima both in the visible region (490 – 528) as well as in the UV region (260 – 280) (Billot, 1965). The extract (0.5 g) to be analyzed is dissolved in 25 mL of methanol (0.01 %) and then filter with a 0.45 µm micro filter. The solution may be diluted to reduce the absorption below 1.5.

- High pressure liquid chromatography spectrometry: The purified samples were analyzed with the help of BrukerMicrO-Tof-Q 2 mass spectrometer made of two quadrupoles and an orthogonal analyzer of time flight and equipped with electrospray sources (Electrospray ionization) or APCI. Methanol was the solvent for analysis.

- Nuclear magnetic resonance spectroscopy: The NMR spectra ^{1}H, ^{13}C, COSY ^{1}H-^{1}H, COSY ^{1}H-^{13}C and HMBQ were recorded on a Jeol brand 400 MHz spectrometer. A few milligrams of the lyophilized samples were dissolved in DMSO-d_6 in 5 mm (diameter) analytical tubes and then analyzed by 1D and 2D NMR.

Results and discussion

***- Identification of anthocyanins*:** In an acid milieu, the purplish-red extract gave a bright red coloration and a blue coloration in an alkaline medium. The extract decolorizes in the presence of sodium bisulfite. These observations reveal the presence of anthocyanins in the extracts. These results corroborate those of Ongoka et al (2006) that reveal the presence of anthocyanins in the aqueous extracts of *Grewia coriacea*. In effect, the variations of color depending on the pH of the medium characterize anthocyanins pigments. The bright red coloration in an acid milieu is due to the formation of the oxonium salt. The blue coloration in the basic medium could be ascribed to either the presence of flavonoid pigments (yellow in an alkaline medium) whose color superposes the anthocyanosids blue coloration or by the isomerization of anthocyanosid into its uncolored form but yellow in a basic medium (Sannie and Sauvain, 1952).

- Fractionation of the hydro ethanolic extract, purification of fractions and isolation of compounds: Three fractions FGC_1, FGC_2 and FGC_3.were obtained using the preparative TLC. It is worth noting the fractions FGC_2 (pink) and FGC_3 (brick red) decolorize in a solution of *meta* sodium bisulfite suggesting the presence of anthocyanins. Fraction FGC_3 was later purified by semi preparative HPLC on a silics plate in the reverse phase and six sub-fractions were obtained and coded GC_1, GC_2, GC_3, GC_4, $GC_5$and GC_6 (figure 4).

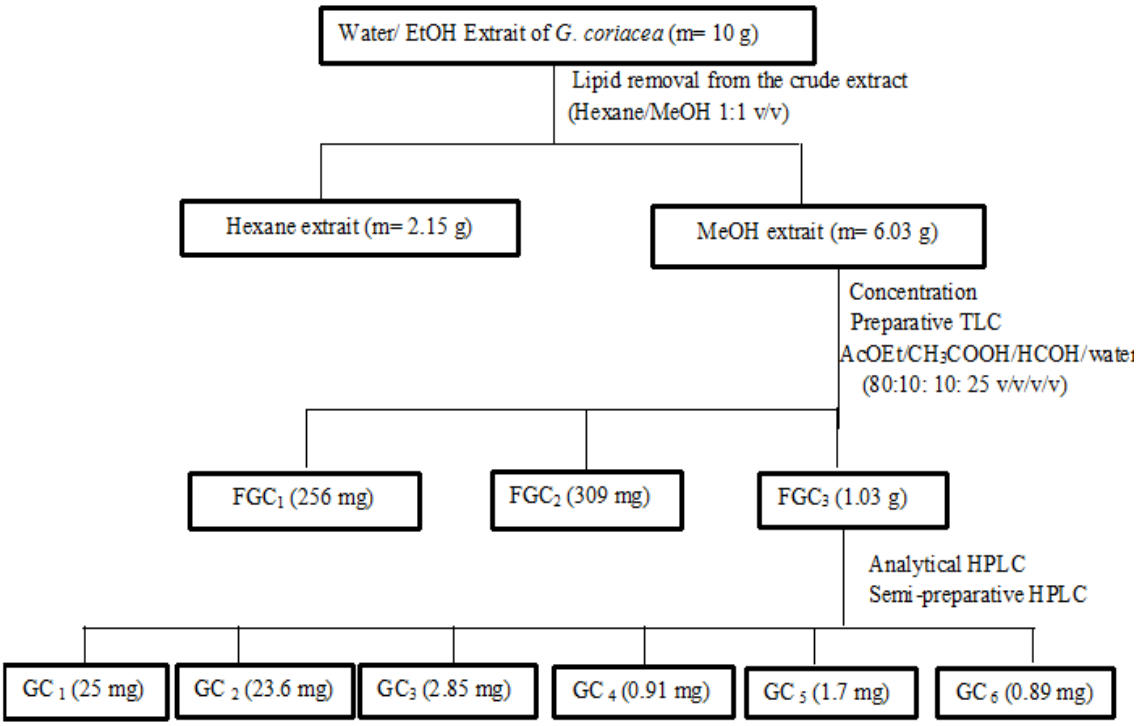

Figure 4: Fractionation, purification and isolationof the hydro alcoholicextract of *Grewiacoriacea.*

HPLC-DAD analysis of fraction FGC_3 gave the chromatographic profile of the extract at 280 nm and the visible UV spectra of some compounds (figure 5 and figure 6).

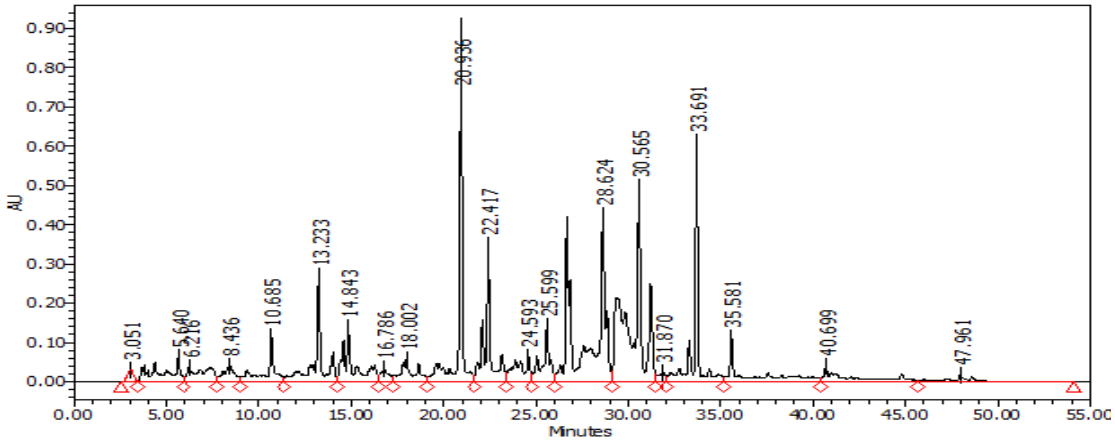

Figure 5: Chromatographicprofile of fraction FGC_3

The UV spectra of the compounds found in fraction FGC_3 show absorption maxima, characteristics of aromatic bands. Other compounds show maxima in the visible, characteristic of molecules of the flavonoid family (figure 7).

On analyzing the above UV spectra, it is obvious that the hydro ethanolic extract contains compounds which absorb in UV and consequently could be of the flavonoid family. Anthocyanins just like flavonoids possess two characteristic bands. One in the UV domain (270 -280 nm) and the other in the visible domain (465 – 560 nm) (Brouillard, 1993).

***- Isolation and characterization of anthocyanins in the GC_3 sub-fraction*:** Sub-fraction GC_3 is found in the form of a soluble powder in methanol. This compound decolorizes in the presence of sodium bisulfite. UV spectroscopy analysis of this sub-fraction shows two absorptions.

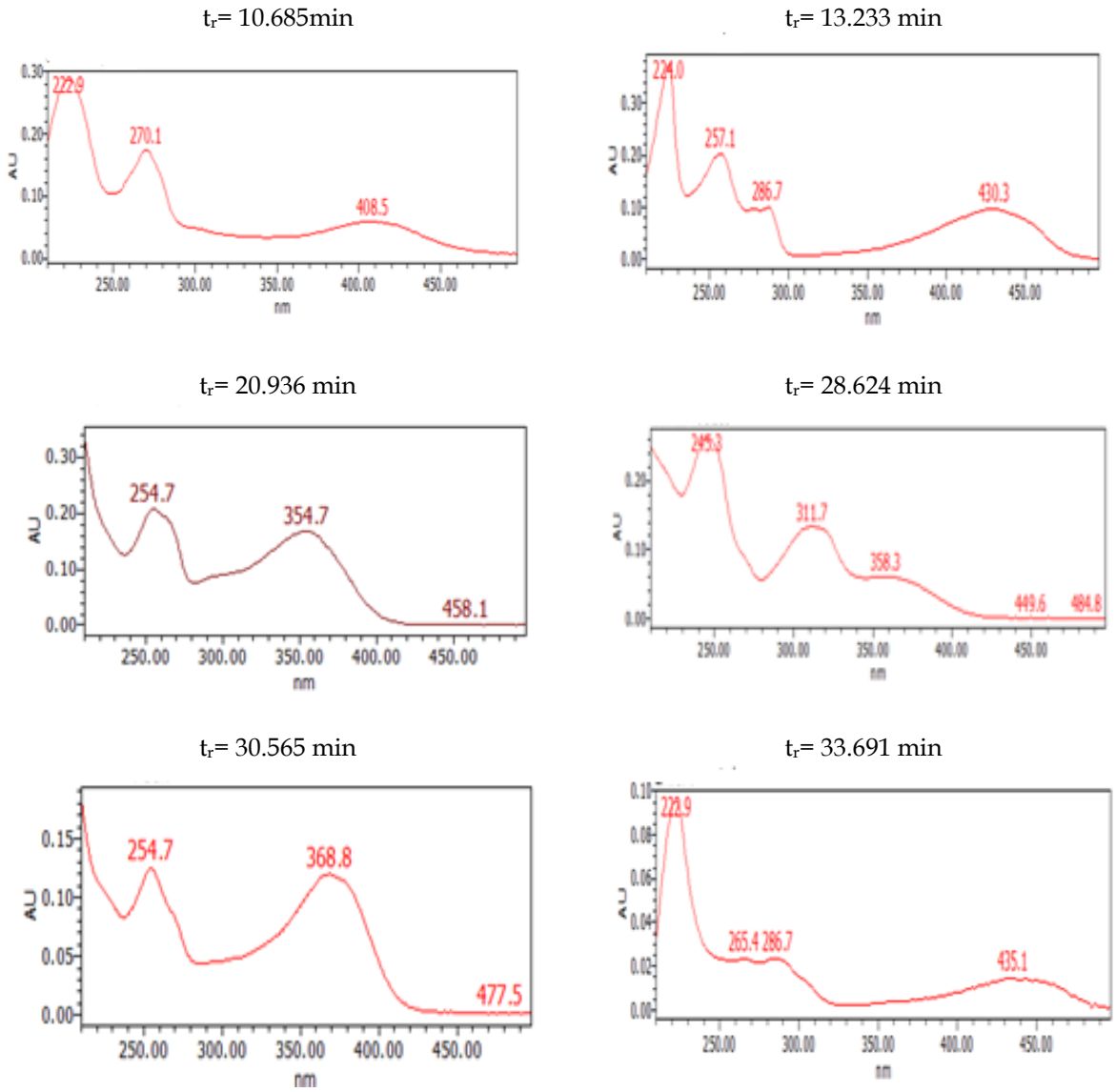

Figure 6: UV absorption spectra of some compounds in FGC3.

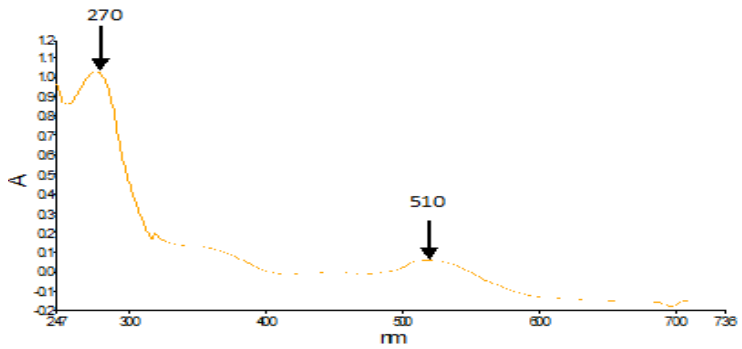

Figure 7: UV-visible spectrum of the compound in GC_3

The peaks at 270 nm and 510 nm give a clue of the presence of an anthocyanin compound. This spectrum is in conformity with that of a compound containing an anthocyanin. Mass spectrometry analysis of compound B shows the presence of a molecular ion $[M+Na]^+$ with a mass to charge ratio of positive ionization m/z = 515, compatible with the crude formula $C_{23}H_{24}O_{12}Na$, a 12 unsaturation compound suggesting the presence of unsaturation and/ or cycle (s).

This spectrum equally shows the presence of an ion at m/z= 497 initially corresponding to a rearrangement of a water molecule thereby confirming the presence of a hydroxyl group in the

molecule. MS-MS fragmentometry was also useful in revealing the successive loss of two fragments of 15 atomic mass units suggesting the presence of two methyl groups in the molecule. The presence of a sugar at C-3 is confirmed by the presence of a peak of m/z = 163.73.

Assessment of the ^{1}H NMR spectrum shows that it an aromatic compound substituted at positions 3, 5, 7, 3′, 4′ and 5′. Two singlets each carry three protons at chemical shifts δ3.52 and 3.57 ppm in the ^{1}H NMR spectrum (Table 1).

Analysis of the ^{13}C NMR spectrum and the DEPT 90 and 135 sequences show signals that are characteristics of aglycone. Six quaternary carbons (one at δC 177.98 ppm two between 100 and 110 ppm, three between 70 and 100. Also are two

Table 1: ^{1}H NMR data for compound B (NMRat 500 MHz; DMSO-*d*$_6$)

	DMSO-d$_6$			DMSO-d$_6$	
Position	**δ_C(ppm)**	**δ_H(ppm)**	**Position**	**δ_C(ppm)**	**δ_H(ppm)**
4	-	9.53, s	1′′	102.1	4.49
6′	-	7.49, s	2′′	71.7	4.18
2′	-	7.48, s	3′′	72.5	4.16
6, 8	-	6.59, s	4′′	73.8	4.08
3′	82.2	3.57, s	5′′	70.5	4.06
5′	78.0	3.52, s	6′′	18.6	4.05

signals at δ 58.66 and 54.94 corresponding to primary carbons of which that of ^{13}C *J*-modulated indicate that its methyl groups belong to two methoxyl groups.

Evaluation of the COSY ^{1}H-^{1}H spectrum shows thatthe H-4 at δ = 4.26 ppm correlates with the two protons at δ = 3.50 ppm and δ = 2.50 ppm. These two nuclei correlate on the HMBQ spectrum with the atom of carbon C-5 to be equally noted is the correlation of the first proton with C-3. These corroboration coupled to those of the COSY ^{1}H-^{1}H permit the attribution of two protons at H-2′and H-6′ respectively. These data lead to an anthocyanin glycosylated at position 3. The oxygen bridge between the flavylium and sugar is confirmed by the signal at δ = 109.73 ppm on the ^{13}C spectrum pertaining to an anomeric carbon in the sugar. The anomeric proton of the glycosyl appear at δ = 4.49 ppm in the form of a doublet. This value suggests a β-type junction of the glycosyl and an axial orientation of H-2′′of the sugar. This excludes mannose as a substitute and glucose which admits C-6′′ in the form of CH_2OH at δ = 61.59 ppm. All the above data lead to the structure proposed here below (figure 8).

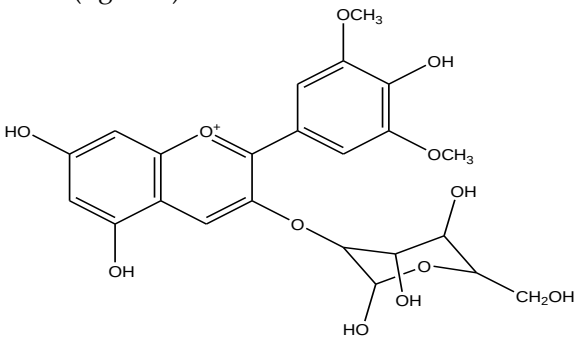

Figure 8: Structure of malvidine-3-*O*-glucoside.

Conclusion

This study carried out on *Grewia coriacea* proves that the species contains anthocyanin compounds. During the course of this study, one anthocyanin was identified as malvidine-3-*O*-glucoside. Characterization of this anthocyanin involved separation by TLC and a purification step by HPLC followed by analyses by high resolution mass spectrometry (HRMS), ultra violet (UV) and nuclear magnetic resonance (NMR) techniques. To the best of our mind, this anthocyanin is the first to be isolated and identified in *Grewia coriacea* of which studies on it are still unknown internationally. This study paves a new way in the diversification research of anthocyanin resources. The presence of anthocyanins in *Grewia coriacea* confers to this species a real cosmetic and dietary potential.

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References

Anonyme, 1963. Flore du Congo Belge, du Rwanda et du Burundi Spermatophytes, **8**(1) et 10, Publ. INEAC, Bruxelles.

Attibayeba L., Ngantsoué L., Essamambo F. et Nkourissa A.C., 2007. Changes in the chemical compostion of the fruits of *Grewia coriacea* Mast during development and ripening. *Fruits*, **62**(6), 369–375.

Attibayeba L., Ngantsoué, L., Massamba et al., 2010. Lipid changes in kernels of the fruits of *Grewia coriacea* (Tilliaceae) during development and ripening. *Rivista Italianadelle Sostanze* Grasse, **87**, 58–62.

Adjanahoun E.J., Ayri A.M.R., Ake Assi L., Baniakina J. & Chibon P., 1988. Contribution aux études ethnobotaniques et floristiques en République Populaire du Congo. Agence de Coopération Culturelle et Technique. Paris. 605p.

Arozarena I., Casp A., Marin R., Navarro M., 2000a. Differenciation of some Spanish wines according to variety and region based on their anthocyanin composition. *Eur. Food Res. Technol.*, **212**, 108–112.

Arozarena I., Casp A., Marin R., Navarro M., 2000b. Multivariate differenciation of some Spanish wines according to region and variety. *J. Sci. Food Agric.* **80**: 1909-1917.

Blank F., 1947. The anthocyanin pigments of plants, *Bot. Review,* **13**, 241–317.

Blank, F., 1958. Antocyanins, flavonones, xanthones, *Handbuch der Pjlanzenphysiologie*, Springer Verlag, Berlin. Gottingen, Heidelberg, **10**, 300–343.

Billot J., 1965. Analyse des pigments anthocyaniques des fleurs de Jacaranda, Annales de l'Université de Madagascar (Sciences), **2**, 41-55.

Brouillard R., 1993. The flavonoids, advances in research since 1986, éd. J.B. Harborne, Chapman and Hall, London, 525-638.

Burns I., Mullen M., Landrault N., Teissedre P.-L., Lean M.E.I., Crozier A., 2002. Variations in the Profile and Content of Anthocyanins in Wines made from cabernet Sauvignon and hybrid grapes. *J. Agric. Food Chem.,* **50**, 4096-4102.

Dalziel K., 1937. The useful plants of West Africa. Crown Agenta, 41 Millbank, West minister, London, S.W.L 98.

Eder R., Wendelin S., Barna J., 1990. Auftrennung der monomeren Rotwein anthocyane mittels Hochdruckflüssigkeits chromatographie (HPLC, Method en vergleich und Vorstellungeinerneu en Methode. Mitt. Klosterneuburg, **40,** 68–75.

Eder R., Wendelin S., Barna J., 1994. Classification of red wine cultivars by means of anthocyanin analysis. *Mitt. Klosterneuburg*, **44**: 201 – 212.

Garcia-Beneytez E., Revilla E., Cabello F., 2002. Anthocyanin pattern of several red grape cultivars and wines made from them. *Eur. Food Res. Technol*. **215**, 32-37.

Heier, A., Blaas, W., Drob, A., Wittkowski R., 2002. Anthocyanin Analysis by HPLC/ESI-MS, *Am. J. Enol. Vitic.*, **915,** 53-60.

Madiélé Mabika A.B., Nkounkou Loumpangou C., Agnaniet H., Moutsamboté J-M., Ouamba J.M., 2013. Les plantes tinctoriales d'Afrique Centrale: enquête ethnobotanique et screening phytochimique, *Journal of Applied Biosciences*, **67**, 5236–5251.

Madiélé A.B., Quio Zhao J.M., Thiery V., Agnaniet H., Brunet C., Graber M., Ouamba J.M., 2015. Caractérisations Analytiques des Extraits Colorants des Plantes Tinctoriales d'Afrique Centrale. *Lebanese Science Journal,* **16**(1), 33-44.

Makany L., 1976. Végétation des Plateaux Téké (Congo). Thèse d'Etat. Paris. Orsay. 301 p.

Malien-Aubert C., Dangles O., Amiot M.J., 2002. Influence of procyanidins on the color stability of oenin solutions. *J. Agric. Food Chemistry*, **50,** 3299-3305.

Mattivi F., Scienza A., Failla O., Vika P., Anzani R., Redesco G., Gianazza E., Righetti P., 1990. *Vitis vinifera*- a chemotaxonomic approach: Anthocyanins in the skin. *Vitis (special issue)*, 119–133.

Nkourissa A.C., 2005. Quelques aspects du métabolisme, de la croissance et la maturation sur l'arbre du fruit de *Grewia coriacea* Mast. Brazzaville, Univ. MarienNgouabi, Mémoire CAPES, 39p.

Ongoka P. R., Matini L., Moutou J.M., Youhouvoulou Ngabé D., 2006. Evaluation des propriétés physico-chimiques et du profil chimique des indicateurs colorés naturels locaux. cas des extraits de : *Hibiscus sabdariffa*, *Amaranthusoleraceus*, *Grewiacoriacea*, *Spinaciaoleracea*et *Bougainvilleaspectabilis*. *Annales de l'UniversitéMarien NGOUABI*, **7**(3), 138–146.

Onyamboko N.V., 2012. Substances naturelles. Note de cours L2 chimie, département de chimie, Faculté des sciences (UNIKIN).

Otteneder H., Holbach B., Marx,R., Zimmer,M., 2002. Rebsortenbestimmung in Rotweinmittels Anthocyan spektrum, *Mitt. KLosterneuburg,* **52**, 187-194.

Otteneder H., Marx R., Olschimke D., 2001. Method-performance study on the determination of nine characteristic anthocyanins in wine by HPLC. O.I.V.F. N° 1130.

Goyal K.P., 2012. Phytochemical and pharmacological properties of *Genus Grewia*: A review. *Int J Pharm Pharm Scie*, **4**(4), 72–74.

Revilla E., Garcia-Beneytez E., Cabello F., Martin-Ortega G., Ryan J-M., 2001. Value of high-performance liquid chromatography analysis of anthocyanins in the differentiation of red grape cultivars and red wines made from them. *J. Chromatogr. A.*, **53,** 78-86.

Roggero I.P., Larice I.L., Rocheville-Divorne C., Archier P., Coen V., 1998. Composition Anthocyanique des cépages. Revue Française d'œnologie, **112**, 41–48.

Sannie Ch. et Sauvain H., 1952. Les couleurs des fleurs et des fruits. Anthocyanes et flavones. *Mémoire du Muséum d'Histoire Naturelle*, Nouvelle Sér, Sér. B, II.

Wulf L.W., and Nagel C.W., 1978. High-Pressure liquid chromatographic separation of Anthocyanins of *Vitis vinifera*. Am. J. Enol. Vitic., **29**, 42-49.