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Evaluation of the antioxidant potential and aglycone coumarins contents of ethereal extracts of 4 medicinal plants from Côte d'Ivoire

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Abstract
Spectrophotometric determination of aglycone coumarins at the maximum wavelength of 275 nm was performed on the ethereal extracts of the leaves of *Zanthoxylum gilletii* (ZG), *Dichrostachys cinerea* (DC), *Vernonia amygdalina* (VA) and *Morinda lucida* (ML), four plant species used in the trade therapy of various ailments in Côte d'Ivoire. ZG leaves with a value of 22.5692±0.0007 mg EC/g recorded the highest content of total aglycone coumarins, followed by VA (14.8808±0.0002 mg EC/g), ML (11.0068±0.0004 mg EC/g) and DC (8.9354±0.0006 mg EC/g) leaves. On the other hand, the ethereal extracts of the leaves of the four plants exhibited significant antioxidant efficacy, with CR_{50} of 0.024010±7x10^{-6}% mg/mL for ZG, 0.01789±0.3x10^{-5} % mg/mL for DC, 0.01995±0.2x10^{-5} % mg/mL for VA, and 0.04831±0.3x10^{-5} % for ML compared to that of vitamin C (0.016150±0.3x10^{-6} %). Thus, the pharmacological properties of these plant species of the Ivorian flora would be due to their richness in coumarins.

Keywords: *Z. gilletii, d. cinerea, v. amygdalina, m. lucida*, dosage, aglycone coumarins, antioxidant potential

1. Introduction
Free radicals (FR) and other reactive oxygen species (ROS) originate either from normal essential metabolic processes in the human body or from external sources. In the biological system, FR are often derived from oxygenated, nitrogenous and sulphurous substances. These unstable and highly reactive chemical particles with respect to their unpaired electron availability play an important role in cell signalling, gene expression, and ion transport [1, 2]. However, excess ROS can have deleterious effects on vital components (proteins, lipids, RNA, DNA, carbohydrates) of living matter [3, 4]. For this reason, there is a craze for the research of new antioxidants from plant sources, which has grown considerably because of their action in the prevention of diseases. Most natural antioxidants come from fruits, vegetables, spices, grains and plants. Among these phytonutrients of interest are antioxidants coumarins that may help protect against cellular damage due to oxidative stress and reduce the risk of chronic diseases [3]. Coumarins are an important subfamily of phyto phenols used to prevent and treat many diseases. These secondary metabolites possess anti-inflammatory, antioxidant [4], anticancer, anticoagulant [5] properties. Coumarins are distributed in free (aglycone) or bound (hetero side) form in various botanical families (Apiaceae, Asteraceae, Fabaceae, Rubiaceae, Solanaceae, rutaceae) [6-9]. They can be distributed to all parts of the plant depending on the growing conditions [10]. The present study focused on the total coumarins of four medicinal plants namely *Zanthoxylum gilletii* (De wild.) P.G. Waterman (Rutaceae), *Dichrostachys cinerea* (L.) Wight & Arn (Fabaceae), *Vernonia amygdalina* Delile (Asteraceae) and *Morinda lucida* (L.) Bent (Rubiaceae). They are traditionally used in Côte d'Ivoire to treat high blood pressure, diabetes, eye pain, sterility, fever, gonorrhoea, malaria, rheumatism [11-14]. They were chosen on the basis of a chemotaxonomic approach. In addition, these plant species come from the Ivorian floristic biodiversity, and seem to have never been the subject of an exclusive study on coumarins, both phyto chemically and biologically.

2. Materials and methods
2.1 Plant material
The plant material consists of aerial parts of the 4 study plants, growing in different localities of Côte d'Ivoire. Leaves of *V. amygdalina* (VA) were collected from Anyama (5° 29′ 40″ north, 4° 03′ 06″ west, a city in the southern of Côte d'Ivoire, autonomous district of Abidjan),

those of *D. cinerea* (DC) at Dabakala (8° 21′ 48″ north, 4° 25′ 43″ west, town in central Côte d'Ivoire in the Bandama Valley region) and those of *Z. gilletii* (ZG) and *M. lucida* (ML) at Alépé (5° 29′ 46″ north, 3° 39′ 49″ west, town northeast of Abidjan of the District des Lagunes). The identification of the plant material was confirmed by the late ASSY Jean of the Centre National de Floristique (CNF) in Abidjan (5° 20′ 11″ North, 4° 01′ 36″ West), in accordance with existing herbaria. Samples are deposited in our laboratory. The plant material is dried under air conditioning (18 °C) for 2 weeks, and then milled with an electric grinder (Moulinex) to obtain powders used to perform the various analyses.

2.2 Methods

2.2.1 Selective extraction of aglycone coumarins

The extraction of coumarins was performed according to Grinkevitch and Safronitch (1993) [15] with some modifications. In a reflux heater, 50 g of each plant powder was hydrolysed in HCl (2N, 300 mL) for 2 h. After cooling, the hydro lysate separated from the pomace by vacuum filtration, is left to dry under an extractor hood for 48 h, then taken up in hexane (24 h). The pomace is exhausted with MeOH (100 mL) in a Soxhlet extractor for 2 h. After removal of the solvent, the extracted mass is successively treated with potash lyes at different concentrations for 1 h: 90 mL KOH (0.5%; w/v) and 20 mL KOH (5%; w/v). The alkaline fractions are then collected and treated with ethyl ether (3 × 100 mL) to remove unwanted compounds. After decantation and separation, HCl (5%; v/v) is added cautiously to the alkaline phase to pH = 2. The reaction mass is subsequently treated with ethyl ether (3 × 100 mL). Ethereal fractions of total aglycone coumarins from the leaves of *V. amygdalina* (VAF), *D. cinerea* (DCF), *Z. gilletii* (ZGF) and *M. lucida* (MLF), are used for the determination of total aglycone coumarins (TAC) and the evaluation of antioxidant potential by spectrophotometry.

2.2.2 Determination of total aglycone coumarins by spectrophotometry

The TAC contents of the ethereal fractions are determined according to Anuj *et al*, (2012) [16]. From a methanolic solution of 2H-1-benzopyran-2-one (coumarin) (100 µg/mL) diluted to 1/10th, 2.5 mL of solution is taken, which was used to scan in the 190-600 nm region to determine the maximum wavelength at which the spectrophotometer is calibrated (Jasco, V-630). To 5 mg of each selective extract is added 10 mL of MeOH (80%), and the mixture is vortexed for 15 min. After filtration, the absorbance is measured at the wavelength of maximum absorption against a blank without extract taken as reference. Quantification of CAT was performed according to the calibration curve of equation $y = ax + b$ performed using coumarin (standard reference) at different concentrations (0.1954-25 µg/mL) under the same conditions as the extracts. TAC contents are expressed as milligram Coumarin Equivalent per gram (mg CE/g) of extract.

2.2.3. Spectrophotometric evaluation of the antioxidant capacity of total aglycone coumarin extracts

The method of Blois (1958) [17], repeated by Kabran *et al*., (2014) [18] was employed to measure the total antioxidant activity of total coumarin extracts. The stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was solubilized in absolute MeOH to obtain a solution of concentration 0.3 mg/mL. Different concentration ranges of ethereal extracts (1; 0.5; 0.25; 0.1; 0.05; 0.025 and 0.01 mg/mL) are prepared in the same solvent. In dry and sterile tubes, 1 mL of extract and 2.5 mL of methanolic solution of DPPH are introduced. After shaking, the tubes are placed in the dark for 30 min. The absorbance of the mixture is then measured at 517 nm against a blank consisting of 1 mL of pure MeOH and 2.5 mL of DPPH solution. Ascorbic acid (vitamin C) is the positive reference control. The reduction percentages (PR) of DPPH are calculated according to the formula

$$PR = ((A_b - A_e)/A_b) \times 100$$

A_b: absorbance of the blank
A_s: absorbance of the sample

For each extract, the concentration needed to reduce 50% of the DPPH radical concentration (CR_{50}), determining the antioxidant effectiveness of an extract, was determined using Origin Pro 9.1 software. The lower the CR_{50}, the more significant the antioxidant efficiency [19].

2.2.4 Statistical analysis

The experimental measurements are performed in triplicate and the results expressed as means plus or minus standard deviations (Mean ± SD). A one-way analysis of variance (ANOVA ONE WAY) is performed using OriginPro 9.1 software. The difference between the means is considered significant at the 5% level. In case it is significant ($p < 0.05$), the data are analysed by Tukey's post-hoc test (multiple comparison test).

3. Results and Discussion

3.1 Extraction yield of total aglycone coumarins

The yields of the extractions are reported in Table 1. It appears that the yields of TAC extracted from leaves vary between 0.22% and 0.25%. In addition, it is reported in the literature that the tonka bean, the fruit of the Cayenne guaiac (Dipteryx odorata) of the botanical family Fabaceae, is very rich in coumarin (1-3.5%) [20]. In view of the above, we believe that *D. cinerea*, *M. lucida*, *V. amygdalina* and *Z. gilletii* are significant sources of coumarins.

Table 1: Selective extraction yields of total aglycone coumarins

Plant species	Organ	Yield (%)
D. cinerea	Leaves	0,22
M. lucida	Leaves	0,23
V. amygdalina	Leaves	0,25
Z. gilletii	Leaves	0,24

3.2 Total aglycone coumarin content

TAC contents were determined at the maximum absorption wavelength 275 nm (Figure 1).

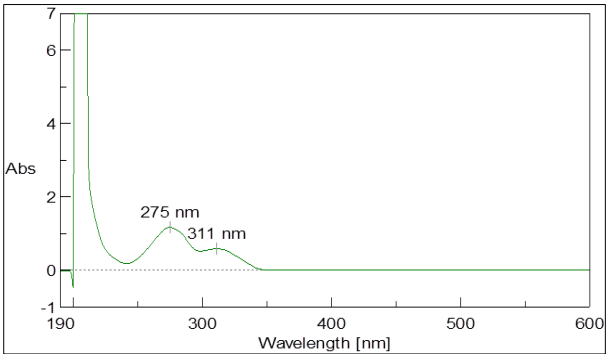

Fig 1: Maximum absorption wavelength of coumarin

The calibration curve for coumarin (Figure 2) shows that the absorbance varies linearly with coumarin concentration, and the equation of the calibration curve is given with a high regression coefficient (R2 = 0.999). The quantification of coumarin was determined based on the calibration curve of equation y = 0.0456 x.

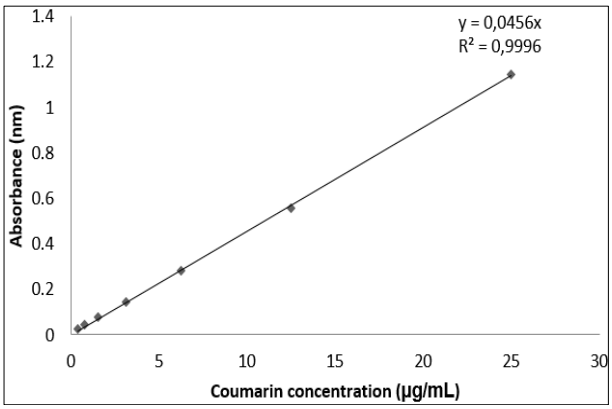

Fig 2: Coumarin calibration curve

The TAC contents of the study plants were subjected to analysis of variance (ANOVA). The ANOVA leads to $p < 0.05$; and showed a significant difference between coumarin contents.

The results (Table 2) show variable TAC contents, depending on the botanical family of the plant species. Furthermore, when comparing the TAC contents of the leaves of the different plants, it is noted that *Z. gilletii* with a TAC content of 22.57 ± 0.0007 mg EC/g, is the plant species with the highest coumarin content. This richness in coumarins observed in Rutaceae, represented by *Z. gilletii* has been reported in the literature by Venugopala *et al*., (2013) [21] in a study conducted on 30 plant families comprising about 150 species. On the other hand, the high levels of coumarins recorded in the leaves (the case of *V. amygdalina* and *Z. gilletii*) could be explained by their increased involvement in the defense system of the said plants. However, the coumarin content of a plant is dependent on environmental conditions; and may undergo variations over time [21]. Furthermore, according to Wittstock and Gershenzon (2002) [22] as cited by Audray Dugrand-Judek *et al*. (2015) [23], frequently attacked plant parts, are usually protected by a constitutive synthesis of defensive compounds. In addition, the TAC content of the leaves could also be explained by the marked photo-sensitizing action of coumarins, exploited by the plant at the leaf level for the stimulation of its pigments, enhancing their actions against the UV rays of the sun [24].

Table 2: Total aglycone coumarin content of ethereal extracts

Plant species	Organ	Total aglycone coumarin content (mg EC/g)
		Ethereal extract
D. cinerea (Fabaceae)	Leaves	8,9354±0,0006
M. lucida (Rubiaceae)	Leaves	11,0068±0,0004
V. amygdalina (Asteraceae)	Leaves	14,8808±0,0002
Z. gilletii (Rutaceae)	Leaves	22,5692±0,0007

3.3. Antioxidant profile by spectrophotometry of total aglycone coumarin extracts

The spectrophotometric method called DPPH in reference to the stable radical 2, 2-diphenyl-1-picrylhydrazyl, is a routine colorimetric test available to quantify the antioxidant potential of plant extracts containing phyto constituents likely to transfer hydrogen atoms. This method was used in this study because of its speed and reproducibility. Figure 3 shows the results of the antioxidant activity evaluation test, which refers to the concentrations of the different samples studied that reduce DPPH, in comparison with Vitamin C, the reference antioxidant. The fractions tested showed DPPH reduction percentages greater than 50% starting at 0.03 mg/mL, with the exception of MLF, which began to trap DPPH at 0.06 mg/mL. ANOVA applied to the mean of the percent reductions at 0.25 mg/mL leads to a $p<0.05$. Therefore, the differences between the values of the percentages of reduction are significant. Thus, at the concentration of 0.25 mg/mL, the ethereal extract of DCF with 94.691% ±0.005 shows the highest percentage reduction, followed by VAF (94.371±0. 004%) and ZGF (94.110±0.007%). The lowest percentage reduction was observed in MLF (87.924±0.009%) at the same concentration. Comparing the results obtained with those reported by N'guessan (2013) [25] from a study conducted on ethyl acetate and n-butanolic extracts of *Z. gilletii* and *M. lucida*, we conclude that the percentages of reduction are relatively close.

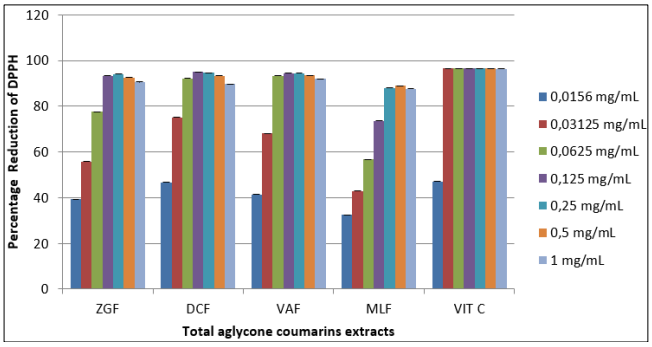

Fig 3: Antioxidant activity of the ethereal extracts of leaves, quantified against DPPH

ZGF: Ethereal extract of *Z. gilletii* leaves; DCF: Ethereal extract of *D. cinerea leaves*; VAF: Ethereal extract of *V. amygdalina* leaves; MLF: Ethereal extract of *M. lucida* leaves; VIT C: vitamin C

The concentrations of the ethereal extracts from the leaves, required to reduce 50% of the DPPH concentration (CR_{50}%) were determined graphically (Table 3). The lower this concentration, the higher the antioxidant efficiency [19, 26].

Table 3: CR_{50} values (mg/mL) of ethereal leaf extracts

	Extract				
	ZGF	DCF	VAF	MLF	VIT C
CR_{50} (mg/mL)	0,024010± 7x10^{-6}%	0,01789±0, 3x10^{-5}%	0,01995±0, 2x10^{-5}%	0,04831±0, 3x10^{-5}%	0,016150±0, 3x10^{-6}%

ZGF: Extrait éthéré de *Z. gilletii* feuilles; DCF: Extrate éthéré de *D. cinerea* feuilles; VAF: Extrait éthéré de *V. amygdalina* feuilles; MLF: Extrait éthéré de *M. lucida feuilles*; VIT C: vitamine C

With respect to CR_{50}, the ethereal extracts of the leaves of the 4 plants showed clear antioxidant efficacy compared to vitamin C. The extracts with CR_{50} values close to that of vitamin C are ZGF (0.024010 ± 7x10^{-6}% mg/mL), DCF (0.01789 ± 0.3x10^{-5}% mg/mL) and VAF (0.01995 ± 0.2x10^{-5}% mg/mL). However, compared to ZGF, DCF, VAF, MLF extract (CR_{50} of 0.04831 ± 0.3x10^{-5}% mg/mL) has the lowest antioxidant efficiency. This appears to be evidenced by the nature of the intrinsic reactivity seat of the aglycone coumarins that MLF contains, as well as the number, position, and nature of the phenolic OH [19, 26, 27].

4. Conclusion

Coumarins are natural compounds present in Dicotyledons and widely distributed in some botanical families such as Rutaceae, Fabaceae, Apiaceae, Oleaceae, Loganiaceae, Solanaceae, Asteraceae and Hippocastanaceae. This family of secondary metabolites is still exploited in the food and pharmaceutical industries and in various applications (flavours, pigments, insecticides). In the present work, we were interested in TAC, which we quantified in the leaves of *Z. gilletii*, *D. cinerea*, *V. amygdalina*, and *M. lucida*. TAC were extracted from leaves with a yield between 0.224% and 0.254%. Their quantification revealed their dominant presence in leaves of *Z. gilletii*, a Rutaceae. The study of the antioxidant activity by spectrophotometry showed that the DPPH radical is trapped by the ethereal extracts of the leaves of the 4 plants, with a reduction percentage varying between 32% and 95%. This leads us to believe that the antioxidant effectiveness of the extracts with regard to the determined CR_{50} would be governed by the presence of antioxidant TAC. Thus, we identify a rational track that would explain the recurrent use of these plants in the traditional treatment of certain pathologies.

5. Source of Funding

None

6. Conflict of Interest

None

7. References

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