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Phytochemical screening and evaluation of antioxidant activity of the stem bark of *Anacardium occidentale* L. and *Vitellaria paradoxa* C.F. Gaertn, two medicinal plants from Poro region (Northern of Côte d'Ivoire)

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Abstract

Anacardium occidentale and *Vitellaria paradoxa* are two medicinal plants traditionally used by Ivorian, particularly in the treatment of diabetes, arterial hypertension and diarrhea. The objective of this work was to evaluate the phytochemical constituents and the antioxidant activity of the stem barks of *A. occidentale L.* and *V. paradoxa*. The stem bark of both plants was used for the aqueous extraction. Major chemical groups were identified using the tube staining method followed by confirmation by thin layer chromatography (TLC). The antioxidant activity of the extracts was evaluated by DPPH method.

The results of phytochemical screening of plant extracts revealed the presence of polyphenols, anthraquinones, tannins, sterols, terpenes and saponins. In addition, the evaluation of the antioxidant activity by spectrophotometry using the DPPH method showed that all extracts had significant and different antioxydant activity compared to the reference molecule (Vitamin C, IC_{50} = 0.80 ± 0.70 µg/mL) with respective IC_{50} of 0.42 ± 0.32 µg/mL for *V. paradoxa* and 0.87 ± 0.77 µg/mL for *A. occidentale*. *V. paradoxa* therefore has the best antioxydant activity. These results could explain the therapeutic virtues of *A. occidentale* and *V. paradoxa* in the treatment of various pathologies.

Keywords: Phytochemical screening; Antioxidant; *Vitellaria paradoxa*; DPPH

1. Introduction

For several decades, men have used medicinal plants to treat various pathologies [1]. According to the World Health Organization (WHO), traditional medicine is defined as the set of all practical knowledge, explicable or not, to diagnose or eliminate a physical and / or mental imbalance based exclusively on lived experience and observation, and transmitted from generation to generation (orally or in writing) [2]. Medicinal plants are a numerically large and economically important group. They still remain one of the most widespread forms of medicine in developing countries

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[3]. Currently, WHO estimates that around 80% of the world's people use traditional herbal medicine as part of primary health care [4]. In addition, worldwide, nearly 25% of prescriptions are based on plant species with therapeutic virtues. Natural substances derived from plants are a potential source of bioactive natural molecules used in the pharmaceutical, food and cosmetic industries [5]. Among these compounds we find to a large extent the secondary metabolites which have been particularly illustrated in therapy [3]. In Africa, traditional medicine is the cheapest and therefore the most accessible health system for the populations, mainly because of the richness of the flora in medicinal plants [6]. Côte d'Ivoire, a country known for its biodiversity, has a particularly rich and varied flora with around 3,000 plant species, 15% of which are endemic and belonging to several botanical families. This floristic potential made up of medicinal plants is little explored from a chemical and pharmacological point of view. However, the use of medicinal plants for therapeutic purposes presents dangers related to lack of dosage, imprecise diagnosis and ignorance of unwanted and toxic side effects. It therefore appears vital to promote the traditional pharmacopoeia through reliable and rational scientific studies in order to provide the populations with Improved Traditional Medicines at a lower cost and accessible to all social classes.

The present study falls within this perspective and has as general objective to carry out the phytochemical study and to evaluate the antioxidant activity of aqueous extracts from the stem bark of of *A. occidentale* and *V. paradoxa*.

2. Material and methods

2.1. Plant material

The plant material consists of the stem bark of two plant species, namely *A. occidental*e and *V. paradoxa*. The stem bark was harvested in Korhogo (Ivory Coast) in February 2020 and identified at the National Floristic Center of the Félix HOUPHOUËT-BOIGNY University (Abidjan, Ivory Coast). After harvest, the bark was cleaned carefully then cut into small pieces to facilitate drying. Then they were dried in the dark at room temperature for two months. At the end of drying, the bark was pulverized using an electric grinder (RETSCH, Type AS 200) to obtain a fine powder. This vegetable powder was stored in sterile jars and was used for the preparation of the aqueous extracts.

2.2. Methods

2.2.1. Preparation of aqueous extracts

The aqueous extracts of the two plants were prepared according to the method described by Zirihi *et al.* (2003) [7] with some modifications. 100 g of vegetable powder were subjected to decoction for 30 min with distilled water (1L). After cooling to room temperature, the decoction was first wrung out in a tissue. Then filtered twice through cotton wool and once through whatman paper (3mm). The filtrate was concentrated in an oven at 50 °C for one week. The resulting extract was weighed and stored in a sterile vial. The extraction yield *R* (%) was determined according to the following formula:

$$R\ (\%) = (m\ /M) \times 100$$

M: mass of the vegetable powder (100 g); m: mass of the crude extract (g).

2.2.2. Phytochemical study of aqueous extracts

The search for phytocompounds was carried out by coloration and tube precipitation tests on plant powders [8,9]. The groups of molecules which have been sought are alkaloids, anthraquinones, flavonoids, polyphenols, tannins and saponins.

Alkaloids were characterized using Dragendorff's and Bouchardat's reagents. Indeed, 1g of each extract of *A. occidentale* or *V. paradoxa* was placed in 10 mL of 90% methanol to obtain stock solutions. The mixture was stirred for 15 min and filtered. Then to 1.5 mL of each filtrate was added 1.5 mLof methanol. The addition of 2 drops of Dragendorff's reagent causes an orange-colored precipitate. Then, adding 2 drops of Bouchardat's reagent to the methanol solution of the extract causes a reddish-brown precipitate indicating a positive reaction.

To characterize anthraquinones, 1g of each extract of *A. occidentale* or *V. paradoxa* was placed in 10 mL of 90% methanol. The mixture is stirred for 15 min and filtered. Then to 1.5 mL of each filtrate was added 1.5 ml of methanol. The addition of 2 or 3 drops of NaOH (0.1 M), after stirring, confirms the presence of anthraquinones by turning the aqueous phase to red or yellow.

Flavonoids were tested by placing 0.5 g of each extract of *A. occidentale* or *V. paradoxa* in 10 mL of distilled water for 10 min. After stirring, the extract was filtered and 5 mL of a dilute ammonia solution was added to it. Finally, 1 mL of aluminum chloride ($AlCl_3$) was added to the mixture. The appearance of a yellow color indicates the presence of flavonoids.

Ferric chloride ($FeCl_3$) made it possible to search for polyphenols. Indeed, 0.8 g of each extract of *A. occidentale* or *V. paradoxa* was placed in 10 mL of distilled water for 10 min. After stirring, the extract was filtered and a drop of 2% alcoholic ferric chloride solution was added. The appearance of a more or less dark blue-blackish or green color indicates the presence of phenolic compounds.

As for terpenes and sterols, they were demonstrated by adding 1mL of chloroform to 2.5 mL of each plant extract, then the mixture is homogenized before adding 1.5 mL of concentrated sulfuric acid (H_2SO_4). The formation of a reddish-brown ring at the interface indicates a positive reaction.

To characterize tannins, 1 g of each extract of *A. occidentale* or *V. paradoxa* was mixed in 10 mL of 90% methanol. The mixture was stirred for 15 min and filtered. Then to 1.5 mL of each filtrate was added 1.5 mL of methanol. The addition of 5 mL of sulfuric vanillin makes it possible to detect the presence or absence of tannins. The solution obtained was brought to the boil for 15 min in a water bath. The formation of a precipitate which turns black blue marks the presence of gallic tannins while the presence of catechetical tannins is indicated by a precipitate turning greenish brown.

To test for saponosides, 10 mL of the total aqueous extract from each plant was poured into two test tubes. Each tube was shaken vigorously for 15 s and then left to stand for 15 min. A height of persistent foam at the level of the tubes, greater than 1 cm, indicates the presence of saponosides.

2.3. Study of phytocompounds by chromatography

The search for phytomolecules was carried out by thin layer chromatography (TLC) according to the method of Harbone (1998) [10] with some modifications. This test required the use of an aluminum TLC plate containing silica gel (stationary phase) on which a start line (deposit) and a front line were marked. Using a capillary tube, a few drops of extracts were placed on the plate which is then dried in the open air. Subsequently, the mobile phase (eluent) was prepared by mixing three solvents: methanol (1.5 mL); ethyl acetate (8 mL) and dicloromethane (0.5 mL) in a chromatography tank. Then, the TLC plates were placed in the tank which is covered to prevent evaporation of the solvent. Once the eluent has reached the front line, the TLC plate is removed and is dried in the open air. The revelation of the different chemical compounds on the plate was made using the revealing reagents presented in Table 1.

Table 1 Reagents for the detection of different chemical compounds

Revelation reagents	Compounds identified
Dragendorff	Alkaloids
Ferric chloride	Polyphenols
Aluminum chloride	Flavonoids
Sodium hydroxide	Anthraquinones

2.4. Evaluation of the antioxidant activity of plants

The antioxidant activity of the aqueous extracts of the two plants was carried out by the 2,2 -diphenyl-1-picrylhydrazyl (DPPH) test according to the method of Huang et *al*. (2011) [11] with some modifications. The reduction of the DPPH radicals in solution by an antioxidant agent leads to a discoloration of this solution. Briefly, 2 mL of a methanol solution of DPPH (100 µM) was mixed with 2 mL of different dilutions of the two extracts (0-100 µg/mL). A range of concentrations (0-100 µg/mL) of vitamin C also prepared was used as a reference. The resulting mixtures were then stored in the dark at room temperature for 30 min. Then, the absorbance (517 nm) was measured against a control (2 mL of the DPPH solution + 2 mL of methanol). For each concentration, three (3) tests were performed. The absorbances thus measured made it possible to calculate the Percentage of inhibition (PI) of the DPPH radicals according to the formula below:

$$PI\ (\%) = (A_0 - A_1) \times 100 / A_0$$

A_0: absorbance of the DPPH solution without extract; A_1: absorbance of the DPPH solution with the extract.

The antioxidant power of the aqueous extracts was evaluated by comparing their IC_{50} to that of vitamin C. The IC_{50}, which is the concentration of extract responsible for 50% inhibition of DPPH radicals, is determined by projection from 50% on the graph of the percentage inhibition of DPPH as a function of the concentrations of extracts and vitamin C.

2.5. Statistical analysis of the results

The graphical representation of the data was performed using Graph Pad Prism 7.0 software (Microsoft U.S.A) and Microsoft Excel. Statistical analysis of the results was performed using Multiple Analysis of Variance (ANOVA). The differences between the means were determined according to Duncan's test at the 5% level ($P < 0.05$ is considered significant). The mean value is accompanied by the standard error on the mean (Mean ± SEM).

3. Results

3.1. Yields of the different extractions

The yields of the various lyophilized aqueous extracts of *A. occidentale* and *V. paradoxa* are shown in Table 2. *A. occidentale* extract gave the highest yield (44.8 %) compared to *V. paradoxa* extract.

Table 2 Yields of the various aqueous extracts

Species	***Anacardium occidentale***	***Vitellaria paradoxa***
Yields **(%)**	44.8	37.7

3.2. Phytochemical composition of the different extracts

The results of the phytochemical analysis of the two plants revealed several secondary metabolites shown in Table 3. Tannins and polyphenols are present in high concentrations in the bark of plants unlike flavonoids which are absent. However, saponins have a moderate concentration in extracts compared to alkaloids and anthraquinones. It should be noted that the same result was confirmed by the TLC test.

Table 3 Phytochemical groups of the two plants (stem bark)

Chemical composition	***A. occidentale***	***V. paradoxa***
Alkaloids	+/-	+/-
Anthraquinones	+/-	+/-
Flavonoids	-	-
Polyphenols	+++	+++
Saponins	++	++
Tannins	+++	+++

(-) : Absent; (+/-) : present; (++) : present but moderate; (+++) : present in high concentration

3.3. Antioxidant power of the aqueous extracts

The absorbances and inhibitory powers of plant extracts and vitamin C made it possible to obtain figure 1. It appears from this graph that compared to vitamin C, the two extracts expressed a strong antioxidant power against DPPH with an increase in the inhibitory power as a function of the concentration in a dose-response manner. However, the aqueous extract of *V. paradoxa* exhibited the best antioxidant activity with an IC_{50} of 0.42 ± 0.32 µg/mL followed by vitamin C (IC_{50} = 0.80 ± 0.72 µg/mL) and *A. occidentale* extract (IC_{50} = 0.87 ± 0.77 µg/mL). Statistical analysis did not show a significant difference between these values.

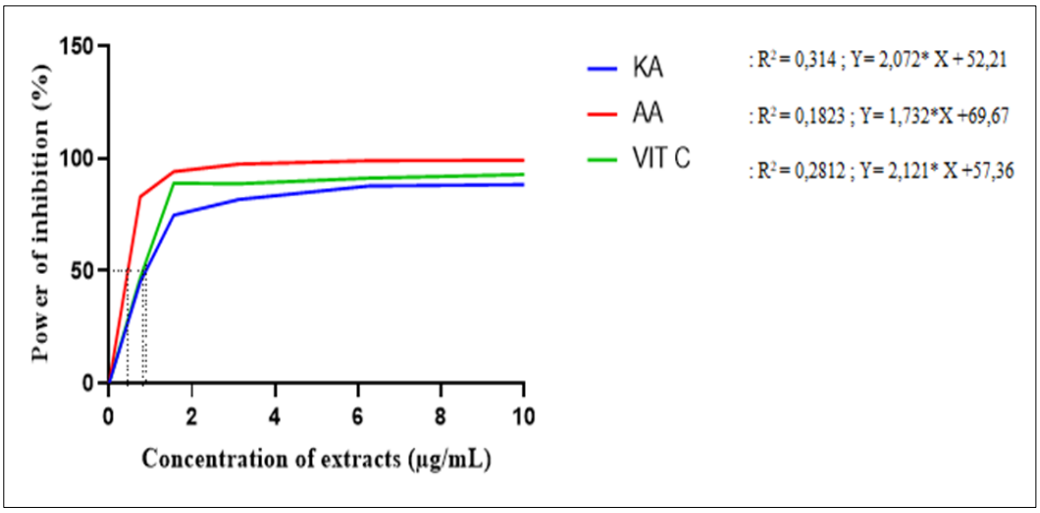

Figure 1 Inhibition of the DPPH radical as a function of the concentration of aqueous extracts of *A. occidentale* and *V. paradoxa*

KA: *V. paradoxa*, AA: *A. occidentale*, Vit C: Vitamin C; Values are expressed as mean ± standard deviation (n = 3); $p < 0,05$: significant difference

4. Discussion

The search for new substances with biological activities from medicinal plants is one of the greatest concerns of the scientific world. It is therefore right that the present study was conducted with the objective of evaluating the phytochemical constituents and the antioxidant activity of the stem barks of *A. occidentale* and *V. paradoxa*.

The study first required the extraction of the bioactive compounds. To do this, the judicious choice of solvent on the one hand and of the extraction method on the other hand represents an essential factor for the optimal evaluation of the pharmacological effects of the plant [12, 13]. There was a difference in yield from the extraction according to the plants. Indeed, the best yield was obtained with the extract of *A. occidentale* (44.8%) compared to *V. paradoxa* (37.7%). This difference in yield could be interpreted by the fact that the solvent (water) better concentrates the chemical constituents of *A. occidentale* compared to *V. paradoxa.*

Regarding the phytochemical sorting of the aqueous extract of the two plants, the results revealed the presence of the same chemical compounds for the two methods used (reaction in tube and TLC plates) with an abundance of polyphenols and tannins. The other chemical groups present are anthraquinones, alkaloids, terpenes and sterols and saponins without forgetting the absence of flavonoids. Our results on *A. occidentale* are in agreement with those of Kane *et al*. (2015) [14]. However, the abundance or absence of certain chemical groups could be explained by a difference in several parameters, be it geographic, physicochemical or biological such as the difference in the harvesting site including the environment of the plant, the light, the precipitation, the season, the type of soil, the harvest period, the genetic heritage, the extraction procedure used, the part of the plant studied [15, 16, 17]. In addition, it should be noted that each secondary metabolite detected in the extracts would be involved in the biological activities of plants or even therapeutic for the treatment of several pathologies [18].

As for the evaluation of the antioxidant activity of the plants aqueous extracts, it emerges from this study that they have a strong antioxidant power compared to vitamin C with a higher IC_{50} for the aqueous extract of *V. paradoxa*. This antioxidant character could be due to the presence of abundant phenolic compounds (polyphenols) in plants [19]. However, the anti-radical potency of the aqueous extract of *V. paradoxa* was higher (lower IC_{50}) than that of *A. occidentale*. This difference would be directly linked to the quantitative and / or qualitative diversity of the phenolic compounds present in the two extracts [20]. In addition, our results are in agreement with those obtained by Allal (2010) on *V. paradoxa* [21].

In addition, the phytochemical screening on the TLC plates made it possible to observe polar compounds. A study by Kang *et al*. (2003) found that the polar molecules present in plant extracts contribute to the increase of anti-free radical activity [22].

The antioxidant properties of aqueous extracts of *A. occidentale* and *V. paradoxa* could constitute a real contribution in the research of bioactive molecules, being able to prevent or treat pathologies linked to oxidative stress, namely cancers,

diseases linked to stress and aging. These results could therefore justify the use of these plants in traditional medicine in the treatment of various pathologies including diabetes, hypertension and diarrhea.

5. Conclusion

This research work is a contribution to the valorization of medicinal plants which focused on the phytochemical sorting and the evaluation of the antioxidant activity of the aqueous extracts of the stem bark of *A. occidentale* and *V. paradoxa* utilised in the region of Korhogo. The study showed on the one hand the presence of various secondary metabolites in the extracts of the two plants and on the other hand an interesting antioxidant activity with the best activity obtained with *V. paradoxa.* These results point to promising prospects for future studies on these two species.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest for this study.

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