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Antioxidant potential of medicinal plants used against liver diseases in Bengassou,
Bocanda department (Central-Eastern, Côte d'Ivoire)

AKPO Siallou Amino Prisca^{1*}, N'GUESSAN Alain Hugues Olivier², KONAN Kouassi
Pierre-Alain², SYLLA Youssouf, KONE Mamidou Witabouna¹, TRA Bi Fézan Honora¹

¹Université Nangui ABROGOUA, UFR Sciences de la Nature, 02 BP 801 Abidjan 02, Côte
d'Ivoire

²Université Nangui ABROGOUA, UFR Sciences Fondamentales Appliquées, 02 BP 801
Abidjan, Côte d'Ivoire

*E-mail : pris_siallou@yahoo.fr

Abstract

Hepatitis, which causes acute liver infections, is a global health problem, especially viral hepatitis B and C, which are major causes of serious morbidity and death. In Côte d'Ivoire, viral hepatitis (78%) is the leading cause of chronic liver disease. Late diagnosis of this condition forces most patients, who are generally poor, to turn to medicinal plants, given the high cost of treatments offered by traditional medicine. Hence the need to evaluate their hepatoprotective potential. To contribute to this, this study focused on the phytochemical composition and antioxidant properties of several medicinal plants used to treat liver disease in Bengassou. Oxidative stress, a state of imbalance between the generation and elimination of free radicals in the body, is considered a major factor in the pathogenesis of liver disease. This study aims to identify the major groups of phytochemicals in hydroethanolic extracts from these plants via colorimetric tests, and to evaluate their antioxidant properties using DPPH radical scavenging and Folin-Ciocalteu assays. Polyphenols, flavonoids, sterols and polyterpenes were found in most of the species studied. Three of them, namely the leaves of *Uncaria africana* (TPC = $1,99 \pm 0,02$ mg GAE/g DM; CR50 = $0,109 \cdot 10^{-4}$ mg/ml), *Entandrophragma angolense* (TPC = $2,58 \pm 0,04$ mg GAE/g DM; CR50 = $0,0026$ mg/ml) and *Vismia guineensis* (TPC = $1,79 \pm 0,01$ mg

GAE/g DM; CR50 = 0,0334 mg/ml) exhibited the best antioxidant profiles. These species represent promising candidates for the development of Improved Traditional Medicines (ITM) against hepatitis.

Keywords: medicinal plants, liver diseases, phytochemicals, antioxidant properties, Bengassou

Introduction

Medicinal plants have been used since ancient times and long constituted the primary therapeutic resource, grounded in empirical knowledge (Catier and Roux, 2007). Their use has expanded considerably worldwide (Kouamé & Koné, 2017), particularly in developing countries where access to conventional medicine remains limited (WHO, 2012). In West Africa, nearly 80% of the population relies on medicinal plants for primary healthcare (WHO, 2002). This reliance persists, especially in rural communities, where traditional remedies are often perceived as more accessible and culturally appropriate (Marshall, 1998).

In Côte d'Ivoire, disparities in access to primary, secondary, and specialized healthcare services continue to pose a significant public health challenge (Goba, 2012). As highlighted by WHO/UNICEF (2005), medicinal plants represent the backbone of primary healthcare for a large proportion of the population due to their geographical availability, affordability, and cultural acceptance. The first volume of the Ivorian Pharmacopoeia, published in 2018, consolidates extensive ethnobotanical knowledge documented by several authors (Adjanooun & Aké Assi, 1979, 2011; Bouquet & Debray, 1974; Vangah-N'Guessan, 1995; Téré, 2000; Zirihi, 2006; Malan, 2008; Tra Bi et al., 2008; Koné et al., 2012; N'Guessan et al., 2015; Orsot et al., 2016). Among the recorded species, several are traditionally employed in the management of liver disorders.

The liver plays a central role in maintaining metabolic homeostasis through its involvement in carbohydrate and lipid metabolism, bile synthesis, and vitamin storage (Ahsan et al.,

2009). Its dysfunction can therefore result in severe pathological conditions, making liver diseases a major global health concern (Asha et al., 1998; Adewusi et al., 2010).

In Côte d'Ivoire, despite ongoing efforts to curb hepatitis through awareness and early screening initiatives, effective treatment remains constrained by the high costs associated with modern medical care. This economic barrier encourages patients to seek alternative therapies based on medicinal plants (Pourette et al., 2014; Anzouan et al., 2022).

Experimental evidence increasingly supports the hepatoprotective potential of several plant species (Akharaiyi et al., 2005; Sourabié et al., 2012; Mallik et al., 2014; Jaiswal et al., 2015; Adesiyun, 2018). Bioactive compounds such as alkaloids, flavonoids, lignans, saponins, and terpenoids have attracted significant scientific interest for their hepatoprotective properties (Domitrović & Potočnjak, 2016; Zhou et al., 2021). Through their antioxidant activity, these phytochemicals may enhance hepatic defense mechanisms and mitigate the progression of liver damage. This is particularly relevant as hepatitis is closely associated with oxidative stress resulting from an imbalance between pro-oxidant and antioxidant systems (Rambaldi et al., 2005; Li et al., 2015).

The present study aims to promote the rational use of medicinal plants in the management of liver diseases in Bengassou, a hamlet located in the Bocanda department (central-eastern Côte d'Ivoire), recognized for its rich ethnomedicinal heritage in treating hepatic disorders (Siallou et al., 2024). Specifically, this study seeks to identify major phytochemical groups using colorimetric screening and to assess their antioxidant potential through DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity and the Folin–Ciocalteu assay.

Materials and methods

Plant selection

The plants were selected following an ethnomedicinal survey conducted by Siallou et al. (2024) in Bengassou. The subsequent literature review, based on the lack of information relating to hepatoprotective properties, led to the selection of 13 medicinal plants (Table I). The organs of these plants were harvested in July 2018, dried in a ventilated room at room

temperature, then crushed in a mortar and blender to obtain fine powders. These powders were stored in Kraft paper at room temperature.

Table I. Medicinal plants used to treat liver diseases that have been identified and selected

Plant extracts

Family

Part used

Albizia adianthifolia (Schumach) W. Wight

Fabaceae

Bark

Aframamum alboviolaceum (Ridl.) K. Schum.

Zingiberaceae

Leaves

Anthocleista nobilis G. Don

Gentianaceae

Leaves, Bark

Bombax buonopozense P.Beauv.

Malvaceae

Leaves

Diospyros monbuttensis Gürke

Ebenaceae

Leaves

Entandrophragma angolense (Welw.) C.DC.

Meliaceae

Leaves, Bark

Ficus sur Forssk.

Moraceae

Leaves

Griffonia simplicifolia (DC.) Baill.

Fabaceae

Leaves

Leonotis nepetifolia (L.) R. Br.

Lamiaceae

Leaves

Oxyanthus unilocularis Hiern

Rubiaceae

Leaves, Bark

Trichilia prieureana A. Juss.

Meliaceae

Leaves

Uncaria africana G. Don

Rubiaceae

Leaves

Vismia guineensis (L.) Choisy

Hypericaceae

Leaves

Preparation of extracts

Fifteen (15) g of powder from each part of the plant were macerated in 100 ml of an ethanol-water mixture (80:20). This maceration was repeated three times, renewing the solvent every 24 hours. The hydroalcoholic macerates were combined, filtered and then evaporated under reduced pressure using a rotary evaporator until a dry hydroalcoholic residue was obtained. Aliquots of the selective extracts obtained were used to perform phytochemical screening and antioxidant profiling.

Phytochemical screening

Polyphenol detection

Polyphenols were detected using the ferric chloride reaction. A drop of 2% ferric chloride

aqueous solution was added to 2 ml of each solution. The presence of polyphenols is indicated by the appearance of a blue-black or green colouration of varying intensity (N'Guessan et al., 2009). If a positive reaction is obtained, coumarins, flavonoids and tannins are sought.

Detection of flavonoids

In a test tube, a few drops of concentrated hydrochloric acid and 2 to 3 magnesium chips were added to 2 ml of the extract. A pink-orange or purplish colour indicates the presence of flavonoids (Auwal et al., 2014).

Detection of coumarins

Coumarins were identified using the lactone ring test. Two (2) millilitres of each solution were examined under UV light at 366 nm. The appearance of blue fluorescence indicates the presence of coumarins. A confirmation test is performed with soda. To do this, 1 g of plant powder is placed in a test tube with a few drops of distilled water. The tube is covered with filter paper soaked in 10% soda (NaOH) and brought to the boil. The paper is removed and examined under UV light at 366 nm. Any yellow fluorescence indicates the presence of coumarins (Auwal et al., 2014).

Detection of tannins

Five (5 ml) of Stiasny's reagent (30% CH₂O in concentrated HCl 2/1(v/v)) was added to an aliquot of the extract taken in methanol and then evaporated. The formation of flakes after cooling indicates a positive reaction. The solution is then filtered and saturated with sodium nitrate (NaNO₃). A few drops of 2% FeCl₃ (m/v) are added to this mixture. The appearance of a blue, blue-black or black colour indicates the presence of gallic tannins, while a green or dark green colour indicates the presence of catechinic tannins (Karumi et al., 2004).

Detection of alkaloids

Alkaloids were detected using Dragendorff's reagent (iodine and bismuth). Six (6) ml of solution were evaporated and the residue was taken up with 6 ml of ethanol. Then, 3 drops of Dragendorff's reagent were added to the tube. The formation of an orange precipitate

indicates the presence of alkaloids (Auwal et al., 2014).

Detection of sterols and polyterpenes

An aliquot amount of hydroethanolic crude extract from each plant sample is dissolved hot in 1 ml of acetic anhydride ($\text{CH}_3\text{CO}_3\text{CH}_3$) in a test tube. Next, 0.5 ml of concentrated sulphuric acid (H_2SO_4) is slowly poured down the side of the test tube. The appearance of a purple colour turning blue and then green indicates a positive reaction (Békro et al., 2007).

Evaluation of antioxidant properties

Total Phenolic Content (TPC)

The total phenolic content (TPC) was determined using the Folin-Ciocalteu method, adapted from Singleton & Rossi (1965). Briefly, 0,005 g of each hydroethanolic crude extract was dissolved in 10 mL of distilled water. 1 mL aliquot of this solution (diluted 1/10) was mixed with 1,5 mL of a Na_2CO_3 solution (17% w/v) and 0,5 mL of Folin-Ciocalteu reagent (0,5 N). The mixture was incubated at 37°C for 30 minutes. Absorbance was then measured at 760 nm against a blank (containing no extract).

Quantification was performed using a linear calibration curve ($y = ax + b$) generated from gallic acid standards at various concentrations under identical conditions. The TPC expressed in micrograms of gallic acid equivalent per gram of dry matter ($\mu\text{g GAE/g DM}$), was calculated using the following formula:

Q: Total phenolic content ($\mu\text{g GAE/g DM}$);

V: Total volume of the extraction solvent (mL);

C: Concentration of gallic acid established from the calibration curve ($\mu\text{g/mL}$)

d: Dilution factor;

m: Mass of the extract used (g).

DPPH radical reduction assay

The radical scavenging activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, following the method described by Blois (1958) with slight modification. Briefly, 0.03 g of each crude hydroethanolic extract was dissolved in 10 mL of rectified ethanol (96%). A series of extract solutions was prepared at various concentrations (1; 0,5; 0,25; 0,125, 0,061, 0,042; 0,031; 0,016; 0,008; 0,004 mg/mL). A 0,03 mg/mL DPPH radical solution was also prepared in the same solvent.

For the assay, 1 mL of each extract dilution was mixed with 2 mL of the DPPH solution. The mixtures were incubated at 37°C for 30 minutes in the dark. Absorbance was measured at 517 nm using a spectrophotometer.

The following controls were used:

Reaction mixture: 1 mL of extract solution + 2 mL of DPPH solution;

Control: 1 mL of ethanol (96%) + 2 mL of DPPH solution;

Blank: 3 mL of ethanol (96%).

The following equation was used to calculate the DPPH radical reduction percentage induced by the extracts (RP):

A_{extract} and A_{control} are the absorbance values of the sample and the control, respectively.

The CR_{50} value, defined as the extract **10 concentration required to reduce 50% of DPPH** radicals, was determined from the linear regression curve of RP (%) versus extract concentration. When the 50% reduction level was not experimentally achieved, the CR_{50} was estimated by interpolation of the values surrounding the 50% reduction threshold. Antioxidant capacity is inversely proportional to the CR_{50} value. Thus, a higher CR_{50} indicates lower antioxidant capacity, and vice versa (Tanoh et al., 2019).

Statistical analyses

The averages of the amounts of the different compounds were calculated using Excel

software and were subjected to a ¹¹ one-way analysis of variance (ANOVA) using GraphPad Prism 5 software. When a difference is observed for each characteristic ($p < 0.05$), the variance is completed by comparing the means using Tukey's multiple comparison test at a threshold of 0.05.

Results

Phytochemicals detected

Phytochemical screening of the 16 hydroethanolic extracts obtained from the leaves and bark of the 13 plants showed that all these extracts have total polyphenols, flavonoids, sterols, and polyterpenes in common, except the bark of *Anthocleista nobilis*. Coumarins were present in the hydroethanolic extracts of the leaves and ⁹ bark of *Entandrophragma angolense*, the leaves of *Trichilia prieureana*, *Uncaria africana*, *Vismia guineensis*, *Bombax buonopozense*, and *Diospyros monbuttensis*. Condensed tannins were present in virtually all hydroethanolic extracts, except for the leaves of *Entandrophragma angolense* and *Ficus sur*, which contain hydrolyzable tannins, and *Albizia adianthifolia*, which contains neither. Alkaloids, ⁶ on the other hand, were not present in any of the extracts (Table II). These results show a diversity of chemical compound groups in the hydroethanolic extracts of the leaves and bark of the plants studied.

Table II. Chemical compounds in hydroethanolic extracts from the leaves and bark of plants used to treat hepatitis in Bengassou

Plant extracts

Organs

Poly

Flav

Cou

Ster/ Polyt

TC

TH

Alc

Albizia adianthifolia

Bark

+

+

-

+

-

-

-

Aframamum alboviolaceum

Leaves

+

+

-

+

+

-

-

Anthocleista nobilis

Bark

+

+

-

-

+

-

-

Leaves

+

+

-

+

+

-

-

Bombax buonopozense

Leaves

+

+

+

+

+

-

-

Diospyros monbuttensis

Leaves

+

+

+

+

+

-

-

Entandrophragma angolense

Bark

+

+

+

+

+

-

-

Leaves

+

+

+

+

-

+

-

Ficus sur

Leaves

+

+

-

+

-

+

-

Griffonia simplicifolia

Leaves

+

+

-

+

+

-

-

Leonotis nepetefolia

Leaves

+

+

-

+

+

-

-

Oxyanthus unilocularis

Leaves

+

+

-

+

+

-

-

Bark

+

+

-

+

+

-

-

Trichilia prieureana

Leaves

+

+

++

+

+

-

-

Uncaria africana

Leaves

+

+

+

+

+

-

-

Vismia guineensis

Leaves

+

+

+

+

+

-

-
Poly. Polyphenols; Flav. Flavonoids; Cou. Coumarins; TC. Condensed tannins; TH.
Hydrolyzable tannins; Alc. Alkaloids; Ster/Polyt. Sterols and polyterpenes

Total phenolic compound content

The results obtained ¹ are shown in Table III. These results show that the phenolic compound content (TPC) varied among the organs of the same plant. The highest TPC was found in hydroethanolic extracts of bark (2.58 ± 0.04 mgEAG/g DM) and leaves (2.42 ± 0.08 mgEAG/g DM) of *Entandrophragma angolense*, followed respectively by the leaves of *Trichilia prieureana* (2.01 ± 0.06 mgEAG/g DM), *Uncaria africana* (1.99 ± 0.02 mgEAG/g DM) and *Vismia guineensis* (1.79 ± 0.01 mgEAG/g DM).

Table III. Total phenolic compound content of hydroethanolic extracts

Plant species

Organs

Polyphenols \pm ET (mg EAG/g DM)

Aframamum alboviolaceum

Leaves

$0,39 \pm 0,02$ ef

Albizia adianthifolia

Bark

$0,43 \pm 0,06$ e

Anthocleista nobilis

Bark

$0,72 \pm 0,05$ d

Leaves

$0,22 \pm 0,01$ efg

Bombax buonopozense

Leaves

1,25±0,05c

Diospyros monbuttensis

Leaves

0,36±0,02ef

Entandrophragma angolense

Bark

2,58±0,04a

Leaves

2,42±0,08a

Ficus sur

Leaves

0,03±0,01g

Griffonia simplicifolia

Leaves

0,36±0,01ef

Leonotis nepetifolia

Leaves

0,30±0,01ef

Oxyanthus unilocularis

Leaves

0,23±0,03efg

Bark

0,08±0,01g

Trichilia prieureana

Leaves

2,01±0,06b

Uncaria africana

Leaves

1,99±0,02b

Vismia guineensis

Leaves

1,79±0,01b

Statistical parameters

DI

15

F

485,335

P

<0,001

SD. Standard deviation; GAE. Gallic acid equivalent; df. Degrees of freedom; F. Statistical test value; P. Probability; DM. Dry matter. Values with the same letters are not significantly different.

Radical scavenging activity

¹ For each extract, the RPs are shown in Figure 1. These results show that RPs increased with the concentration of the plant extract. In addition, the hydroethanolic extracts of the leaves of *Vismia guineensis*, *Uncaria africana* and *Entandrophragma angolense*, with RPs between 50 and 95%, appear to have the highest anti-radical properties of all the plant extracts studied. The EC₅₀ ¹ are shown in Table IV. The hydroethanolic extract of *U. africana* leaves (EC₅₀ = 0.109 10⁻⁴ mg/ml) had the greatest DPPH radical reduction capacity. It is followed by the hydroethanolic extracts of the leaves of *E. angolense* (EC₅₀ = 0.0026 mg/ml) and *V. guineensis* (EC₅₀ = 0.0334 mg/ml). (0,0026 mg/mL), which shows an activity nearly identical to Vitamin C, and *V. guineensis* (0,0334mg/mL). These findings confirm that among all studied samples, the leaves of *U. africana*, *E. angolense*, and *V. guineensis* possess the most potent antioxidant properties.

Table IV. Reducing concentrations (EC50) of crude hydroethanolic extracts

Plant extracts

Organs

EC50 (mg/ml)

Vitamine C

0,002456

Aframamum alboviolaceum

Leaves

0,805

Albizia adianthifolia

Leaves

0,716

Anthocleista nobilis

Bark

0,159

Leaves

>1

Bombax buonopozense

Leaves

0,0842

Diospyros monbuttensis

Leaves

0,717

Entandrophragma angolense

Bark

0,287

Leaves

0,0026

Ficus sur

Leaves

0,683

Griffonia simplicifolia

Leaves

0,212

Leonotis nepetifolia

Leaves

0,582

Oxyanthus unilocularis

Leaves

0,495

Bark

0,854

Trichilia prieureana

Leaves

0,175

Uncaria africana

Leaves

0,109 10⁻⁴

Vismia guineensis

Leaves

0,0334

Discussion

Phytochemical screening revealed a diversity of chemical compound groups in the 16 hydroethanolic extracts studied. Polyphenols and flavonoids, tannins (except the bark of

Albizia adiantifolia), sterols, and polyterpenes (except in the bark of *Anthocleista nobilis*) are present in all extracts.

Coumarins were present in the hydroethanolic extracts of the leaves and ⁹ bark of *Entandrophragma angolense*, the leaves of *Trichilia prieureana*, *Uncaria africana*, *Vismia guineensis*, *Bombax buonopozense* and *Diospyros monbuttensis*. Alkaloids are absent in all the extracts studied. The presence of this group of secondary metabolites was reported by several authors. This is the case of Sieniawska et al. (2022) and Akoto et al. (2020), who detected alkaloids in the methanolic extracts of *Ficus sur* bark and *Griffonia simplicifolia* leaves, respectively.

Similarly, Sima Obiang et al. (2015) reported the presence of the above-mentioned compounds in the hydroethanolic extract of *A. nobilis* bark, while Kangbéto et al. (2022) found these phytochemicals in the ethanolic extract of *T. prieureana* leaves. However, alkaloids absent in all the extracts studied were identified by Kangbéto et al. (2022) in the ethanolic extract of *T. prieureana* leaves, by Iroka et al. (2014) in the aqueous and ethanolic extracts of *B. Buonopozense* bark, and by Lagou et al. (2016) in the ethanolic extract of *E. angolense* bark. These contradictory results could be explained, on the one hand, by the solubility of any alkaloids in aqueous or organic solvents and, ⁶ on the other hand, by the polarity of the extraction solvents. Indeed, due to their extremely complex and varied chemical structures, the solubility of alkaloids and their respective salts can vary considerably depending on the polarity of the extraction solvents (Kumar, 2014) and the pH of the reaction medium. Furthermore, a positive test for alkaloids may be due to the presence of other nitrogenous constituents in the plant, such as purines, proteins, betaines, and ammonium salts (Kumar, 2014). Conversely, the presence of flavonoids in all hydroethanolic extracts compared to other secondary metabolites is evidence of the widespread distribution of flavonoids in the plant kingdom, as reported by Macheix et al. (2005). In addition to flavonoids and tannins, coumarins observed in hydroethanolic extracts from the bark and leaves of *E. angolense*, and the leaves of *T. prieureana*, *U. africana*, *V. guineensis*, and *B. buonopozense* are reported ⁶ to be responsible for the

high total phenol content of these extracts compared to other plant extracts. Phenolic compounds are the most important group of phytochemicals in plants. They are divided into ten chemical classes characterized by the presence of a benzoic nucleus to which **one or more hydroxyl groups** are directly linked (Macheix et al., 2005; Stalikas, 2007). 12

The evaluation of antioxidant properties showed that, of all the extracts studied, the leaves of *U. africana*, *E. angolense*, and *V. guineensis* exhibited the best antioxidant properties (higher RP with low EC50).

The strong antioxidant activity attributed to the hydroethanolic extracts of the leaves of *U. africana*, *E. angolense* and *V. guineensis* **1 can be explained by the** high quantity of phenolic compounds contained in these plant organs. The presence of sterols and polyterpenes detected in these plants could also explain this activity. Terpenoids (monoterpenes and carotenoids) have strong antioxidant properties (Gutiérrez-Del-Río et al., 2021). The antioxidant activity of certain plants, notably *B. buonopozense*, *G. simplicifolia*, *A. nobilis*, *A. adianthifolia* and *E. angolense*, has been reported in the literature (Ngwoke et al., 2015; Sonibare et al., 2017; Akoto et al., 2020; Tilaoui et al., 2021; Oyawaluja et al., 2019). However, the difference in EC50 values **1 can be explained by the** influence of the extraction solvent (Xie & Schaich., 2014; Sharma & Bhat, 2009). In this study, a hydroethanolic solvent was used, whereas most previous studies used methanol or ethanol (100%) as the extraction solvent.

Conclusion

This study highlighted the antioxidant potential of 16 plant extracts studied and their major groups of phytochemicals. Three of them stood out for their remarkable antioxidant activities and significant phenolic compound content. These are the leaves of *E. angolense*, *U. africana* and *V. guineensis*. These results open up promising prospects for the promotion of the pharmacopoeia used in Côte d'Ivoire. The next step in this work will be to evaluate the safety of these extracts (acute and subacute toxicity) in order to guarantee their safe use. Furthermore, confirmation of their hepatoprotective properties in vivo would be a crucial step towards the development of improved traditional medicines

(ITMs) for the treatment of liver diseases.

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