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RESEARCH ARTICLE

ANTIOXIDANT POTENTIAL OF MEDICINAL PLANTS USED AGAINST LIVER DISEASES IN BENGASSOU, BOCANDA DEPARTMENT (CENTRAL-EASTERN, CÔTE D'IVOIRE)

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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 ± 0,02 mg GAE/g DM; CR_{50} = 0,109 10^{-4} mg/ml), *Entandrophragma angolense* (TPC = 2,58 ± 0,04 mg GAE/g DM; CR_{50} = 0,0026 mg/ml) and *Vismia guineensis* (TPC = 1,79 ± 0,01 mg GAE/g DM; CR_{50} = 0,0334 mg/ml) exhibited the best antioxidant profiles. These species represent promising candidates for the development of improved traditional medicines against hepatitis.

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,

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2012). 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 (Adjanohoun & 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

Medicinal plants	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% CH_2O 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 ($NaNO_3$). A few drops of 2% $FeCl_3$ (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 ($CH_3CO_3CH_3$) 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 (µg GAE/g DM), was calculated using the following formula:

$$Q(\mu g\ GAE/g\ DM) = \frac{V \times C \times d}{m}$$

Q: Total phenolic content (µg GAE/g DM);
V: Total volume of the extraction solvent (ml);
C: Concentration of gallic acid established from the calibration curve (µg/ ml)
d: Dilution factor;
m: Mass of the dry 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):

$$RP\ (\%) = \left(1 - \frac{A_{extract}}{A_{control}}\right) \times 100$$

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

The CR_{50} value, defined as the extract 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

Data were expressed as means ± standard deviation (SD). Statistical analyses were performed using Excel (Microsoft, USA) for initial data processing, followed by a one-way analysis of variance (ANOVA) using GraphPad Prism 5 software. When significant differences were observed ($p < 0.05$), Tukey's multiple comparison test was conducted at a 5% significance level to compare the means.

Results:-

Phytochemicals screening

The phytochemical screening of the 16 hydroethanolic extracts obtained from the leaves and bark of the 13 selected plants revealed that all extracts contained total polyphenols, flavonoids, sterols, and polyterpenes, with the exception of *Anthocleista nobilis* bark. Coumarins were identified in the hydroethanolic extracts of *Entandrophragma angolense* (leaves and bark), as well as in the leaves of *Trichilia prieureana, Uncaria africana, Vismia guineensis, Bombax buonopozense,* and *Diospyros monbuttensis*. Condensed tannins were present in nearly all extracts, except for the leaves of *Entandrophragma angolense* and *Ficus sur*, which contained hydrolysable tannins, and *Albizia adianthifolia*, which contained neither. In contrast, alkaloids were not detected in any of the extracts (Table II). These results demonstrate a diversity of secondary metabolites within the hydroethanolic extracts of the studied plant organs.

Table II. Phytochemical constituents of hydroethanolic extracts from the leaves and bark of medicinal plants used against liver diseases in the Bengassou Sub-prefecture

Plant species	Plant part	Poly	Flav	Cou	Ster/ Polyt	TC	TH	Alk
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 nepetifolia	Leaves	+	+	-	+	+	-	-
Oxyanthus unilocularis	Leaves	+	+	-	+	+	-	-
	Bark	+	+	-	+	+	-	-
Trichilia prieureana	Leaves	+	+	++	+	+	-	-
Uncaria africana	Leaves	+	+	+	+	+	-	-
Vismia guineensis	Leaves	+	+	+	+	+	-	-

(+): presence; (++): strong presence; (-): absence. Poly: Total polyphenols; Flav: Flavonoids; Cou: Coumarins; CT: Condensed tannins; HT: Hydrolysable tannins; Alk: Alkaloids; Ster/Polyt: Sterols and polyterpenes.

Evaluation of antioxidant properties

Total Phenolic Content (TPC)

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

Table III. TPC of the hydroethanolic extracts from studied plants

Plant species	Plant part	TPC ± SD (µg GAE/g DM)
Aframamum alboviolaceum	Leaves	$0,39\pm0,02^{ef}$
Albizia adianthifolia	Bark	$0,43\pm0,06^{e}$
Anthocleistanobilis	Bark	$0,72\pm0,05^{d}$
	Leaves	$0,22\pm0,01^{efg}$
Bombax buonopozense	Leaves	$1,25\pm0,05^{c}$
Diospyros monbuttensis	Leaves	$0,36\pm0,02^{ef}$
Entandrophragma angolense	Bark	$2,58\pm0,04^{a}$
	Leaves	$2,42\pm0,08^{a}$
Ficus sur	Leaves	$0,03\pm0,01^{g}$
Griffonia simplicifolia	Leaves	$0,36\pm0,01^{ef}$
Leonotis nepetifolia	Leaves	$0,30\pm0,01^{ef}$
Oxyanthus unilocularis	Leaves	$0,23\pm0,03^{efg}$
	Bark	$0,08\pm0,01^{g}$
Trichilia prieureana	Leaves	$2,01\pm0,06^{b}$
Uncaria africana	Leaves	$1,99\pm0,02^{b}$
Vismia guineensis	Leaves	$1,79\pm0,01^{b}$

Statistical parameters	Dl	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

The DPPH radical reduction percentages (RP) for each extract across the concentration range (0,03125 to 1 mg/ml) are illustrated in Figure 1. The results demonstrate that the hydroethanolic extracts from the leaves of *Vismia guineensis, Uncaria africana* and *Entandrophragma angolense* exhibited the highest DPPH radical reduction potential, with RP values ranging between 50% and 95%.

These observations are confirmed by the CR_{50} values presented in Table IV. Since the CR_{50} is inversely proportional to the antioxidant activity, lower values indicate higher potency. The hydroethanolic extract of *U. africana* leaves showed the most significant radical reduction capacity, with a CR_{50} of 0,109 10-4 mg/ml, which even surpasses the activity of the reference standard, Vitamin C (0,002456 mg/ml). It is followed by the leaf extracts of *E. angolense* (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

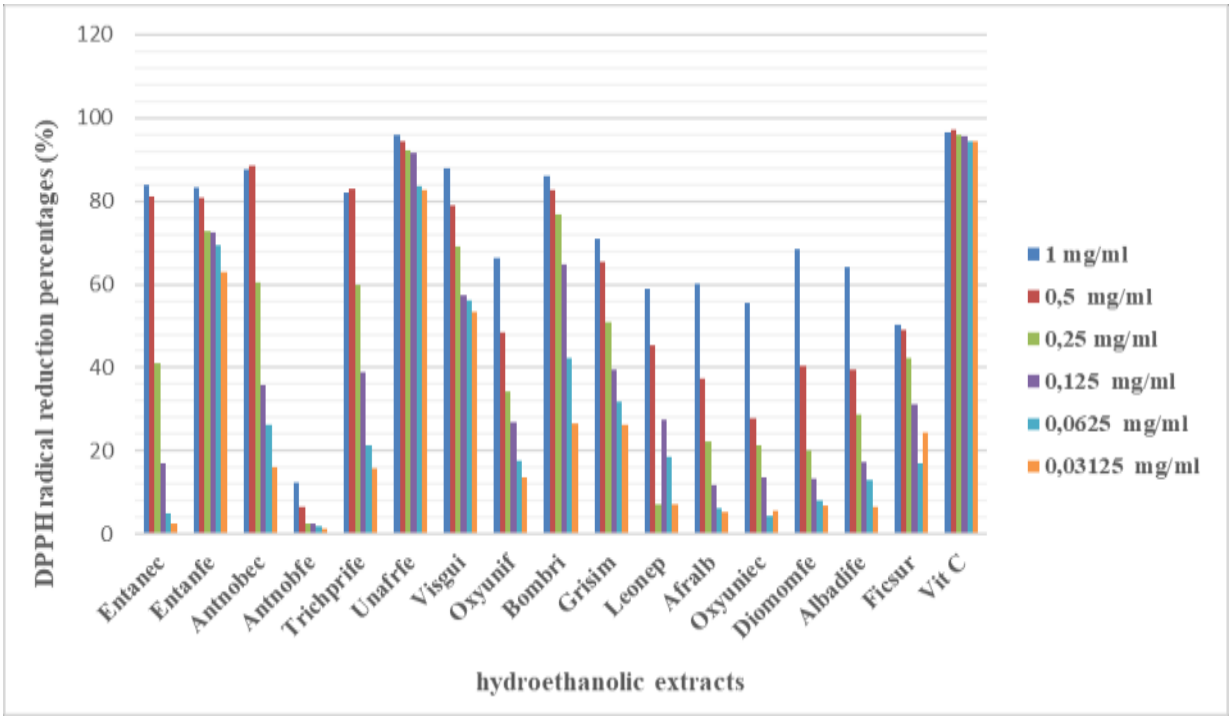

Figure 1. Histogram of the DPPH radical reduction percentages (RP) of the hydroethanolic extracts and vitamin C at different concentrations

Entanec. *Entandrophragma angolense* bark; Entanfe. *Entandrophragma angolense* leaves; Antnobec. *Anthocleista nobilis* bark; Antnofe. *Anthocleista nobilis* leaves; Trichprife. *Trichilia prieureana* leaves; Unafrife. *Uncaria Africana*; Visgui. *Vismia guineensis*; Oxyunif. *Oxyanthus unilocularis* leaves; Bombuo. *Bombax buonopozense*; Grisim. *Griffonia simplicifolia*; Leonep. *Leonotis nepetifolia*; Afralb. *Aframamum alboviolaceum*; Oxyuniec. *Oxyanthus unilocularis* bark; Diomomfe. *Diospyros monbuttensis*

Table IV. CR_{50} of hydroethanolic extracts from studied plants

Plant species	Plant part	CR_{50} (mg/ml)
Vitamine C *(positif control)*		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 secondary metabolites across the sixteen hydroethanolic extracts analyzed. Polyphenols, flavonoids, tannins (except in *Albizia adiantifolia* bark), and sterols/polyterpenes (except in *Anthocleista nobilis* bark) were ubiquitous. Coumarins were specifically detected in the hydroethanolic extracts of *Entandrophragma angolense* (leaves and bark) and in the leaves of *Trichilia prieureana*, *Uncaria africana*, *Vismia guineensis*, *Bombax buonopozense*, and *Diospyros monbuttensis*.

Interestingly, alkaloids were absent from all analyzed extracts, in contrast with several previous studies. For example, Sieniawska *et al.* (2022) and Akoto *et al.* (2020) reported the presence of alkaloids in methanolic extracts of *Ficus sur* bark and *Griffonia simplicifolia* leaves, respectively. Similarly, Sima Obiang *et al.* (2015) detected alkaloids in hydroethanolic extracts of *A. nobilis* bark, while Kangbéto *et al.* (2022) reported them in ethanolic extracts of *T. prieureana* leaves. Additional discrepancies were noted by Iroka *et al.* (2014) regarding *B. buonopozense* bark and by Lagou *et al.* (2016) for *E. angolense* bark. These variations may be attributed to differences in solvent polarity and alkaloid solubility, as the extraction efficiency of alkaloids depends on the solvent's dielectric constant and the pH of the medium (Kumar, 2014). False-positive results in alkaloid tests can also occur due to the presence of other nitrogenous compounds, such as purines, proteins, or quaternary ammonium salts (Kumar, 2014). The presence of flavonoids in all extracts underscores their near-universal distribution in the plant kingdom (Macheix *et al*., 2005). Beyond flavonoids and tannins, the coumarins detected in *E. angolense*, *T. prieureana*, *U. africana*, *V. guineensis*, and *B. buonopozense* likely contribute to the higher total phenolic content observed in these species. Phenolic compounds, characterized by at least one aromatic ring bearing one or more

hydroxyl groups, represent a critical group of metabolites for plant defense and therapeutic potential (Macheix *et al*., 2005; Stalikas, 2007).

Evaluation of antioxidant activity demonstrated that leaf extracts of *Uncaria africana*, *Entandrophragma angolense*, and *Vismia guineensis* exhibited the strongest antioxidant potential. This was evidenced by their high TPC, superior DPPH radical reduction capacity, and remarkably low CR_{50} values. In particular, the CR_{50} of *U. africana* (0.109×10^{-4} mg/ml) indicates exceptional radical reduction efficiency, even surpassing the reference standard, vitamin C. This pronounced activity is primarily attributed to the high content of phenolic compounds, which act as hydrogen donors to neutralize free radicals. The presence of sterols and polyterpenes may also synergistically enhance this effect, as various terpenoids are known for their potent antioxidant properties (Gutiérrez-Del-Río *et al.*, 2021).
While antioxidant activity has been previously reported for *B. buonopozense*, *G. simplicifolia*, *A. nobilis*, *A. adiantifolia*, and *E. angolense* (Ngwoke *et al.,* 2015; Sonibare *et al.*, 2017; Akoto *et al.*, 2020; Tilaoui *et al.*, 2021; Oyawaluja *et al.*, 2019), the variations in CR_{50} values observed between our study and the literature are likely due to the extraction solvent. In this study, a hydroethanolic mixture (ethanol/water) was employed, which often optimizes the recovery of both polar and semi-polar antioxidant compounds compared to absolute ethanol or methanol alone (Xie & Schaich, 2014; Sharma & Bhat, 2009).

Conclusion:-

This study demonstrated the antioxidant potential of sixteen plant extracts traditionally used in the Bengassou Sub-prefecture for the treatment of liver disorders. Phytochemical screening confirmed the presence of secondary metabolites known for their antioxidant properties, including polyphenols, flavonoids, and tannins, as well as sterols, polyterpenes, and coumarins. Among the investigated species, particularly *Entandrophragma angolense*, *Uncaria africana*, and *Vismia guineensis* exhibited outstanding antioxidant activity, as evidenced by their high phenolic content and strong DPPH radical scavenging capacity. 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 for the treatment of liver diseases.

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