Effect of the association of *Chromolaena odorata* or *Combretum micranthum* on the antioxidant activity of *Cochlospermum tinctorium*

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Abstract :
Cochlospermum tinctorium is a medicinal plant recognized for the treatment of various diseases. In Benin, *C. tinctorium* (wild or cultivated form) alone or its combination with *Chromolaena odorata* or *Combretum micranthum*, used in the treatment of liver diseases. The present study aimed to evaluate the association of *Chromoleana odorata* or *Combretum micranthum* to *C. tinctorium* on its phenolic composition and antioxidant activity. The aqueous, ethanolic and hydroethanolic extracts of *Cochlospermum tinctorium* preparations were prepared. The antioxidant activity of the extracts was explored by the DPPH radical scavenging test. Their polyphenol and total flavonoid contents were determined by the spectrophotometric method. Results showed that there was no difference in phenolic composition and antioxidant activity between wild and cultivated forms of *C. tinctorium*. However, recipes combining *Cochlospermum tinctorium* with *Chromolaena odorata* or *Combretum micranthum* enhance the antioxidant activity of the aqueous extracts but do not influence the antioxidant activity of the hydroethanolic and ethanolic extracts of the plant. Similarly, the polyphenol and flavonoid contents did not vary with the combination. Hydroethanolic extracts presented the best antioxidant activities and the highest polyphenols contents. This study showed that the cultivation of *C. tinctorium* does not alter its phenolic composition and antioxidant activity. The combination of *Chromolaena odorata* or *Combretum micranthum* does not significantly enhance the antioxidant activity of *Cochlospermum tinctorium*.

Key words: Antioxidant activity, Phenolic composition, *Cochlospermum tinctorium, Chromolaena odorata, Combretum micranthum*.

Effet de l'association de *Chromolaena odorata* ou *Combretum micranthum* sur l'activité antioxydante de *Cochlospermum tinctorium*

Résumé :
Cochlospermum tinctorium est une plante médicinale reconnue pour le traitement de diverses maladies. Au Bénin, *C. tinctorium* (forme sauvage ou cultivée) seul ou en association avec *Chromolaena odorata* ou *Combretum micranthum*, est utilisée dans le traitement des maladies hépatiques. Cette étude visait à évaluer l'effet de l'ajout de *Chromolaena odorata* ou de *Combretum micranthum* sur la composition phénolique et l'activité antioxydante de *C. tinctorium*. Les travaux ont porté sur les extraits aqueux, éthanolique et hydroéthanolique de différentes préparations (100 %, 80 % et 50 %) à base de *Cochlospermum tinctorium*. L'activité antioxydante des extraits a été explorée par le test de piégeage du radical DPPH. Leurs teneurs en polyphénols et en flavonoïdes totaux ont été déterminées par la méthode spectrophotométrique. Les résultats ont montré qu'il n'y a pas de différence de composition phénolique et d'activité antioxydante entre les formes sauvages et cultivées de *C. tinctorium*. Cependant les recettes combinant *Cochlospermum tinctorium* avec *Chromolaena odorata* ou *Combretum micranthum* renforcent l'activité antioxydante des extraits aqueux mais n'influencent pas celle des extraits hydroéthanolique et éthanolique de la plante. De même, les teneurs en polyphénols et en flavonoïdes n'ont pas varié avec l'association. Les extraits hydroéthanoliques ont présenté les meilleurs pouvoirs antioxydants et les teneurs en polyphénols les plus élevées. Cette étude a montré que la culture de *C. tinctorium* n'altère pas sa composition phénolique et son activité antioxydante. La combinaison de *Chromolaena odorata* ou de *Combretum micranthum* ne renforce pas significativement l'activité antioxydante de *Cochlospermum tinctorium*.

Mots clés: Activité antioxydante, composition phénolique, *Cochlospermum tinctorium, Chromolaena odorata, Combretum micranthum*.

Introduction

Cochlospermum tinctorium Perrier ex A.Rich. (*C. tinctorium*) is one of the plant species belonging to the Bixaceae family (The Plant List, 2012). It is a sub-shrub (woody plant at the base with herbaceous branches) up to 80 cm high, with a woody underground rhizome producing annual shoots. It is a medicinal plant with several ethnopharmacological applications. The medicinal virtues of various parts of C. *tinctorium*, particularly its rhizome, are recognized in several African pharmacopoeias (Ballin et al., 2002). In West Africa, the rhizome of *C. tinctorium* is used in the treatment of jaundice and liver diseases (Ahmad et al., 2021). It is also used to treat oedema, urinary incontinence, dysmenorrhoea, epilepsy, schistosomiasis, pneumonia, bronchial diseases, conjunctivitis, gastric problems, diarrhoea, indigestion, stomach ache and skin diseases (Ahmad et al., 2021).
In Burkina Faso, rhizome extracts of the plant are

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indicated for the treatment of malaria (Zerbo et al., 2007). In Nigeria, a recipe based on its fruits and tamarind is indicated to treat snake bites (Igoli et al., 2004; Maiha et al., 2009). The rhizome is used in the treatment of leprosy (Igoli et al., 2004; Maiha et al., 2009). The decoction of the branches or rhizome is used in Ivory Coast for the treatment of genito-urinary disorders, renal or intercostal pain (Ballin et al., 2002). In Mali, the plant is used to treat gastrointestinal diseases, jaundice, malaria and schistosomiasis (Nergard et al., 2005).

The frequent use of the plant's rhizome in the traditional treatment of diseases could pose a threat to the species, although it is not listed as threatened. In Benin, apart to the use of wild plant, populations used also cultivated species for food and medicinal purposes (Dossa et al., 2021). From this observation, emerges the following question: Is there a difference between the composition of secondary metabolites (total polyphenols and flavonoids) and the antioxidant activity of these two forms (wild and cultivated) of *C. tinctorium*?

Furthermore, according to some medicinal indications, *C. tinctorium* is often used in association with other plant species in the traditional treatment of various pathologies (Dossa et al., 2021). In Benin, *Cochlospermum tinctorium* is associated with *Chromolaena odorata* or *Combretum micranthum* in the traditional treatment of liver diseases. Moreover, liver diseases are pathologies in which oxidative stress is at the center of their physiopathology (Li et al., 2015; Singh et al., 2020). The therapeutic success of modern drugs or medicinal formulations of phytotherapy is often related to their antioxidant properties (Casas-Grajales and Muriel, 2015; Li et al., 2017). These observations suggest the need to investigate the influence of plants associated with *C. tinctorium* on its antioxidant activity and phenolic composition. The present study aimed to evaluate the effect of the association of *Chromoleana odorata* or *Combretum micrathum* to *C. tinctorium* on its phenolic composition and antioxidant activity.

Material and Methods

1. Le matériel végétal

Plant material

Plant material used was the rhizome of *Cochlospermum tinctorium* and the leaves of *Chromolaena odorata* and *Combretum micranthum*. *C. tinctorium* was collected in its natural habitat and in plantations maintained by some populations. *Chromolaena odorata* and *Combretum micranthum* were collected, identified and certified at the National Herbarium of Benin. The certification numbers are YH 356 /HNB for *Combretum micranthum* G.Don; YH621/HNB for *Chromolaena odorata* (L.) R.M. King and YH 622/HNB for *Cochlospermum tinctorium* ex A.Rich.

Chemicals

Chemical material used in this study were ethanol, vitamin C, DPPH (2,2-Diphenyl-1-picrylhydrazyl), rutin, Folin Ciocalteu reagent, gallic acid, iron chloride ($FeCl_3$), sodium carbonate (Na_2CO_3), aluminium trichloride ($AlCl_3$) etc. These reagents and solvents were provided by the Research Unit in Applied Microbiology and Pharmacology of natural substances (URMAPha) of the University of Abomey-Calavi (UAC-Benin).

Formulation of medicinal recipes and preparation of extracts plants

Based on the indications of Beninese traditional medicine practitioners, ten medicinal formulations of recipe from the powder of each of the three plants studied were used (Table I) (Dossa et al., 2021).

Table I: Formulation of medicinal recipes based of *Cochlospermum tinctorium*

Numbers recipes/ Composition of the recipes	Percentage of the powder of *C. tinctorium* (wild form)	Percentage of the powder of *C. tinctorium* (cultivated form)	Percentage of the powder of *Chromolaena odorata*	Percentage of the powder of *Combretum micranthum*
1	100%	-	-	-
2	-	100%	-	-
3	80%	-	20%	-
4	-	80%	20%	-
5	50%	-	50%	-
6	-	50%	50%	-
7	80%	-	-	20%
8	-	80%	-	20%
9	50%	-	-	50%
10	-	50%	-	50%

Each medicinal formulation was used for the preparation of the three extracts types (aqueous, hydroethanolic and ethanolic) following the protocol described by Klotoé et al. (2020). Briefly, 50 g of powder were macerated in 500 mL of distilled water for the aqueous extraction. For ethanolic extraction 50 g of powder were macerated in 500 mL of ethanol and finally for hydroethanolic extraction 50 g of powder were macerated in 500 mL of the mixture of distilled water and ethanol in equal volume (v/v). After 72 h of continuous agitation, the homogenate obtained was then filtered three times on absorbent cotton and once of paper wattman N°2. The filtrate was evaporated with the drying oven with 40 °C until obtaining a mass dries representing the extract. The extract obtained has been weighed and used to evaluate the extraction yield (EY) and then kept in the refrigerator at 4°C.

$$EY = \frac{\text{mass of the extract after evaporation of the solvent}}{\text{mass of the powder of the plant used for the extraction}} \times 100$$

Determination of the total polyphenol and flavonoid contents of the extracts of the preparations studied

Total polyphenols were quantified following the method of Singleton et al. (1999) using the commercial Folin Ciocalteu reagent (Klotoé et al., 2020). The quantification of flavonoids in the plant extracts studied was carried out by a method adapted using aluminium trichloride (AlCl3) as reagent (Kim et al., 2003; Zhishen et al., 1999).

Antioxidant activity of extracts from different medicinal preparations

The DPPH free radical scavenging assay was used to assess the antioxidant activity of the extracts. DPPH (2,2- Diphenyl-1-picrylhydrazyl) is a stable purplish colored free radical which in the presence of anti-free radical compounds is reduced and changes its color to yellow. The method adopted in this study is that of Klotoé et al. (2020). The percentage of DPPH radical scavenging was determined by the formula:

$$P = \frac{(Ab - Ae).}{Ab} \times 100,$$

With P, the percentage of trapping; Ab, the absorbance of the control and Ae, the absorbance of the sample.

Statistical data analysis

Data obtained were subjected to statistical analysis using SPSS 26.0 software. Quantitative variables are presented as mean and standard deviation. Probit analysis was used for the determination of the IC_{50}. The Student's t-test was used to compare the data of the different parameters (extraction yield, total polyphenol and flavonoid content and antioxidant activity) of the wild type to the cultivated type. A single factor analysis of variance (ANOVA) was used to assess the influence of plant associations on extraction yield, total polyphenol and flavonoid content and antioxidant activity. The significance level was set at 5%.

Results and Discussion

Extraction yields of wild and cultivated forms of *C. tinctorium* and the formulations studied.

Figure 1 shows the extraction yields of the wild and cultivated forms of *C. tinctorium*. Analysis of the data in this figure showed that the comparison between the same types of extracts of both forms revealed that the hydroethanolic and ethanolic extracts of the wild form of *C. tinctorium* showed significantly (P<0.05) better yield compared to those of the cultivated form. Concerning the aqueous extracts, a better extraction yield was obtained for the wild form of C. tinctorium. Tables II and III show the extraction yields of *Cochlospermum tinctorium* alone (wild and cultivated forms) and of its association with *Combretum micranthum* and with *Chromolaena odorata*, respectively.

From the analysis of the data in Table II, it appears that the association of Combretum micranthum with *Cochlospermum tinctorium* had no significant effect on the extraction yield (P>0.05). The same observation was noted concerning the recipe combining *Cochlospermum tinctorium* with *Chromolaena odorata* (Table III). However, the hydroethanolic extracts of different formulations have presented the best extraction yields. These observations reflect that the mixture (water-ethanol) offers a better quantitative bioavailability of the active ingredients of the studied plants. Several studies have pointed out

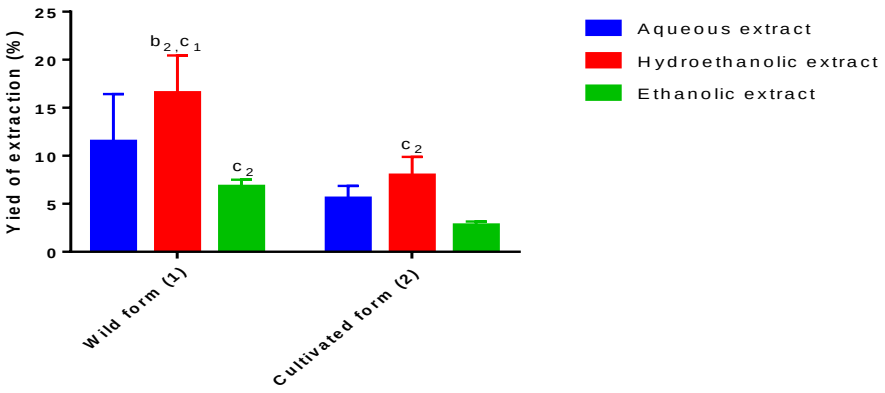

Figure 1: Extraction yield of wild and cultivated forms of *C. tinctorium*.

Legend: 1: Wild form; **2:** Cultivated form; **a1:** Aqueous extract (wild); **b1:** hydroethanolic extract (wild); **c1:** ethanolic extract (wild); a2: aqueous extract (cultivated); **b2:** hydroethanolic extract (Cultivated) **c2:** ethanolic extract (Cultivated). Extracts marked with different letters reflect a significant difference (P<0.05). The letters not mentioned on the graph translate that there is no significant difference in relation with their extract (P>0.05). The marked extracts of two letters translate a significant difference between this extract and two other extracts identifiable according to the legend.

Table II: Extraction yield of *Cochlospermum tinctorium* alone and its association with *Combretum micranthum*

C. tinctorium forms	Type of extract	Recipes based of *C. tinctorium*	Average (%)	Ecart type	ANOVA
Wild form	Aqueous	100 % of *C. tinctorium*	11.49	4.93	NS
	Aqueous	80 % of *C. tinctorium* and 20% *C. micranthum*	9.57	0.64	
	Aqueous	50% of *C. tinctorium* and 50% *C. micranthum*	9.42	0.71	
Cultivated form	Aqueous	100 % of *C. tinctorium*	5.58	1.28	NS
	Aqueous	80 % of *C. tinctorium* and 20% *C. micranthum*	6	0.14	
	Aqueous	50% of *C. tinctorium* and 50% *C. micranthum*	12.09	0.13	
Wild form	Hydroethanolic	100 % of *C. tinctorium*	16.55	3.9	NS
	Hydroethanolic	80 % of *C. tinctorium* and 20% *C. micranthum*	13.48	1.61	
	Hydroethanolic	*50% of C. tinctorium and 50% C. micranthum*	14.62	0.56	
Cultivated form	Hydroethanolic	100 % of *C. tinctorium*	7.96	1.93	NS
	Hydroethanolic	80 % of *C. tinctorium* and 20% *C. micranthum*	10.73	1.71	
	Hydroethanolic	50% of *C. tinctorium* and 50% *C. micranthum*	12.71	0.15	
Wild form	Ethanolic	100 % of *C. tinctorium*	6.8	0.72	NS
	Ethanolic	80 % of *C. tinctorium* and 20% *C. micranthum*	4.21	0.13	
	Ethanolic	50% of *C. tinctorium* and 50% *C. micranthum*	3.11	0.1	
Cultivated form	Ethanolic	100 % of *C. tinctorium*	2.77	0.38	NS
	Ethanolic	80 % of *C. tinctorium* and 20% *C. micranthum*	7.33	6.15	
	Ethanolic	50% of *C. tinctorium* and 50% *C. micranthum*	6.03	0.15	

Legend: **NS:** No significant difference, **a:** Significant difference with 100% form, **b:** Significant difference with 80% combination and **c:** Significant difference with 50% combination. Statistical analysis (ANOVA) was carried out by type of extract and the form of *C tinctorium*, between the receipts with 100% of *C. tinctorium*, 80% of C. *tinctorium* and 20 % of C. *micranthum* and 50% of C. *tinctorium* and 50 % of *C. micranthum*.

Table III: Extraction yield of *Cochlospermum tinctorium* alone and its association with *Chromolaena odorata*

C. tinctorium forms	Type of extract	Recipes based of *C. tinctorium*	Average (%)	Ecart type	ANOVA
Wild form	Aqueous	100 % of *C. tinctorium*	11.49	4.93	NS
	Aqueous	80 % of *C. tinctorium* and 20% *C. odorata*	10.92	1.03	
	Aqueous	50% of *C. tinctorium* and 50% *C. odorata*	11.41	4.74	
Cultivated form	Aqueous	100 % of *C. tinctorium*	5.58	1.28	NS
	Aqueous	80 % of *C. tinctorium* and 20% *C. odorata*	10.12	1.49	
	Aqueous	50% of *C. tinctorium* and 50% *C. odorata*	14.87	0.3	a
Wild form	Hydroethanolic	100 % of *C. tinctorium*	16.55	3.9	NS
	Hydroethanolic	80 % of *C. tinctorium* and 20% *C. odorata*	16.49	1.27	
	Hydroethanolic	*50% of C. tinctorium* and *50% C. odorata*	16.13	0.44	
Cultivated form	Hydroethanolic	100 % of *C. tinctorium*	7.96	1.93	b.c
	Hydroethanolic	80 % of *C. tinctorium* and 20% *C. odorata*	16.25	1.67	NS
	Hydroethanolic	50% of *C. tinctorium* and 50% *C. odorata*	15.67	0.44	
Wild form	Ethanolic	100 % of *C. tinctorium*	6.8	0.71	NS
	Ethanolic	80 % of *C. tinctorium* and 20% *C. odorata*	7.01	1.31	
	Ethanolic	50% of *C. tinctorium* and 50% *C. odorata*	8.96	0.17	
Cultivated form	Ethanolic	100 % of *C. tinctorium*	2.78	0.38	NS
	Ethanolic	80 % of *C. tinctorium* and 20% *C. odorata*	4.83	1.46	
	Ethanolic	50% of *C. tinctorium* and 50% *C. odorata*	8.39	0.2	

Legend: **NS:** No significant difference, **a:** Significant difference with 100% form, **b:** Significant difference with 80% combination and **c:** Significant difference with 50% combination. Statistical analysis (ANOVA) was carried out by type of extract and the shape of *C. tinctorium,* between the receipts with 100% of *C. tinctorium,* 80% of *C. tinctorium* and 20 % of *C. odorata* and 50% of *C tinctorium* and 50 % of *C. odorata*

that the mixed solvent (water-ethanol) improves the extraction yield more than pure solvents (Do et al., 2014; Vieito et al., 2018; Złotek et al., 2016).

Determination of total polyphenol and flavonoids contents of extracts of wild and cultivated forms of C. tinctorium and the formulations studied

Figure 2 shows the total polyphenol and flavonoid contents of extracts from wild and cultivated forms of *C. tinctorium*. The analysis of the data presented in this figure showed that there is no significant difference (P>0.05) between the total polyphenol contents of the same types of extracts of wild and cultivated forms of *C. tinctorium*. However, there is a variation according to the type of extract. Thus, the hydroethanolic and ethanolic extracts compared to the aqueous extract, presented a better content of total polyphenols. For total flavonoids, the comparison between the same types of extracts of the two forms of *C. tinctorium* revealed that the aqueous extract of the cultivated form presented a significantly high content (P<0.05) compared to that of the wild form. On the other hand, the comparison between the extracts of the same form of *C. tinctorium* showed that the ethanolic extract of the wild type presented a significantly high content (P<0.05) compared to the aqueous and hydroethanolic extracts. The same observation was obtained for the cultivated form. These observations showed that the culture of this vegetable species could

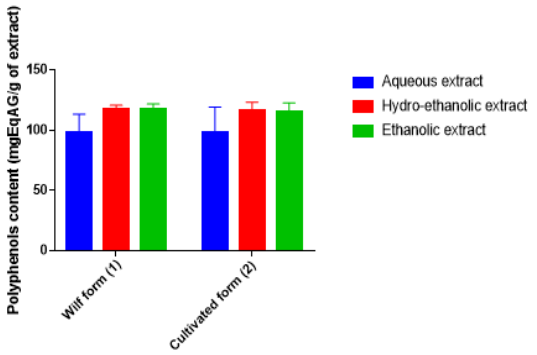

Fig 2a. Total polyphenols content

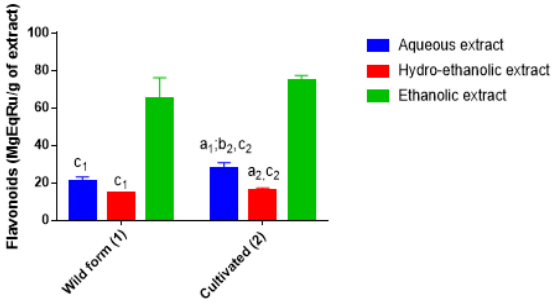

Fig 2b. Total flavonoids content

Figure 2: Total polyphenol and flavonoid contents of extracts from wild and cultivated forms of *C. tinctorium*.

1: Wild form ; **2:** Cultivated form; a_1: aqueous extract (wild); $\mathbf{b_1}$**:** hydroethanolic extract (wild); $\mathbf{c_1}$**:** ethanolic extract (wild); $\mathbf{a_2}$**:** Aqueous extract (cultivated); $\mathbf{b_2}$**:** hydroethanolic extract (cultivated) $\mathbf{c_2}$**:** Ethanolic extract (cultivated). Extracts marked with different letters reflect a significant difference (P<0.05). The letters not mentioned on the graph translate that there is no significant difference in relation with their extract (P>0.05). The marked extracts of two or three letters translate a significant difference between this extract and two or three other extracts identifiable according to the legend.

constitute an option to the threats of disappearance of *Cochlospermum tinctorium*. Some scientific data have emphasized that the cultivation of plant species allows the permanent availability of these species in response to the threats of their disappearance due to the uses of their non-renewable organs (roots) in traditional medicine (Carrillo-Galván et al., 2020; Ekiert et al., 2021; Vodouhè and Dansi, 2012). Moreover, the similarity of phenolic composition and antioxidant activity observed for the two forms of *C. tinctorium* allows predicting the same therapeutic potentialities. However, certain authors reported that the cultivation of medicinal plants does not always allow the conservation of their phytochemical composition and therapeutic virtues (Sher et al., 2010).

Tables IV and V present respectively the total polyphenol contents of the extracts of *Cochlospermum tinctorium* and its association with *Chromolaena odorata* and its association with *Combretum micranthum*. The data of these tables proved that the association of *Chromolaena odorata* or *Combretum micranthum* to *C. tinctorium* did not have a significant influence on its total polyphenol content (P>0.05). However, a few improvement of this content was observed for the extracts aqueous and hydroethanolic of the association of *Chromolaena odorata* to *C. tinctorium*. In addition, the comparison of these data with those of the literature indicated that *C. tinctorium* of this study was richer in total polyphenols (112,96 mg/g) that *Chromolaena odorata* (56 mg/g) (Alara et al., 2019) and *Combretum micranthum* (17,17 mg/g) (Tine et al., 2019). The association with *C. tinctorium* to *Chromolaena odorata* (115.32 mg/g) or to *Combretum micranthum* (104.51mg/g) was richer in total polyphenols than either of these plants (Ndouyang et al., 2018; Tine et al., 2019) . These observations could be explained by the combined effect of the richness in polyphenolic compounds of the two plants studied such as reported by several authors (Ahmad et al., 2021; Ndouyang et al., 2018; Tine et al., 2019; Vijayaraghavan et al., 2018).

Determination of total flavonoids of the extracts of the studied formulations

Total flavonoid contents of *Cochlospermum tinctorium* extracts and its association with *Chromolaena odorata* are presented in table VI. The analysis of the data presented in this table reveals that the association of *Cochlospermum tinctorium* (80%) (wild form) with *Chromolaena odorata* (20%) induced an increase in the total flavonoid content in the aqueous and ethanolic extracts. Concerning the cultivated form, only the aqueous and hydroethanolic extracts obtained from the 80% association showed a better flavonoid content compared to *Cochlospermum tinctorium* alone (100%). Comparison of these data with the literature reveals that the C. tinctorium species in this study was richer in total flavonoids (36.36 mg/g) than that studied (4 mg/g) by Ndouyang et al. (2018). Similarly, the combination of Chromolaena odorata with C. tinctorium showed

high total flavonoids (36.43 mg/g) compared to C. tinctorium alone (4 mg/g) (Ndouyang et al., 2018) and Chromolaena odorata alone (22.39 mg/g) (Alara et al., 2019).

Table IV: Total polyphenol content of *Cochlospermum tinctorium* extracts and its association with *Chromolaena Odorata.*

C. tinctorium forms	Type of extract	Recipes based of *C. tinctorium*	Polyphenols content mgEqAG/g of extract	Ecart type	ANOVA
Wild form	Aqueous	100 % of *C. tinctorium*	98.84	14.68	NS
	Aqueous	80 % of *C. tinctorium* and 20% *C. odorata*	114.9	1.38	
	Aqueous	50% of *C. tinctorium* and 50% *C. odorata*	121.18	11.74	
Cultivated form	Aqueous	100 % of *C. tinctorium*	98.34	21.1	NS
	Aqueous	80 % of *C. tinctorium* and 20% *C. odorata*	117.16	0.99	
	Aqueous	50% of *C. tinctorium* and 50% *C. odorata*	125.73	4.67	a
Wild form	Hydroethanolic	100 % of *C. tinctorium*	118.12	2.83	NS
	Hydroethanolic	80 % of *C. tinctorium* and 20% *C. odorata*	123.24	2.91	
	Hydroethanolic	*50% of C. tinctorium* and *50% C. odorata*	121.59	2.84	
Cultivated form	Hydroethanolic	100 % of *C. tinctorium*	116.82	6.61	NS
	Hydroethanolic	80 % of *C. tinctorium* and 20% *C. odorata*	122.23	3.44	
	Hydroethanolic	50% of *C. tinctorium* and 50% *C. odorata*	131.62	3.16	
Wild form	Ethanolic	100 % of *C. tinctorium*	117.23	4.79	NS
	Ethanolic	80 % of *C. tinctorium* and 20% *C. odorata*	109.73	4.08	
	Ethanolic	50% of *C. tinctorium* and 50% *C. odorata*	116.2	15.32	
Cultivated form	Ethanolic	100 % of *C. tinctorium*	115.12	7.78	NS
	Ethanolic	80 % of *C. tinctorium* and 20% *C. odorata*	104.69	4.03	
	Ethanolic	50% of *C. tinctorium* and 50% *C. odorata*	117.68	3.85	

Legend: **NS:** No significant difference, **a:** Significant difference with 100% form, **b:** Significant difference with 80% combination and **c:** Significant difference with 50% combination. Statistical analysis (ANOVA) was carried out by type of extract and the shape of *C. tinctorium*, between the recipes with 100% of *C. tinctorium*, 80% of *C. tinctorium* and 20 % of *C. odorata* and 50% of *C. tinctorium* and 50 % of *C. odorata*

Table V: Total polyphenol content of *Cochlospermum tinctorium* extracts and its association with *Combretum micranthum*.

C. tinctorium forms	Type of extract	Recipes based of *C. tinctorium*	Polyphenols mgEqAG/g of extract	Ecart type	ANOVA
Wild form	Aqueous	100 % of *C. tinctorium*	104.49	4.92	NS
	Aqueous	80 % of *C. tinctorium* and 20% *C. micranthum*	98.76	1.32	
	Aqueous	50% of *C. tinctorium* and 50% *C. micranthum*	95.68	6.19	
Cultivated form	Aqueous	100 % of *C. tinctorium*	106.41	7.4	NS
	Aqueous	80 % of *C. tinctorium* and 20% *C. micranthum*	103.04	3.86	
	Aqueous	50% of *C. tinctorium* and 50% *C. micranthum*	95.04	8.12	
Wild form	Hydroethanolic	100 % of *C. tinctorium*	119.2	1.06	NS
	Hydroethanolic	80 % of *C. tinctorium* and 20% *C. micranthum*	114.12	3.15	
	Hydroethanolic	*50% of C. tinctorium and 50% C. micranthum*	107.84	3.65	
Cultivated form	Hydroethanolic	100 % of *C. tinctorium*	119.26	2.8	NS
	Hydroethanolic	80 % of *C. tinctorium* and 20% *C. micranthum*	107.4	0.25	
	Hydroethanolic	50% of *C. tinctorium* and 50% *C. micranthum*	111.26	1.07	
Wild form	Ethanolic	100 % of *C. tinctorium*	116.15	3.99	NS
	Ethanolic	80 % of *C. tinctorium* and 20% *C. micranthum*	108.21	1.72	
	Ethanolic	50% of *C. tinctorium* and 50% *C. micranthum*	109.21	3.38	
Cultivated form	Ethanolic	100 % of *C. tinctorium*	112.28	3.48	NS
	Ethanolic	80 % of *C. tinctorium* and 20% *C. micranthum*	95.54	50.11	
	Ethanolic	50% of *C. tinctorium* and 50% *C. micranthum*	81.62	16.44	

Legend: NS: No significant difference, a: Significant difference with 100% form, b: Significant difference with 80% combination and c: Significant difference with 50% combination. Statistical analysis (ANOVA) was carried out by type of extract and the form of *C tinctorium*, between the receipts with 100% of *C. tinctorium,* 80% of *C. tinctorium* and 20 % of *C. micranthum* and 50% of *C. tinctorium* and 50 % of *C. micranthum*.

Table VI: Total flavonoid content of *Cochlospermum tinctorium* extracts and its association with *Chromolaena odorata.*

C. tinctorium forms	Type of extract	Recipes based of *C. tinctorium*	Flavonoids MgEqRu/g of extract	Ecart type	ANOVA
Wild form	Aqueous	100 % of *C. tinctorium*	20.5	2.73	NS
	Aqueous	80 % of *C. tinctorium* and 20% *C. odorata*	32.09	3.04	
	Aqueous	50% of *C. tinctorium* and 50% *C. odorata*	42.44	10.76	a
Cultivated form	Aqueous	100 % of *C. tinctorium*	27.99	2.82	NS
	Aqueous	80 % of *C. tinctorium* and 20% *C. odorata*	33.7	1.01	
	Aqueous	50% of *C. tinctorium* and 50% *C. odorata*	23.69	0.5	
Wild form	Hydroethanolic	100 % of *C. tinctorium*	14.24	0.18	b,c
	Hydroethanolic	80 % of *C. tinctorium* and 20% *C. odorata*	25.19	0.24	c
	Hydroethanolic	*50% of C. tinctorium* and 50% *C. odorata*	3.32	0.4	
Cultivated form	Hydroethanolic	100 % of *C. tinctorium*	15.78	1.44	
	Hydroethanolic	80 % of *C. tinctorium* and 20% *C. odorata*	27.57	0.42	c
	Hydroethanolic	50% of *C. tinctorium* and 50% *C. odorata*	4.17	0.38	
Wild form	Ethanolic	100 % of *C. tinctorium*	64.78	11.44	b
	Ethanolic	80 % of *C. tinctorium* and 20% *C. odorata*	50.04	0.16	
	Ethanolic	50% of *C. tinctorium* and 50% *C. odorata*	73.85	0.01	b
Cultivated form	Ethanolic	100 % of *C. tinctorium*	74.97	2.52	b,c
	Ethanolic	80 % of *C. tinctorium* and 20% *C. odorata*	50.3	0.39	NS
	Ethanolic	50% of *C. tinctorium* and 50% *C. odorata*	55.12	1.22	

Legend: **NS:** No significant difference, **a:** Significant difference with 100% form, **b:** Significant difference with 80% combination and **c:** Significant difference with 50% combination. Statistical analysis (ANOVA) was carried out by type of extract and the shape of *C. tinctorium,* between the receipts with 100% of *C. tinctorium*, 80% of *C. tinctorium* and 20 % of *C odorata* and 50% of *C. tinctorium* and 50 % of *C. odorata.*

Table VII provide the information on the quantification of total flavonoids of the extracts of *Cochlospermum tinctorium* and its association with *Combretum micranthum*. The analysis of the data in this table shows that except for the hydroethanolic extract, there is no significant difference between the total flavonoid content of the aqueous and ethanolic extracts of *Cochlospermum tinctorium* alone compared to its association with *Combretum micranthum*. However, the combination of *Combretum micranthum* with *C. tinctorium* showed a higher content of total flavonoids than either plant (Ndouyang et al., 2018; Tine et al., 2019).

Table VII: Total flavonoids content of *Cochlospermum tinctorium* extracts and its association with *Combretum micranthum*.

C. tinctorium forms	Type of extract	Recipes based of *C. tinctorium*	Flavonoids MgEqRu/g of extract	Ecart type	ANOVA
Wild form	Aqueous	100 % of *C. tinctorium*	20.5	2.73	NS
	Aqueous	80 % of *C. tinctorium* and 20% *C. micranthum*	33.38	5.67	
	Aqueous	50% of *C. tinctorium* and 50% *C. micranthum*	23.61	1.1	
Cultivated form	Aqueous	100 % of *C. tinctorium*	27.99	2.82	NS
	Aqueous	80 % of *C. tinctorium* and 20% *C. micranthum*	11.24	7.81	
	Aqueous	50% of *C. tinctorium* and 50% *C. micranthum*	26.03	2.6	
Wild form	Hydroethanolic	100 % of *C. tinctorium*	14.24	0.18	
	Hydroethanolic	80 % of *C. tinctorium* and 20% *C. micranthum*	34.96	2.23	a
	Hydroethanolic	*50% of C. tinctorium and 50% C. micranthum*	34.41	3.2	
Cultivated form	Hydroethanolic	100 % of *C. tinctorium*	15.78	1.44	
	Hydroethanolic	80 % of *C. tinctorium* and 20% *C. micranthum*	26.12	2.82	c
	Hydroethanolic	50% of *C. tinctorium* and 50% *C. micranthum*	52.97	12.74	
Wild form	Ethanolic	100 % of *C. tinctorium*	64.78	11.44	NS
	Ethanolic	80 % of *C. tinctorium* and 20% *C. micranthum*	66.95	8.41	
	Ethanolic	50% of *C. tinctorium* and 50% *C. micranthum*	74.05	1.2	
Cultivated form	Ethanolic	100 % of *C. tinctorium*	74.97	2.52	NS
	Ethanolic	80 % of *C. tinctorium* and 20% *C. micranthum*	73.76	2.34	
	Ethanolic	50% of *C. tinctorium* and 50% *C. micranthum*	66.11	16.68	

Legend: **NS:** No significant difference, **a:** Significant difference with 100% form, **b:** Significant difference with 80% combination and **c:** Significant difference with 50% combination. Statistical analysis (ANOVA) was carried out by type of extract and the form of *C. tinctorium*, between the recipes with 100% of *C. tinctorium*, 80% of C. *tinctorium* and 20 % of *C. micranthum* and 50% of *C. tinctorium* and 50 % of *C. micranthum*.

Antioxidant activity of extracts of wild and cultivated forms of C. tinctorium and the formulations studied

Figure 3 presents the antioxidant activity of extracts of wild and cultivated forms of *C. tinctorium*. The data analysis of this figure reveals that there is no significant variation ($P<0.05$) in the antioxidant activity of the same types of extracts of both forms of *C. tinctorium*. However, the ethanolic and hydroethanolic extracts showed

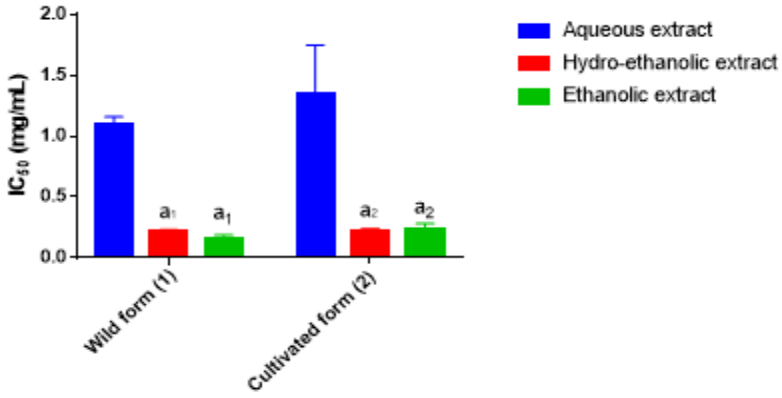

Figure 3: Antioxidant activity of extracts from wild and cultivated forms of *C. tinctorium*.
1: Wild; **2:** Cultivated; **a_1:** aqueous extract (wild); **b_1:** hydroethanolic extract (wild); **c_1:** ethanolic extract (wild); **a_2:** aqueous extract (cultivated); **b_2:** hydroethanolic extract (Cultivated) **c_2:** ethanolic extract (Cultivated). Extracts marked with different letters reflect a significant difference (P<0.05). The letters not mentioned on the graph translate that there is no significant difference in relation with their extract (P>0.05). The marked extracts of two letters translate a significant difference between this extract and two other extracts identifiable according to the legend.

significantly (P<0.05) better antioxidant activity compared to the aqueous extract for both the wild and cultivated forms of *C. tinctorium*. The overall antioxidant activities of the different extracts of the two forms of *C. tinctorium* are low compared to that of the vitamin C used as reference molecule. Such antioxidant activity of *C. tinctorium* has been reported as in our study by the works of Ndouyang et al. (2018) and Nergard et al. (2005). These observations prove that *C. tinctorium* is a medicinal plant endowed with antioxidant property.

Table VIII presents the results of the antioxidant activity of *Cochlospermum tinctorium* and its combination with *Chromolaena odorata*. The data in this table showed that the combination of *Chromolaena odorata* enhanced the antioxidant activity of the aqueous extracts of *C. tinctorium* in contrast to the ethanolic and hydroethanolic extracts. The best antioxidant activities were obtained for the hydroethanolic extracts. Similar results were obtained for the association of *Combretum micranthum* with *Cochlospermum tinctorium* (Table IX). These observations could be explained by the synergy of antioxidant effect of Chromolaena odorata (Maulida et al., 2019; Melinda et al., 2010; Putri and Fatmawati, 2019), *C. tinctorium* (Ndouyang et al., 2018; Nergard et al., 2005) and *Combretum micranthum* (Kpemissi et al., 2019). Similar data is reported by Jain et al. (2011) for tea obtained from the combination of *Vitis vinifera, Phyllanthus emblica L., Punica granatum, Cinnamomum cassia, Ginkgo biloba* L., and *Camellia sinensis* Linn. These authors explain this observation by evoking the synergy of action, which could exist between the bioactive molecules endowed with antioxidant activities of which these plants are constituted. This study revealed that the *C. tinctorium*-based recipes tested have antioxidant properties and are rich in total polyphenols. This property could be the basis of the recognized effectiveness of these recipes in the treatment of liver diseases in Benin (Mouzouvi et al., 2014). Indeed, liver diseases are pathologies in which oxidative stress is at the center of their physiopathology (Li et al., 2015; Singh et al., 2020). In addition, drugs with therapeutic success in the treatment of the hepatic diseases are generally endowed with antioxidant properties (Casas-Grajales and Muriel, 2015; Li et al., 2017). In perspective, in vivo studies are necessary to explore the antioxidant properties in animal model. Similar scientific investigations need to be conducted on medicinal recipes as used by traditional medicine practitioners to establish a scientific basis for this approach.).

Table VIII: Antioxidant activity of *Cochlospermum tinctorium* extracts and its association with *Chromolaena odorata*

C. tinctorium forms	Type of extracts	Recipes based of *C. tinctorium*	IC_{50} (mg/mL)	Ecart type	ANOVA
Wild form	Aqueous	100 % of *C. tinctorium*	1.09	0.06	b,c
	Aqueous	80 % of *C. tinctorium and* 20% *C. odorata*	0.61	0.05	NS
	Aqueous	50% of *C. tinctorium and* 50% *C. odorata*	0.5	0.01	
Cultivated form	Aqueous	100 % of *C. tinctorium*	1.34	0.41	b,c
	Aqueous	80 % of *C. tinctorium and* 20% *C. odorata*	0.68	0.1	NS
	Aqueous	50% of *C. tinctorium and* 50% *C. odorata*	0.8	0.12	
Wild form	hydroethanolic	100 % *of C. tinctorium*	0.22	0.01	
	hydroethanolic	80 % of *C. tinctorium and* 20% *C. odorata*	0.25	0.05	NS
	hydroethanolic	50% of *C. tinctorium and* 50% *C. odorata*	0.47	0.01	
Cultivated form	hydroethanolic	100 % *of C. tinctorium*	0.22	0.01	
	hydroethanolic	80 % of *C. tinctorium and* 20% *C. odorata*	0.32	0.05	NS
	hydroethanolic	50% of *C. tinctorium and* 50% *C. odorata*	0.53	0.01	
Wild form	Ethanolic	100 % *of C. tinctorium*	0.16	0.02	b
	Ethanolic	80 % of *C. tinctorium and* 20% *C. odorata*	0.61	0.11	NS
	Ethanolic	50% of *C. tinctorium and* 50% *C. odorata*	0.42	0.25	
Cultivated form	Ethanolic	100 % of *C. tinctorium*	0.23	0.05	c
	Ethanolic	80 % of *C. tinctorium and* 20% *C. odorata*	0.47	0.13	c
	Ethanolic	50% of *C. tinctorium and* 50% *C. odorata*	0.97	0.05	

Legend: **NS:** No significant difference, **a:** Significant difference with 100% form, **b:** Significant difference with 80% combination and **c:** Significant difference with 50% combination. Statistical analysis (ANOVA) was carried out by type of extract and the shape of *C. tinctorium,* between the recipes with 100% of *C. tinctorium,* 80% of *C. tinctorium* and 20 % of *C. odorata* and 50% of *C. tinctorium* and 50 % of *C. odorata*

Table IX: Antioxidant activity of *Cochlospermum tinctorium* extracts and its association with *Combretum micranthum*.

C. tinctorium forms	Type of extracts	Recipes based of *C. tinctorium*	IC_{50} (mg/mL)	Ecart type	ANOVA
Wild form	Aqueous	100 % of *C. tinctorium*	1.09	0.06	c
	Aqueous	80 % of *C. tinctorium* and 20% *C. micranthum*	0.72	0.05	NS
	Aqueous	50% of *C. tinctorium* and 50% *C. micranthum*	0.53	0.12	
Cultivated form	Aqueous	100 % of *C. tinctorium*	1.34	0.41	b,c
	Aqueous	80 % of *C. tinctorium* and 20% *C. micranthum*	0.45	0.06	NS
	Aqueous	50% of *C. tinctorium* and 50% *C. micranthum*	0.42	0.08	
Wild form	hydroethanolic	100 % of *C. tinctorium*	0.22	0.01	b
	hydroethanolic	80 % of *C. tinctorium* and 20% *C. micranthum*	0.34	0.08	NS
	hydroethanolic	*50% of C. tinctorium and 50% C. micranthum*	0.1	0.01	
Cultivated form	hydroethanolic	100 % of *C. tinctorium*	0.22	0.01	NS
	hydroethanolic	80 % of *C. tinctorium* and 20% *C. micranthum*	0.25	0.06	
	hydroethanolic	50% of *C. tinctorium* and 50% *C. micranthum*	0.23	0.04	
Wild form	Ethanolic	100 % of *C. tinctorium*	0.16	0.02	NS
	Ethanolic	80 % of *C. tinctorium* and 20% *C. micranthum*	0.22	0.1	
	Ethanolic	50% of *C. tinctorium* and 50% *C. micranthum*	0.22	0.04	
Cultivated form	Ethanolic	100 % of *C. tinctorium*	0.23	0.05	NS
	Ethanolic	80 % of *C. tinctorium* and 20% *C. micranthum*	0.59	0.41	
	Ethanolic	50% of *C. tinctorium* and 50% *C. micranthum*	0.55	0.13	

Legend: **NS:** No significant difference, **a:** Significant difference with 100% form, **b:** Significant difference with 80% combination and **c:** Significant difference with 50% combination. Statistical analysis (ANOVA) was carried out by type of extract and the form of *C. tinctorium*, between the recipes with 100% of *C. tinctorium*, 80% of *C. tinctorium* and 20 % of C. *micranthum* and 50% of *C. tinctorium* and 50 % of *C. micranthum*.

Conclusion

This study aimed at studying the influence of *Chromolaena odorata* or *Combretum micranthum* on the phenolic composition and the antioxydant activity of *C tinctorium*. The results obtained showed that the association of each one of these two plants does not influence the content total polyphenols of *C tinctorium*. However, the association of *Chromolaena odorata* and *Combretum micranthum* reinforces the antioxydant activity of the aqueous extracts of *C. tinctorium* but does not influence the antioxidant activity of its hydroethanolic and ethanolic extracts.

Author contributions

Klotoé J.R., Dossa A.K. and Agbodjento E.,

participated at the conception of the study. Ohouko H.F., Amadou A., Vodounon K., Dossa A.K. and Agbodjento E. participed at the collect and analysis of the Data. Klotoé J.R., Agbodjento E. wrote the draft of the manuscript. Dougnon V. and Loko F. ensured the scientific direction of the study. All the authors have read and approved the manuscript.

Declaration of interest

There is no conflict of interest.

Références

Ahmad M.H., Jatau A.I., Khalid G.M., Alshargi O.Y., 2021, Traditional uses, phytochemistry, and pharmacological activities of Cochlospermum tinctorium A. Rich (Cochlospermaceae): a review., *Future Journal of Pharmaceutical Sciences*, 7(1), 1–13.

Alara O., Nour A., Abdul mudalip S.K., 2019, Screening of microwave-assisted-batch extraction parameters for recovering total phenolic and flavonoid contents from Chromolaena odorata leaves through two-level factorial design, *Indonesian Journal of Chemistry*, 19(2), 511-2-521 DOI: 10.22146/ijc.40863.

Ballin N.Z., Traore M., Tinto H., Sittie A., Mølgaard P., Olsen C.E., Kharazmi A., Christensen SR., 2002, Antiplasmodial compounds from Cochlospermum tinctorium, *Journal of Natural Products*, 65(9), 1325–1327.

Carrillo-Galván G, Bye R, Eguiarte LE, Cristians S, Vergara-Silva F., Luna-Cavazos M., 2020, Domestication of aromatic medicinal plants in Mexico: Agastache (Lamiaceae)—an ethnobotanical, morpho-physiological, and phytochemical analysis, *Journal of Ethnobiology and Ethnomedicine*, 16(1): 1-16.

Casas-Grajales S., Muriel P., 2015, Antioxidants in liver health, *World Journal of Gastrointestinal Pharmacology and Therapeutics*, 6(3), 59–72. DOI: 10.4292/wjgpt.v6.i3.59.

Do Q.D., Angkawijaya AE., Tran-Nguyen P.L., Huynh LH, Soetaredjo FE., Ismadji S, Ju Y., 2014, Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica, *Journal of Food and Drug Analysis*, 22(3), 296–302.

Dossa AK, Klotoe J, Agbodjento E, Dougnon V, Sassa Sinkou J, Loko F., 2021, Usages de poudres à base de rhizomes de Cochlospermum tinctorium Perrier ex A.Rich au Bénin : fréquence, formes et indications, *International Journal of Biological and Chemical Sciences*, 15(4), 1511–1523

Ekiert HM, Ramawat KG, Arora J., 2021, Medicinal plants: domestication, biotechnology and regional importance. Sustainable development and biodiversity. *Springer International Publishing*., DOI: 10.1007/978-3-030-74779-4.

Igoli J.O., Igwue I.C., Igoli N.P., 2004, Traditional medicinal practices among the igede people of Nigeria. Journal of Herbs, Spices & Medicinal Plants 10(4), 1–10.

Jain D., Pancholi S., Patel R., 2011, Synergistic antioxidant activity of green tea with some herb, *Journal of advanced pharmaceutical technology & research*, 2(3), 177–183.

Kim D-O ., Chun O.K., Kim Y.J., Moon H.Y., Lee C.Y., 2003, Quantification of polyphenolics and their antioxidant capacity in fresh plums, *Journal of Agricultural and Food Chemistry*, 51(22), 6509–6515.

Klotoé J, Agbodjento E., Dougnon V., Yovo M., Sacramento TI., Deguenon E., Dougnon J., Atègbo JM., 2020, Exploration of the chemical potential and antioxidant activity of some plants used in the treatment of male infertility in southern Benin, *Journal of Pharmaceutical Research International*, 32(4), 1–12.

Kpemissi M, Eklu-Gadegbeku K, Veerapur VP, Potârniche A.V., Adi K., 2019, Antioxidant and nephroprotection activities of Combretum micranthum: A phytochemical, in-vitro and ex-vivo studies, *Heliyon* 5(3), 1 –31.

Li S., Tan H-Y., Wang N., Li HY., Feng Y., Al-Gubory KH., Laher I., 2015, The role of oxidative stress and antioxidants in liver diseases, *International Journal of Molecular Sciences*, 16(11), 26087–26124. DOI: 10.3390/ijms161125942.

Li S, Wang N, Feng Y-G., Wang N., Zhang Z.J., Lao L., Wong C.W., Feng Y., 2017, Antioxidants in the prevention and treatment of liver diseases. In: Al-Gubory KH and Laher I (eds) Nutritional Antioxidant Therapies: Treatments and Perspectives, *Cham: Springer International Publishing*, pp. 467–491. DOI: 10.1007/978-3-319-67625-8_18.

Maiha vii., Magaji M, Yaro A, Hamza A., Magaji R., 2009, Anticovulsant studies on Cochlospermum tinctorium (Cochlospermaceae) and Paullinia pinnata (Sapindaceae) extracts in laboratory animals, *Nigerian Journal of Pharmaceutical Sciences*, 8(1), 102–108.

Maulida PA., Putri DA., Fatmawati S., 2019, Free radical scavenging activity of Chromolaena odorata . Leaves. *IPTEK The Journal for Technology and Science*, 30(3), 73–75.

Melinda KP., Rathinam X., Marimuthu K., Diwakar A., Ramanathan S, Kathiresan S., Subramaniam S., 2010, A comparative study on the antioxidant activity of methanolic leaf extracts of Ficus religiosa L, Chromolaena odorata (L.) King & Rabinson, Cynodon dactylon (L.) Pers. and Tridax procumbens L., *Asian Pacific Journal of Tropical Medicine*, 3(5), 348–350.

Mouzouvi R, Djégo JG, Lalèyè A, Priuli F, Bigot A., 2014, Effet de l'association Combretum micranthum G. Don (Combretaceae) et Cochlospermum tinctorium A. Rich. (Cochlospermaceae) dans la prise en charge de l'hépatite virale B. *Pharmacopée et médecine traditionnelle africaine*, 17(1), 10-14.

Ndouyang C., Kuaté Giscard K., Nguimbou R., Scher J., Gaiani J., Facho B., 2018, Relationship between secondary metabolites, antiradical activities, and colour characteristics of Cochlospermum tinctorium A. Rich. (Bixaceae) Root. *Ghana Journal of Science*, 59, 79–92

Nergard C., Diallo D., Inngjerdingen K., Michaelsen TE, Matsumoto T., Kiyohara H., 2005, Medicinal use of Cochlospermum tinctorium in Mali, anti-ulcer, radical scavenging and immunomodulating activites of polymers in the aqueous extracts of the roots, *J. Ethnopharmacol*, 96(1-2), 255–269.

Putri D.A., Fatmawati S., 2019, A new flavanone as a potent antioxidant isolated from Chromolaena odorata L. leaves. *Evidence-Based Complementary and Alternative Medicine*, 2019, 1–12

Sher H., Alyemeni M., Faridullah F., 2010, Cultivation and domestication study of high value medicinal plant species (its economic potential and linkages with commercialization), *African Journal of Agricultural Research*, 5(18), 2462–2470.

Singh L., Majhi S., Pabreja K., Negi P., Goyal R., Gupta G., Chellappan DK., Dua K., Maurya Pk., 2020, Oxidative stress in liver diseases. In: Maurya PK and Dua K (eds) Role of Oxidative Stress in Pathophysiology of Diseases., *Singapore: Springer*, pp. 209–228. DOI: 10.1007/978-981-15-1568-2_13.

Singleton VL., Orthofer R., Lamuela-Raventós RM., 1999, Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In: Methods in Enzymology. Oxidants and Antioxidants Part A., *Academic Press*, pp. 152–177.

The Plant List., 2012 , Cochlospermum tinctorium Perrier ex A.Rich. — The Plant List. available at: http://www.theplantlist.org/tpl1.1/record/kew-44552 (accessed 31 May 2021).

Tine D., Fall A., Dieng S., Sarr A., Bassene E., 2019, Total polyphenol, tannin and flavonoid contents of Combretum micranthum leaves harvested in three regions of Senegal: Diass, Sandiara and Essyl. *International Journal of Biological and Chemical Sciences*, 13(3), 1817–1820.

Vieito C., Fernandes É., Velho M., Pires P., 2018, The Effect of different solvents on extraction yield, total phenolic content and antioxidant activity of extracts from Pine Bark (Pinus pinaster subsp. atlantica), *Chemical Engineering Transactions,* 64(2018), 127-132.

Vijayaraghavan K., Rajkumar J., Seyed MA., 2018, Phytochemical screening, free radical scavenging and antimicrobial potential of Chromolaena odorata leaf extracts against pathogenic bacterium in wound infections– a multispectrum perspective, *Biocatalysis and Agricultural Biotechnology*, 15, 103–112.

Vodouhè R, Dansi A. 2012. The "Bringing into Cultivation" phase of the plant domestication Process and its contributions to in situ conservation of genetic resources in Benin, *The Scientific World Journa,l* 2012, 1–13.

Zerbo P., Millogo-Rasolodimey J., Nacoulma-Ouerdraogo OG., Damme PV., 2007, Contribution à la connaissance des plantes médicinales utilisées dans les soins infantiles en pays San, au Burkina Faso, *International Journal of Biological and Chemical Sciences,* 1(3), 262–274.

Zhishen J., Mengcheng T., Jianming W., 1999, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals, *Food Chemistry*, 64(4), 555–559.

Złotek U., Mikulska S., Nagajek M., Świeca M., 2016, The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (Ocimum basilicum L.) extracts., *Saudi Journal of Biological Sciences,* 23(5), 628–633.