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Research Paper

Photochemical screening and antioxydant activity of bark extracts from 2 species endemic Commiphora (Burseraceae) : *C.humbertii* and *C.brevicalyx*.

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ABSTRACT: *The current research concerns essentially the study of antioxidant molecules of natural origin. This study falls into this context and consists in making at first a phytochemical screening of bark extracts from 2 species endemic Commiphora (Burseraceae): C. humbertii and C. brevicalyx, we estimated the antioxidant activity of these extracts. The phytochemical study allowed to highlight the existence of, polyphenols and flavonoids in hydroalcoolic extracts and unsaturated steroids. The method used to measure the antioxidant activity was the free radical scavenging by using DPPH•(2,2-diphenyl-1-picrylhydrazyl). Scavenging capacity of DPPH free radical is very interesting with 0.14 mg/ml for Commiphora humberti and 0.22 mg/ml for Commiphora brevicalyx, while that of ascorbic acid is 0.25 mg/ml.*

Key words*: phytochemical screening, antioxidant activity, Commiphora (Burseraceae),* southern *Madagascar*

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I. Introduction

Medicinal plants used in folk medicine contain a wide range of substances that can prevent and treat many illnesses because they contain natural active ingredients used for human or animal therapeutic purposes. According to the WHO (2013), they are defined as plants whose one or more organs contain substances useful for treating or making medicines. Phytotherapy, a branch of medicine that uses plant extracts in the form of teas, powders, or essential oils, offers a natural approach to treating diseases (Bruneton, 2009).

According to the World Health Organization, 80% of people living in developing countries mainly use traditional medicine. It mainly relies on medicinal plants. The safety and effectiveness of medicinal plants used in traditional medicine must therefore be assessed. (Eloff, 2005).

Within the Burseraceae, the genus Commiphora includes about 190 species worldwide (Schatz, 2001). Madagascar has 26 described species but 44 well-defined taxa (Gostel, 2016). In Madagascar, Commiphora is one of the thirty most widely used plant genera (Rafidison and *al*., 2019), and traditional healers use several species of this genus to treat various ailments. According to an ethnobotanical survey conducted in the southwestern region of the country, decoctions prepared from two Commiphora species are traditionally used for medicinal purposes. —**C. humbertii** and **C. brevicalyx** (family Burseraceae)—are used notably by women two months postpartum to strengthen their health; they are also used to treat childhood diarrhea, traumatic injuries, and poisoning, and—as noted by Boiteau in 1975—for their wound-healing properties. Furthermore, disorders of the reproductive system are treated with decoctions of the bark of the **Commiphora brevicalyx* H. Perrier species (Bost, 1961; Boiteau, 1986). Numerous active substances have been identified in these extracts of whole plants or the bark of certain *Commiphora* species, such as flavonoids and tannins (Moctar Chaibou and *al*., 2020), polysaccharides (Limam Hala, 2016), terpenes, beta-caryophyllene, and steroids (Eitan Amiel and *al*., 2012)

Our research aims to assess the presence of secondary metabolites found in the bark extracts of Commiphora (Burseraceae) (*C. humbertii* and *C. brevicalyx*) and to study the antioxidant activity of the methanolic extract of these extracts by the technique of DPPH (2,2-diphenyl-1-picrylhydrazyl).

II.MATERIALS AND METHODOLOGY

2.1 Materials

2.1.1 Description of the study material

Origin and source of the samples

The plants of these two species were collected during the dry season, in the Ampanihy District, Atsimo Andrefana Region, in southern Madagascar, which is located roughly between 24°41′46″ South latitude and 44°44′46″ East longitude, 180 m altitude

- **Systematic Classification of Commiphora**

The two species of *Commiphora* belong to the classification system as follow (Cronquist A., 1988).
Phylum: Angiosperms
Class: Dicotyledones
Sous- Class: Rosideae
Order: Sapindales
Family: Burseraceae
The two target species are ***Co****mmiphora humbertii* H. Perrier *and C. brevicalyx* H. Perrier. They are known locally daromena and darosiky respectively.

2.1.2 Reactive chemicals

Methanolic solution of FeCl3 10%, Gelatin solution 1%, Sodium chloride 1%, FeCl3 aqueous solution (10%), Glacial acetic acid, Concentrated HCl, Concentrated sulfuric acid, Isoamylalcohol, Acetic anhydride, Ammonia solution 25%, Reagents from MAYER, WAGNER and DRAGENDORFF, Methanol solution of DPPH (8%), methanol, Ascorbic acid (vitamin C)

2.2 Methodology

2.2.1- Preparation of extracts

We used the bark of *C. humbertii* and *brevicalyx*, dried in the shade at room temperature for a few weeks and ground into powder using an electric grinder.
We carried out the phytochemical tests in the Molecular Biology laboratories at the University of Fianarantsoa and at the National Research Center on environment (CNRE). Extraction by maceration in aqueous methanol (solid/liquid extraction100 grams of plant powder are mixed into 1000ml of aqueous methanol and left to stir for 24 hours. The mixture is filtered using a filter pump. The filtrate is set aside for phyto-chemical tests. The maceration is repeated twice on the remaining residue.

2.2.2-Phyto-chemical screening

We have characterized the different chemical groups by referring to the techniques described in the work of Békro Y. A. and *al*., (2007); Badiaga M., (2011). The phyto-chemical screening is carried out in order to search for the following active elements:
The detection of saponins is based on their ability to give foamy aqueous solutions (foam index) (N'Guessan K, and *al*., 2009). Tannins and polyphenols: The following tests are carried out on the aqueous extract: Ferric chloride test - Gelatin test 1% - Saline gelatin test:(Duncan and *al*. 1999)
Flavonoids: WILSTATER test (Fong and *al*., 1977). The presence of flavonoids is characterized by the turning of the color of the upper phase: from orange to red for flavones and from red to purple for flavonols, to purplish red for flavonones.
Leucoanthocyanans: The search for leucoanthocyanin is performed by the BATE-SMITH Test. (Fong and *al*., 1977)
Steroids and Triterpenes: LIEBERMANN-BURCHARD Test (Fong and *al*., 1977)
Unsaturated sterols: SALKOWSKI test (Fong and *al*., 1977)
Anthraquinones: BORNTRAGER test (Fong and *al*., 1977)
Alkaloids: pH partitioning for alkaloids was performed according to Brimer and *al*. (1989). Dragendorff's reagent (Wagner and *al*, 1984) was added to one part of the prepared extracts, and Mayer's reagent (Wagner and *al*., 1984) to the other. Observations were made for the development of a red-orange precipitate on the addition of Dragendorff's reagent, and a white precipitate on the addition of Mayer's reagent
Polysacccharides (Fong and *al*., 1977)

2.2.3- Qualitative test by DPPH revelation

The in vitro antioxydant activity of bark extracts from *Commiphora humbertii* and *Commiphora brevicalyx* was evaluated by measuring their ability to scavenge the DPPH free radical (2,2-diphenyl-1-picrylhydrazyl).

The qualitative test was carried out using thin-layer chromatography (TLC). Hydroalcoholic extracts from each species were spotted on a silica plate and then developed with an eluent system made of n-hexane/diethyl ether (Et_2O) (9 :1 ; v/v). After migration, the TLC plate was dipped for a few seconds in a methanolic solution of DPPH at 0.004%, then dried at room temperature.

Compounds showing antioxidant activity appear as pale yellow to light yellow spots on a purple background, indicating local reduction of the DPPH radical (Congo M., (2012).

2.2.4- DPPH free radical scavenging test.

The DPPH radical scavenging activity was determined using a method adapted from Wang and *al*. (2002) and Kivrak and *al*. (2009).

The DPPH solution was prepared by dissolving 2 mg of DPPH in 50 mL of methanol. A 20 µL volume of extract, prepared at different concentrations, was added to 2 mL of the 0.004% methanolic DPPH solution ($0.2 < DO < 0.6$ at 517 nm). The reaction mixture was then mixed thoroughly by vigorous shaking and incubated for 30 minutes in the dark at room temperature.

At the end of the incubation, the absorbance was measured at 517 nm using a UV-Visible spectrophotometer, using a solution with just methanol and DPPH as the blank.

The negative control is composed of 1 ml of the methanol solution of DPPH and 1 ml of methanol. The positive control is represented by a solution of a standard antioxidant: ascorbic acid whose absorbance was measured under the same conditions as the samples and for each concentration. All analyses were performed in triplicate.

III.RESULTS

3.1-The result of the phyto-chemical screening is shown in Table 1.

We have characterized the presence of saponins, polyphenols and flavonoids in aqueous extracts and unsaturated steroids in chloroform extracts

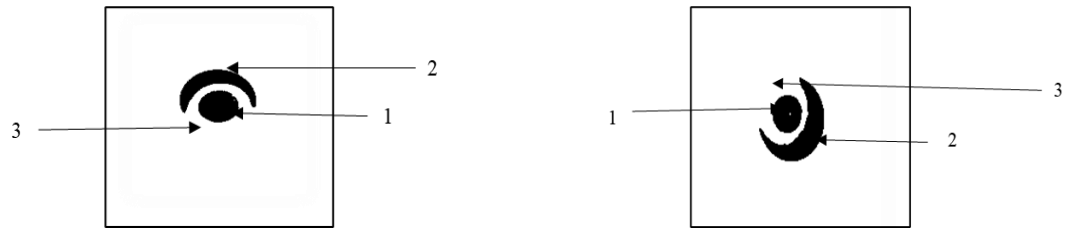
Table 1: Result of the phyto-chemical screening

Chemical components	Bark					
	Hydroalcoholic solution		Cyclohexane solution		Ethereal solution	
	Bcb	Bch	Bcb	Bch	Bcb	Bch
Alkaloids	+	+	-	-	-	-
Alkaloids in quaternary base form	+	+	-	-		
Coumarins	-	-	+	+	+	+
Flavonoids	+	+	+	+	-	-
Anthracenosides	-	-	-	-	+	+
Tannin (catechol type)	+	+	-	-	-	-
Reducing compounds	+	+	+	+	+	+
Saponin	-	-	-	-	-	-
Polysaccharides	+	+				
Cyanogenic heteroside	-	-				
Flavanone & Flavonolone	+	+				
Leucocyanes	+	+				
Anthocyanins	+	+				
Polyphenols	+	+				
Triterpene	-	-				
Steroids	+	+				
Unsaturated sterols	+	+				
Anthraquinone	-	-				

+ present, - absent, ▭ test not done

3.2- Qualitative test

The antioxidant activity of the extracts was observed on the plate by the appearance of yellow spots on the purple background (**Fig 1**)



C humberti. *C. brevicalyx*

***Figure 1** : Chromatographic profile of the screening of antioxidant activity of the bark extract of C. humbertii and C. brevicalyx humbertii*

1 : spot deposited on a TLC plate of the extract to be tested
2 : 0.004% methanolic DPPH solution sprayed
3 : spot appears in pale yellow or light yellow on a purple background

3.3-DPPH assay

The inhibition percentages (%) of the DPPH radical ● are calculated from the following formula:
I% = [1- (Abs Sample-Abs Negative Control)] X 100
I%: percentage of anti-radical activity
Abs Sample: Sample Absorbance
Abs Negative control: Absorbance of the negative control. (Meddour, 2013).

The following curves and tables show the variation of Concentration and percentage of inhibition of our extracts. We also determined graphically the concentration corresponding to 50% inhibition (IC50). The IC50 is inversely proportional to the antioxidant capacity of a compound, because it expresses the amount of antioxidant required to decrease the free radical concentration by 50%.

a. Ascorbic acid

Table 2: Concentration and inhibition percentage of Ascorbic acid

C°(mg/ml)	1,25	0,625	0,312	0,156	0,078	0,039
pAC(%)	92,4	77,19	55,07	31,57	14,52	7,84

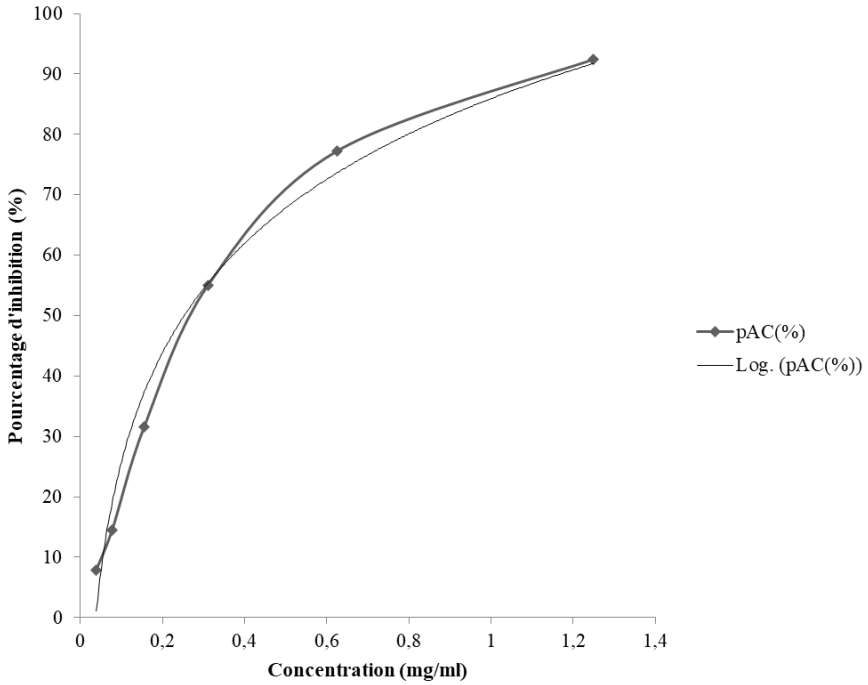


Figure 2: Representative curve of the antioxidant activity of Ascorbic Acid IC50 = 0.25 mg/ml

b. *C. humbertii*

Table 3: Concentration and inhibition percentage of *C. humbertii*

C°(mg/ml)	0,48	0,24	0,12
P%	81	67	44

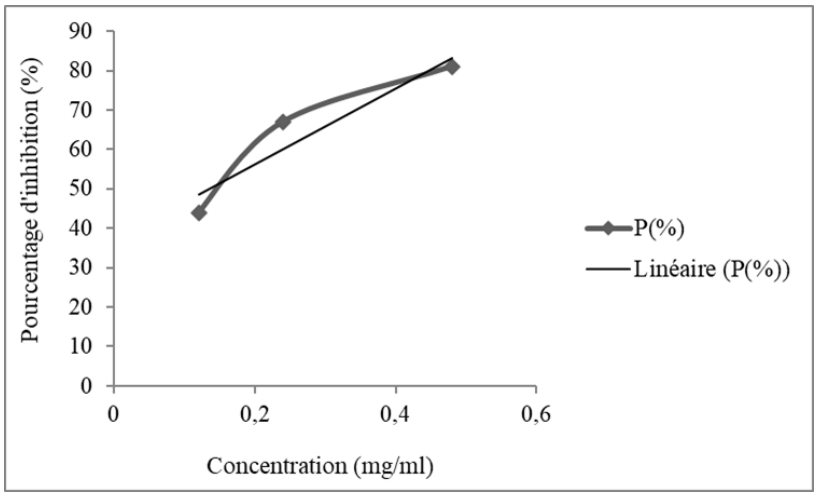


Figure 3: Antioxidant activity of *C. humbertii* bark extract, ascorbic acid by DPPH measurement and IC50 value IC50 = 0.14 mg/ml

c. ***C. brevicalyx***

Table.4: Concentration and inhibition percentage of C. brevicalyx

C(mg/ml)	3,15	1,57	0,78
P%	81	79	63

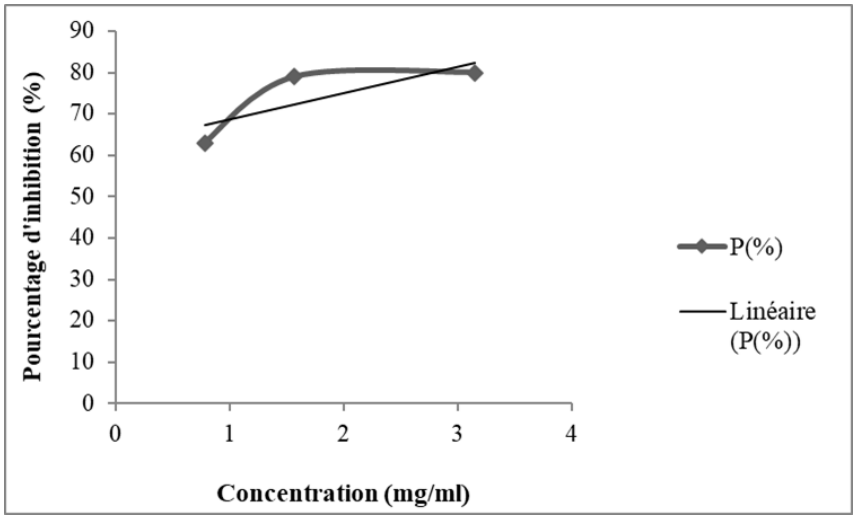


Figure 4 : Antioxidant activity of C. brevicalyx bark extract, ascorbic acid measured by DPPH and IC50 value IC50 = 0.22 mg/ml

Table 5 : Antioxidant activities of ascorbic acid, *C. humbertii*, and *C. brevicalyx*

Nom	C.h	C.b	Asc
IC50	0,14	0,22	0,25

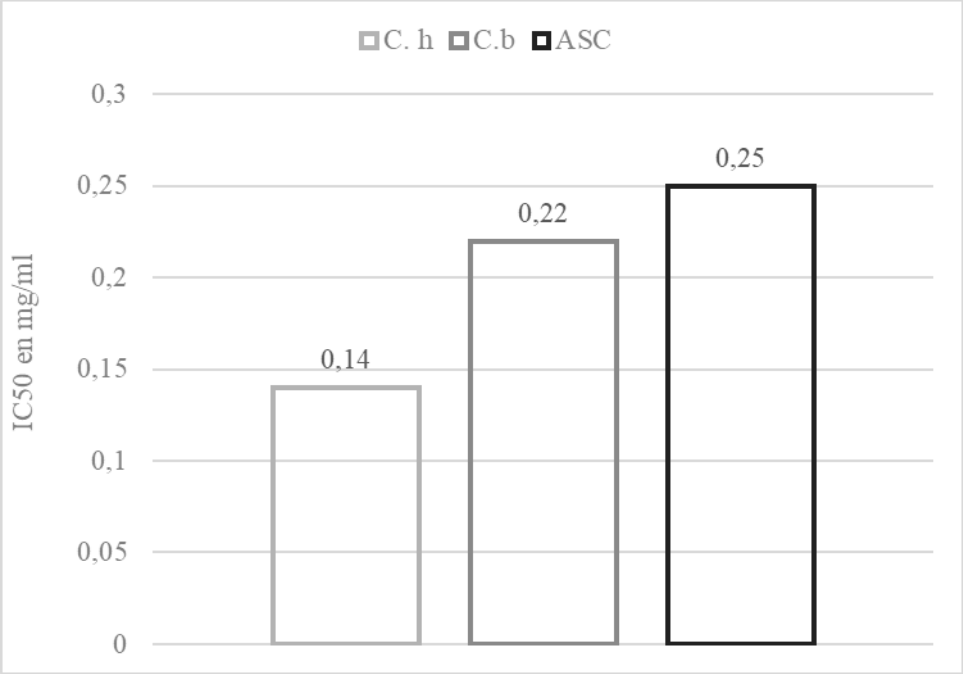


Figure 5 : Antioxidant activities of *C. humbertii*, *C. brevicalyx*, and Ascorbic Acid

IV.DISCUSSION AND CONCLUSION

The phytochemical screening carried out using characterization reactions reveals the richness of our plant in secondary metabolites in the studied extracts i.e. polyphenols and flavonoids. The **Salkowski test** reveals the presence of unsaturated sterols in the Hydroalcoholic extracts of the 2 species of Commiphora to the results obtained in this study, we can say that this analysis will find an important application in the pharmaceutical industry as well as a potential utility in the food industry.

Many methods are currently used to evaluate antioxidant activity. The appearance of a light yellow color on the background of the thin-layer chromatography (TLC) plate indicates that the extracts from both species have antioxidant activity. The large extent of this coloration suggests a strong presence of antioxidant compounds. It could also reflect a synergistic effect between the different bioactive molecules present in the extracts, there by enhancing their antioxidant capacity.

The IC50 values obtained for *Commiphora humbertii* and *Commiphora brevicalyx* are 0.14 mg/mL and 0.22 mg/mL respectively, while that of ascorbic acid is 0.25 mg/mL.

The DPPH radical has been widely used for studying the antiradical activity of various plant extracts. The chemical 2,2-diphenyl-1-picrylhydrazyl was one of the first free radicals used to study the structure-antioxidant relationship of phenolic compounds (Brand-Williams W., and *al*.,1995). Similar results are reported from studies of corn and oregano, respectively, ethanolic extracts are very effective at trapping DPPH radicals due to their high content of phenolic acids and flavonoid glycosides (Ksouri and *al*., 2008). A study by Kang and *al*. (2003) suggested that the polar molecules present in the plant extracts contribute to the increase of the antiradical activity. It is thus obvious that the strong activity of the crude extracts is attributed to its richness to the phenolic compounds, which possess the strongest content of polyphenols, flavonoids.

According to Falleh and *al*. (2008), the lower the IC_{50} value, the higher the antioxidant activity. So, *C. humbertii* shows the strongest antioxidant activity, followed by *C. brevicalyx*, and then ascorbic acid.

These results show that extracts from both species have higher free radical scavenging activity than ascorbic acid under the experimental conditions of this study. This performance could be attributed to the richness of the extracts in natural bioactive compounds, particularly polyphenols and other secondary metabolites, whose effects can be additive or synergistic. On the other hand, it is not possible to conclude that ascorbic acid generally has low antioxidant activity, since it is a widely recognized reference antioxidant. The differences observed are specific to the experimental conditions and the concentrations tested.

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