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Phytochemical Study and Evaluation of the Antioxidant Activity of *Dichapetalum madagascariense* (Dichapetalaceae)

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Abstract: ***Dichapetalum madagascariense is a medicinal plant of the Dichapetalaceae family, widely used in traditional medicine in Benin. In the present work we have prepared the hydro ethanolic extract from the dried leaves of this plant and the yield of crude extract (hydro ethanolic) is about 23%. Quantitative estimation of flavonoids and total phenols by colorimetric method and Folin Ciocalteux method showed that the extract is very rich in these compounds. The evaluation of the antioxidant power which was carried out by using the DPPH free radical scavenging method and the FRAP iron reduction method revealed that the hydroethanolic extract of Dichapetalum madagascariense has a higher reducing power than that of ascorbic acid.***

Keyword: *Dichapetalum madagascariense* flavonoids, antioxidant activity, Polyphenols

1. Introduction

Oxidation reactions are necessary for life, but they can also be destructive: plants and animals use and produce many antioxidants to protect themselves, such as glutathione, vitamin C and vitamin E, or enzymes such as catalase, superoxide dismutase, certain peroxidases, and polyphenols. Polyphenols are natural compounds widely distributed in the plant kingdom which have an increasing importance in particular thanks to their beneficial effects on health. Indeed, their role as natural antioxidants is of increasing interest in the prevention and treatment of cancer, inflammatory, cardiovascular and neurodegenerative diseases. They are also used as additives for the food, pharmaceutical and cosmetic industries on health.

Polyphenols seem to play an important role in the protection against cancer and cardiovascular diseases. The protective action against cancer would be explained by a mechanism quite similar to that of prebiotics by their capacity to select a particular type of microbiota, in particular for cancers of the digestive system (stomach, colon, etc.).

Plants rich in polyphenols are unfortunately not valued as an alternative to synthetic antioxidant products. We wanted to study this plant with antioxidant potential.

Dichapetalum madagascariense belongs to the family Dichapetalaceae; it is a shrub or tree reaching more than 20 m high and 1.70 m in circumference, of the savannah and forest, from Sierra Leone to Nigeria, and perhaps as far as the Congo basin. The bark gives off a bit of brownish gum when trimmed. The freshly cut wood is white and turns brownish. The smaller stems are used as chewing sticks and the larger pieces have unspecified domestic uses.

It is a plant species originally described from Madagascar but now reported from many parts of mainland tropical Africa.

It is used in traditional medicine as a decoction to treat malaria; diabetes; liver disease; haemorrhage; and salmonellosis.

2. Materials and Methods

Plant material

The plant material consists of dried leaves of *Dichapetalum madagascariense* collected in December 2020 in Abomey-Calavi. This plant was identified at the National Herbarium of the University of Abomey Calavi. The leaves of the harvested plant were washed and then dried at room temperature in a ventilated room of the Pharmacognosy laboratory for three weeks before being reduced to powder.

Extraction

The extraction was done for the hydro ethanolic extract by mixing 50g of powder in 500 ml of a hydro ethanolic mixture (40V/60V respectively) for 48 hours with the aim of extracting as much as possible the polar compounds such as polyphenols.
After filtration on Whatman No.1 paper, the filtrates obtained were evaporated using a rotary evaporator at 40°C. The residues of this filtrate were dried in an oven for 48 hours at 40°C to obtain the dry extracts.

Phytochemical Screening

The presence of the main chemical groups in the extracts was investigated using the tests described by Bassene (2012): flavonoids (Shibata test) tannins (Stiasny reaction followed by ferric chloride reaction), carotenoids (Carr-Price reaction), anthracenes (Dragendorff reagent), sterols (Libermann-Buchard reaction), cardiotonic heterosides

'Baljet, Kedde and Raymond-Marthoud reaction) and saponosides (Foam index).

Polyphenol content

The polyphenol content of the extracts is determined by the Folin-Ciocalteu method.1 mL of Folin's reagent is added to 1 mL of the solution of each extract, then 3 minutes later 1 mL of 25% sodium carbonate. After 2 hours of incubation, the samples were centrifuged at 4000 rpm for 4 minutes. The absorbances were then read with a spectrophotometer at 670 nm. Three tests were performed for each concentration of product tested.

A calibration curve based on a dilution series of tannic acid (0.005-0.01-0.015-0.02-0.025-0.03-0.025-0.03-0.035-0.04 mg/mL) was treated in the same way as the extracts. The results are expressed as milligram equivalent of tannic acid per gram of dry extract 'mg ETA/g).

Determination of Flavonoids

The flavonoid content of the extracts was determined using the aluminium trichloride colorimetric method. A quantity of 100µL of the extract was mixed with 0.4 mL of distilled water and subsequently with 0.03 mL of 10% ALCL3 solution was added. To the mixture, 0.2 mL of 1M NaNO2 solution and 0.25 mL of distilled water were added after 5 min of rest. The mixture was vortexed and the absorbance was measured at 510 nm. The results are expressed as milligrams of catechin equivalent per g of dry plant material.

Antioxidant activity DPPH test

Determination of free radical scavenging activity by DPPH assay was carried out using the method described by Molyneux (2003) slightly modified. An ethanolic solution of DPPH was prepared by dissolving 4mg of this product in 100ml of ethanol. Then to 50uL of extract at a given concentration 950ul of the DPPH solution was added. The extracts as well as the reference (ascorbic acid) are tested at different concentrations (250-125-62.5-31.25-15.62-7.81 ug/mL); then the absorbances were measured at 517 nm after 30 min of incubation in the dark. Three tests were carried out for each concentration of product tested.

The antioxidant activity related to the DPPH radical scavenging effect is expressed in percentage inhibition (PI) using the following formula:

PI = 100 (A0-A1) /A0; A0: DPPH absorbance; A1: sample absorbance

The IC50 (concentration of the sample required to neutralize 50% of the free radicals) was obtained using the statgraphics Plus 5/0 software.

FRAP Test

The reducing power of the extracts is determined by FRAP method (Bassène 2012). Thus 0.4 mL of sample at different concentrations is mixed with 1mL of phosphate buffer (0.2M; pH=6.6) and 1mL of 1% potassium hexacyanoferrate [H3Fe (CN) 6]. After incubating the mixture at 50°C for 30 minutes, 1mL of 10% trichloroacetic acid was added, then the tubes were centrifuged at 3000 rpm for 10 minutes. Then, 1mL of the supernatant from each tube was mixed with 0.2 mL of 0.1% FeCl3 solution and allowed to stand in the dark for 30 minutes before measuring absorbances at 700 nm. The antioxidant activity related to the reducing power of the extracts is expressed as Reducing Power (RP) using the following formula: RP = 100 (Aa-Ab) /Aa; Aa: absorbance of the extract, Ab: absorbance of the blank.

Statistical analysis

Significance tests are performed by Fisher's test using Stat View software. A p-value < 0.05 was considered statistically significant.

3. Results

Phytochemical screening

Phytochemical screening revealed the presence of flavonoids, tannins and saponosides in the extracts of both plant species. Alkaloids, anthracenes, triterpenes, coumarins are also present but cardiotonic heterosides were not found in the hydro ethanolic extract of the plant which is the subject of the present study.

Table 1: Phytochemical Screening of Hydroethanolic Extract

Classes	*Dichapetalum madagascariense*
Flavonoids	+
Tannins	+
Glycosylated coumarins	+
Triterpenes	+
Alkaloids	+
Anthracene derivatives	+
Cardiotonic glycosides	-
Saponosides	+
Lignans	+
	Quantity of polyphenols in µg Eq AG/mg extract
Dichapetalum madagascariense	147.879307

Table 2: Polyphenols content in the hydro ethanolic extract

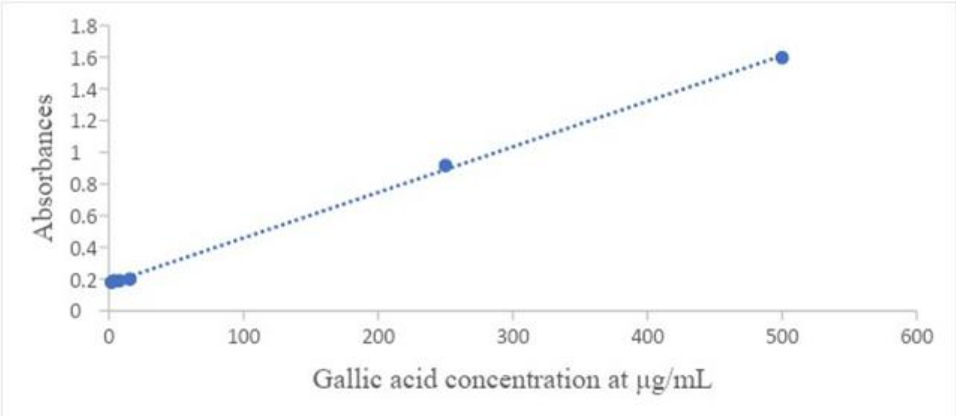
	Quantity of polyphenols in µg Eq AG/mg extract
Dichapetalum madagascariense	147.879307

Determination of Flavonoids

The flavonoid content determined by aluminium trichloride method for each extract was reported as mg catechin equivalent/g dry plant material. The results reveal that the hydroethanolic extract has a moderate content of Flavonoids (Table 3).

Table 3: Yield and content of total polyphenols and flavonoids

Extracts	Yield (%)	Total polyphenol content in µg Eq GA/mg extract	Flavonoid content in mgE
Dichapetalum madagascariense	23%	147.879307	23.4



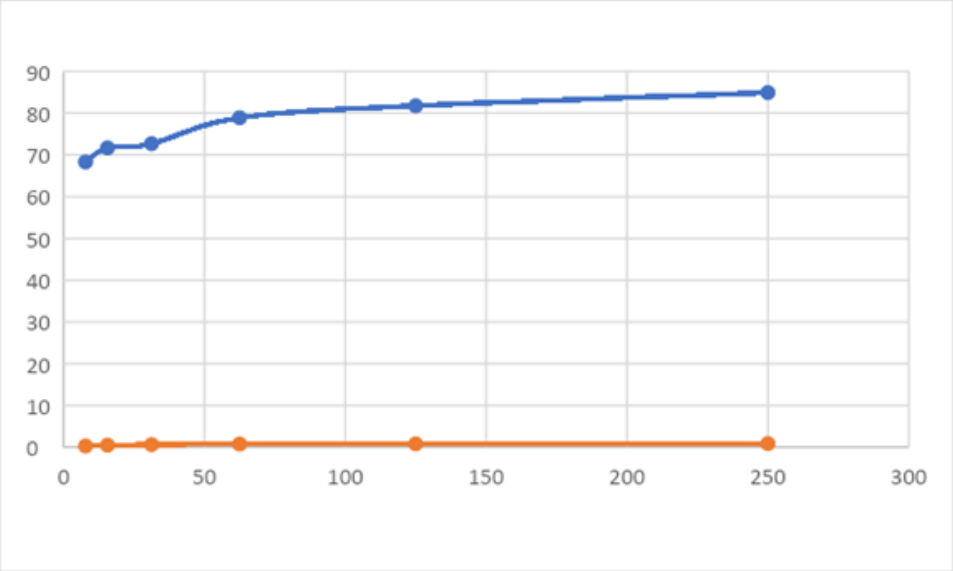
Picture 1: Gallic acid calibration line Total polyphenol content

The determination of the total polyphenol content in the extract was done by the Folin-Ciocalteux method. The content was reported in mg gallic acid equivalent/g dry plant material. It is an extract rich in total polyphenols. This is confirmed by phytochemical screening which reveals the presence of flavonoids, tannins and saponosides in the extract. The use of hydro ethanolic solvent allowed the extraction of polar compounds such as polyphenols which are among the main components of plants with antioxidant activity from the dried leaves (Fall et al., 2015; Sarr et al., 2015)

Antioxidant activity DPPH test

The antioxidant activity of the hydroethanolic extract of the plant and the standard antioxidant (ascorbic acid) towards the DPPH radical was evaluated with a spectrophotometer by following the reduction of this radical which is accompanied by its change from the violet colour (DPPH°) to the yellow colour (DPPH-H) measurable at 517nm. This reduction capacity is determined by a decrease in absorbance induced by antiradical substances.

The results of the antioxidant power of the tested hydro ethanolic extract shows that the percentage of inhibition of the extract is more than 80% at concentrations in the range of 125µg/mL to 250µg/mL. The EC50 value determined in mg/ml expressing the effective concentration of the antioxidant extract required for the entrapment and reduction of 50% moles of DPPH dissolved in methanol (Table 4).



Picture 2: % DPPH inhibition as a function of different concentrations of extract of *Dichapetalum madagascariense* and Vitamine C

According to the results recorded, the extract is endowed with an antioxidant power; the IC50 is 135, 21 ±10, 44 clearly stronger than that of ascorbic acid whose value is of the order of 0, 235mg/mL.

The polyphenols contained in the hydroethanolic extract

are responsible for the antioxidant activity of these extracts.

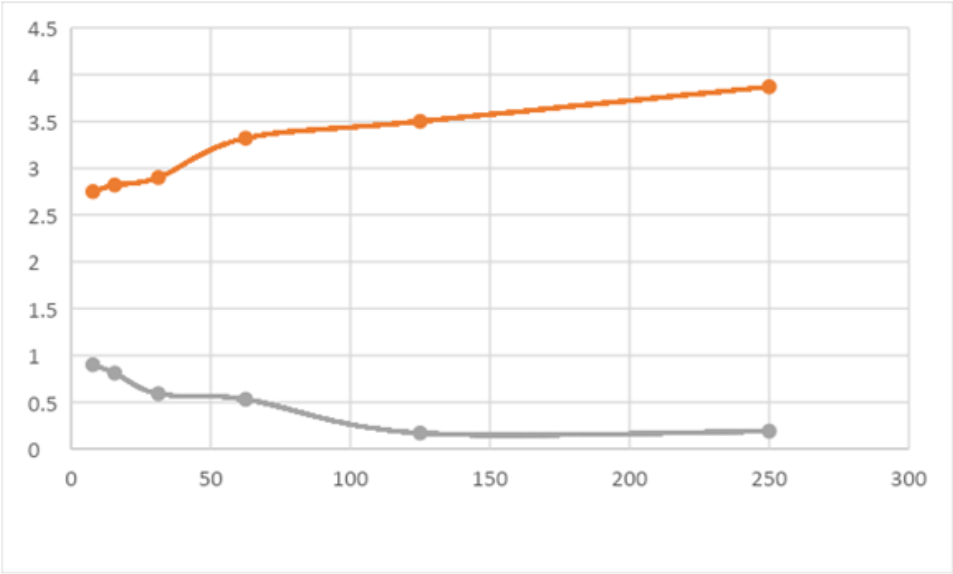
Table 4: IC 50 value of extract and ascorbic acid

Extraits/Standard	IC50 ± Ecart type
Dichapetalum madagascariense	135, 21 ± 10, 44
Acide ascorbique	0, 235 ± 8, 01

FRAP Test

The antioxidant activity of the extract was evaluated by FRAP iron reduction method. The presence of reductants in the plant extract causes the reduction of Fe3+ ferricyanide complex to the ferrous form. ¨Therefore, Fe2+ can be evaluated by measuring the increase in the density of blue colour in the reaction environment at 700nm.

The reducing power of the plant extract is dose dependent (concentration dependent). The evaluation of the reducing power of the extract showed a better activity of the hydro ethanolic extract compared of *Dichapetalum madagascariense* that of vitamin C as shown in figure 2 Indeed, the hydro ethanolic extract of *Dichapetalum madagascariense*, at concentrations of 7.8-15.62-31.25-62.5-125-250 µg/ml gave respective reducing powers of 2, 75%, 2, 82%, 2, 9%, 3, 32%, 3, 5%, 3, 87%. At the same concentrations, respective reducing powers of 0, 9%, 0, 81% 0, 59%, 0, 53%, 0, 17%, 0, 19%, were observed for vitamin C.



Picture 3: Reducing power of the extract of *Dichapetalum madagascariense* and Vitamine C of differents concentrations

4. Conclusion

The study of the antioxidant activity of the hydro ethanolic extract of *Dichapetalum madagascariense* and *according to* the method of iron reduction and the method of free radical scavenging DPPH showed that the hydro ethanolic extract has a proven antioxidant activity. The extract could therefore be an alternative to some synthetic antioxidant additives. Further research is needed to identify, isolate and purify these constituents.

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