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DETERMINATION OF TOTAL PHENOLS AND ANTIOXIDANT ACTIVITY IN AQUEOUS AND ETHANOLIC EXTRACTS FROM THE LEAVES OF LECANIODISCUS CUPANIOIDES PLANCH (SAPINDACEAE)

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ABSTRACT

The objective of this study was to determine the polyphenol content and evaluate the antioxidant activity of aqueous extracts (EAq) and 70% ethanolic extracts (EEth70%) from the leaves of *Lecaniodiscus cupanioides* PLANCH. (Sapindaceae), a plant used in the treatment of diabetes in the Man Department (western Côte d'Ivoire). Quantitative determination of total phenolic content using the colorimetric method showed that the EAq contained 228.3 ± 0.67 mg GAE/g of extract, while the EEth 70% contained 226 ± 13.69 mg GAE/g of extract. As for flavonoids, the amount determined for the EAq was 48 ± 8.50 mg QE/g of extract, while the 70% EEth yielded 87 ± 2.40 mg QE/g. The results indicate that the EAq of Lecaniodiscus cupanioides contains more polyphenolic compounds than the 70% EEth. The antioxidant activity of the various extracts was assessed using the DPPH free radical scavenging assay. The antioxidant capacity of the EAq yielded an IC50 of 01.45 ±

0.15 µg/mL, similar to that of vitamin C (IC50= 01.30 ± 0.02 µg/mL) and higher than that of the 70% EEth, which had an IC50 of 02.40 ± 0.60 µg/mL. This antioxidant activity could therefore represent an additional benefit for the use of *Lecaniodiscus cupanioides* in the treatment of certain conditions related to oxidative stress.

KEYWORDS: *Lecaniodiscus cupanioides*, antioxidant, polyphenols, Cote d'Ivoire.

INTRODUCTION

The development of new antioxidants with superior antioxidant capacity and lower toxicity is essential for combating oxidative stress. This is because free radicals are believed to be responsible for certain chronic diseases (cardiovascular, cancer, and neurodegenerative) as well as aging **(Pastre, 2005).** They are thought to cause cellular dysfunction and various pathologies such as cardiovascular disease, cancer, diabetes, aging, and neurodegenerative diseases such as Alzheimer's and Parkinson's **(Aruoma, 2003).** Similarly, certain synthetic antioxidants pose a potential health risk, including a possible carcinogenic effect. It is therefore necessary to identify new sources of natural, inexpensive, and low-risk antioxidants for use in foods and pharmaceutical preparations to replace synthetic antioxidants.

This is why new sources of natural antioxidants are being actively sought. Natural products are an important source for the discovery of new active compounds used to treat many diseases. Numerous studies have been conducted on the biological properties of extracts from certain plants, leading to the discovery of many active ingredients used in modern medicine for drug synthesis. Thus, it is estimated that more than half of the bioactive molecules used in clinical practice are derived from plants **(Samarth *et al.*, 2008).** Plants are therefore natural reservoirs of antioxidant substances designed to protect them from stress **(Sarr *et al.*, 2015).** Scientific studies have demonstrated the importance of *Lecaniodiscus cupanioides*, particularly for the treatment of cysts, myomas, fibroids, and dental caries in Benin. In Côte d'Ivoire, the Abbey and Krobou peoples use a decoction made from the bark of its stems to facilitate childbirth.

This study aims to determine the total phenolic content and antioxidant activity of aqueous and ethanolic extracts from the leaves of *Lecaniodiscus cupanioides*.

MATERIALS AND METHODS

Plant material

The plant material consists of *Lecaniodiscus cupanioides* leaves collected in December 2024 in Kassiapleu, a village located 7 km from the city of Man on the Man-Danané axis, facing the University of Man (Côte d'Ivoire).

Technical equipment

The technical equipment includes a mechanical grinder type IKAMAG, a magnetic stirrer RCT type IKAMAG, a rotating evaporator Heidolph Type; a UV-Vis spectrophotometer (biomérieux), a P-type oven SELECTA and a precision balance (Denver Instrument).

Reagents

The reagents used are mainly the Folin-Ciocalteu reagent, sodium carbonate, methanol, aluminum trichloride, potassium acetate, 2,2-diphenylpicrylhydrazyl (DPPH), phosphate buffer, ethanol, hydrochloric acid, ferrous chloride, trichloroacetic acid, the ferrocene, potassium ferricyanide, quercetin and gallic acid provided by RYCA-PHARMA and CLE (Chemical Laboratory Equipment).

Preparation of plant extracts Preparation of aqueous extract

100g powder of the leaves of *Lecaniodiscus cupanioides* were macerated for 24 hours in 1L of distilled water **(Olakunle *et al.*, 2005)**. The macerate has been wrung into a square of sterile tissue, filtered successively on cotton wool and one-fold on filter paper (Whatman paper® 2mm). The filtrate was dried slowly in the stove at 50°C. The powder obtained was stored in a hermetically sealed jar and refrigerated at 4 °C **(Zirihi *et al.*, 2003).**

Preparation of ethanolic extract

It was carried out using modified method **(Olakunle *et al.*, 2005)**. A mass of 100 g of plant powder was added in 100ml of ethanol and subjected to maceration for 72 hours. The macerate was treated according to the same procedure like the aqueous extract.

Doses of polyphenols Determination of total phenols

The total phenolic contents of two extracts of *Lecaniodiscus cupanioides* were determined by the Folin-Ciocalteu method **(McDonald *et al.*, 2001)**. To 0.5 mL of each plant extract of concentration 0.1 mg / mL respectively were added 5 mL of Folin-ciocalteu diluted 1/10 in distilled water and 4 mL of sodium carbonate (1M). The whole is incubated at room

temperature for 15 minutes. The optical densities (OD) are then read in a spectrophotometer at 765 nm against a blank. Gallic acid was used as standard and prepared under the same conditions as above with a solvent mixture of methanol / water (50:50, V / V) at concentrations ranging from 0 to 0.5 mg / mL. The total phenolic contents of the extracts are expressed in milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract).(**Graph 1)**

Determination of total flavonoids

The technique used for the determination of the levels of total flavonoids extracted from *Lecaniodiscus cupanioides* is the colorimetric method of aluminum trichloride described by **(Yi *et al.*, 2007)**. Thus, 0.1 mL of 5 mg / mL of each extract plant are collected, to which are successively added 1.5 mL of methanol, 0.1 mL of 10 % aluminum trichloride, 0.1 mL of potassium acetate (1M) and 2.5 mL of distilled water. After incubation at room temperature for 30 minutes, the optical densities were measured in a spectrophotometer at 415 nm. A methanolic solution of quercetin with concentrations ranging from 0 to 100 mg / mL is used as a standard. The contents of flavonoids extracts are expressed in milligrams of quercetin equivalent per gram of extract (mg QE / g extract). **(Graph 2)**

***In vitro* evaluation of the antioxidant activity**

Measurement of anti-radical power

The measurement of the antiradical activity of plant extracts was performed by testing the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) according to the method of **(Parejo *et al.*, 2000)**. From a stock solution of each plant extract to 0.1 mg / mL, a concentration range is prepared by successive doubling dilution of 1.56 mg / mL to 100 mg / mL. Then, to each extract concentration, the same volume of a methanolic solution of DPPH is added. After 30 minutes of incubation at room temperature (37°C) and protected from light, the absorbance is read in a spectrophotometer at 517 nm against a blank sample (0 mg / mL of extract). Vitamin C (100 mg / mL) which is the reference material is prepared in the same conditions. (Graph 3)

The percentage inhibition of DPPH radicals are calculated by the following formula:
Inhibition (%) = [(white ABS - ABS sample / white ABS)] x 100. Where: **Inhibition (%)**: the percentage of inhibition of DPPH radical; **White ABS**: the absorbance of the blank;
ABS sample: the absorbance of the leave extracts of the plant and vitamin C.

Statistical Analysis

Statistical analysis was performed by Graph Pad Prism 11 statistical software. Results are expressed as mean ± SD and analyzed by ANOVA and Tukey tests with univariate rate determination of significance with $P \leq 0.05$ considered statistically significant.

RESULTS AND DISCUSSION

Contents of total phenols and flavonoids leaves extracts of *Lecaniodiscus cupanioides***:**

The levels of total phenols and total flavonoids of leaves extracts of *Lecaniodiscus cupanioides* are determined from the calibration line y = 0.004 x + 00; R^2 = 0.998 and y = 0.037 x + 00; R^2 = 0.997 plotted using standard as gallic acid and quercetin, respectively**.**

The determination of total phenols and flavonoids in the aqueous and ethanolic extract of *Lecaniodiscus cupanioides leaves* was done using separately the colorimetric methods (Folin-Ciocalteu and aluminium trichloride ($AlCl_3$). **(Table 1)**.

The total phenol content estimated by the Folin-Ciocalteu method for each extract was reported in mg gallic acid equivalent/g dry plant material. The results show that the ethanolic root extract has a high total phenol content (EAq of leaves = 228.3 ± 0.67 mg GAE /g extract) compared to that of the aqueous extract (EEth of leaves = 226 ± 13.69 mg GAE /g extract).

Table 1: Levels of total phenols and total flavonoids of leaves extracts of *Lecaniodiscus cupanioides* (mean ± SD of three trials).

Extracts	Total Phenols mg GAE/ g d'extract	Flavonoids mg EQ / g d'extract
EAq	228.3 ± 0.67 [a]	87 ± 2.40 [a]
EEth	226 ± 13.69 [a]	48 ± 8.50 [a]

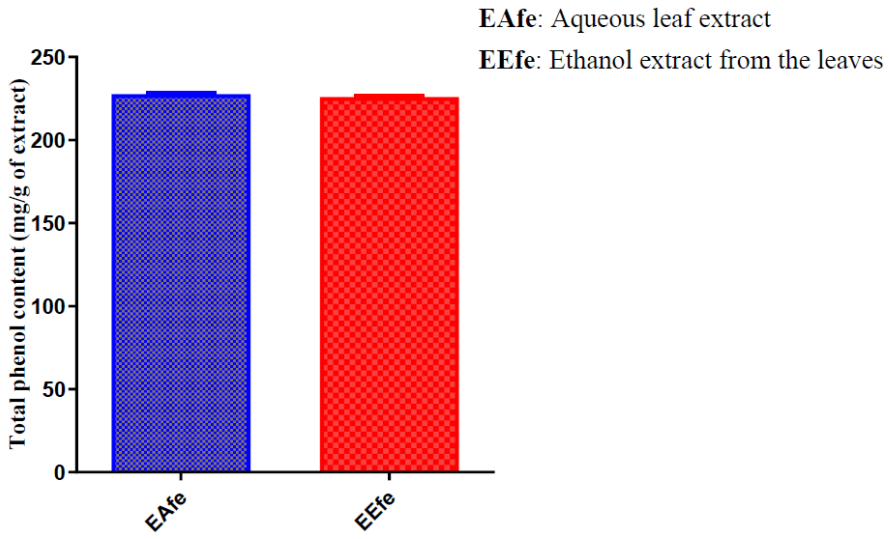
Medium extracts the same column with different letters (a, b) with superscript are significantly different from the smallest to the largest average at P ≤ 0.05.

The flavonoid content determined by the aluminium trichloride method for each extract was reported in mg quercetin equivalent/g extract. The results show that the two extracts have different contents (Table 1). The results showed that EAq of leaves with 87 ± 2.40 mg EQ/g extract was richer in flavonoids than EEth of leaves = 48 ± 8.50 mg EQ/g extract.

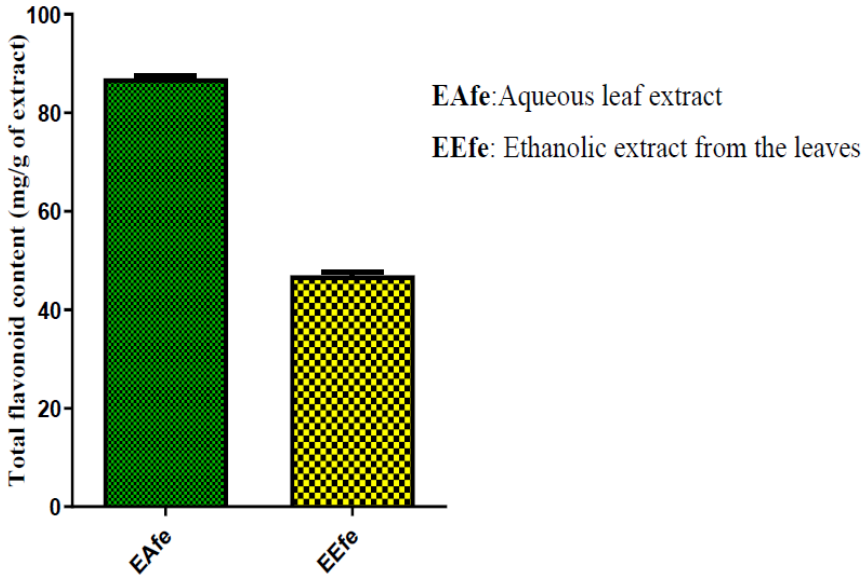
The EAq of leaves of *Lecaniodiscus cupanioides* represents the highest contents of total phenols and flavonoids. This result confirms the high richness of the leaves in phenolic

compounds. The results obtained confirm that the different extraction solvents used differ in their ability to extract phenolic compounds from *Lecaniodiscus cupanioides.*

This difference can be attributed to the polarity of the solvents used; water promotes the extraction of water-soluble flavonoids, while ethanol extracts more of the less polar phenolic compounds. These observations are consistent with the findings of **(Yi et al., 2007)** and **(Samarth et al., 2008),** who demonstrated that the nature of the solvent strongly influences the recovery of bioactive metabolites and their antioxidant activity.



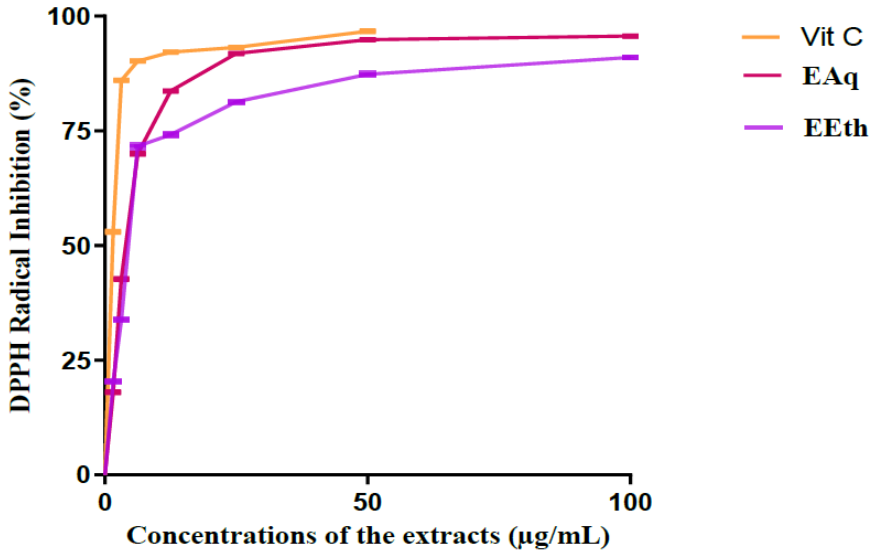
GRAPH 1: Levels of total phenols of leaves extracts of *L. cupanioides* (mg/QE/g of extract) (Mean ± SD of Tree Trial.



GRAPH 2: Levels of total flavonoids of aqueous and ethanolic extracts of *L. cupanioides* leaves (mg/QE/g of extract) (Mean ± SD of Tree Trial)

Table 2: Anti-radical and chelating properties of *L. cupanioides* leaves extracts and vitamin C resulting in 50% reduction of DPPH radicals and chelation of the ferrous ion.

Extracts and Compounds	Anti-radical Anti-free radical activity IC50 (µg/mL)
EAq of leaves	01.45 ± 0,15[a]
EEth of leaves	02.40 ± 0,60[a]
Vit C	01.30 ± 0.01[a]



Graph 3: Evolution of the antiradial activity of the aqueuos and ethanolic extract of the *L. cupanioides* leaves.

The antioxidant activity measured by the DPPH assay confirms this richness: the aqueous extract has an IC50 value of 1.45 µg/mL, close to that of vitamin C (1.30 µg/mL), indicating efficacy comparable to that of a reference antioxidant. This result is consistent with the work of Tokoudagba and Gbaguidi (2022), who reported antioxidant activity superior to that of ascorbic acid in certain fractions of L. cupanioides. Furthermore, several recent studies **(Aruoma, 2003; Sarr *et al.*, 2015)** emphasize that polyphenols and flavonoids play a key role in neutralizing free radicals, reducing oxidative stress, and preventing chronic diseases such as diabetes, cancers, and neurodegenerative disorders. The results thus reinforce the pharmacological significance of this plant, which is already used in traditional medicine to treat various conditions (fibroids, myomas, infections).

CONCLUSION

Aqueous extracts of Lecaniodiscus cupanioides are characterized by high levels of phenols and flavonoids and antioxidant activity comparable to that of vitamin C. These findings

confirm that this plant is a promising natural source of antioxidants that could replace synthetic compounds often associated with adverse effects. The incorporation of L. cupanioides into herbal medicine could contribute to the prevention of diseases linked to oxidative stress, particularly diabetes and cardiovascular diseases. However, further in vivo and clinical studies are needed to validate its efficacy and safety, thereby paving the way for its use in pharmaceutical formulations.

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