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Research Article

Chemical Screening, Determination of Polyphenols and Total Flavonoids, Antioxidant activity of extracts from the leaves of Dischistocalyx hirsutus (Acanthaceae)

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ABSTRACT

This article presents the phytochemical composition, the polyphenols and total flavonoids content, and the antioxidant activity of the extracts of the leaves from Dischistocalyx hirsutus. The phytochemical test was carried out thanks to the colorimetry and precipitation reactions. The total phenol content was determined using the Folin-Ciocalteu reagent and that of the flavonoids by reaction with $AlCl_3$. There were three extracts: aqueous, hydro-ethanolic and ethanolic extracts. Antioxidant activity was assessed by DPPH methods. The results showed that the extracts of Dischistocalyx hirsutus contain large chemical groups, such as flavonoids, tannins, anthocyanins and saponosides. The total phenol contents are 76.5 ± 0.2; 66.5 ± 0.1 and 55.3 ± 0.1 mg EGA/gMS, respectively for the hydro-ethanolic, ethanolic and aqueous extracts. the contents of total flavonoids are 5.5 ± 0.1; 4.5 ± 0.1 and 3.3 ± 0.1 mg EC/gMS. Color intensities (IC_{50}) of 27.5, 17 and 17.5 are obtained, respectively for the aqueous, hydro-ethanolic and ethanolic extracts. These results highlight the interest of this plant and justify its use in traditional medicine for the treatment of certain pathologies.

Keywords: Dischistocalyx hirsutus, Chemical Screening, polyphenolic content, flavonoid content, Antioxidant activity

INTRODUCTION

Despite advances in medicine, many drug treatments are still insufficient to address the scourges such as: anemia, sickle cell anemia, malaria, cancer, Alzheimer's, and many other viral and bacterial infections. To remedy this, it is essential to search for new therapeutic substances. Thus, the research on plants represents an invaluable potential for the discovery of new substances more effective in fighting certain infections, in particular those linked to blood and cardiovascular diseases [**1, 2, 3]**. Although a large part of the 20th century was devoted to the development of synthetic molecules, the modern pharmaceutical industry relies on the diversity of primary and secondary metabolites obtained from the world plant, in order to find out new molecules with new pharmacological properties. Thus, the research for new active pharmacological agents "lead compound" via screening of plants has resulted in the discovery of a large number of

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useful drugs which are starting to play a major role in the treatment of many diseases [**4, 5]**. It is with the aim of contributing to this research, that we propose to carry out the phytochemical study of the leaves of *Dichistocalyx hirsutus*, a plant used in Republic of Congo for the treatment of anemia and sickle cell anemia.

This article presents phytochemical screening, qualitative and quantitative analysis of total polyphenols and flavonoids, and qualitative and quantitative evaluation of anti-radical activity of aqueous (AQ), hydro-ethanolic (HE) and ethanolic (ET) extracts from the leaves of *Dischistocalyx hirsutus*.

MATERIAL AND METHODS

Plant Matter: *Dischystocalyx hirsutus* is a medicinal plant of the African pharmacopoeia which belongs to the family of *Acanthaceae*. The plant is found in Central Africa, mainly in Congo, Gabon and Equatorial Guinea Continental Region [**6, 7, 8, 9]**. *Dischistocalyx hirsutus* is used in northern Congo and southeast Gabon to treat anemia, sickle cell disease, and arterial hypertension. The leaves of *Dischistocalyx hirsutus* were harvested in the sub-prefecture of Kelle in the department of the Western Basin (Congo), in September 2017. The identification was done at the Institut National de Recherche en Sciences Exactes et Naturelles (IRSEN) and a specimen was registered at the National Herbarium under number 7658.

Preparation of extracts: The leaves were dried at room temperature, for about a week. The dry vegetable matter is ground with an IKA-WERKE Gmbh-CO-KG, D-79219, Staufen-type device, with a sieve of granulometry 0.25mm. To make the measurement, Three (03) extracts: Water (100%), hydro-ethanolic (EtOH-H_2O, 50:50 v/v) and ethanolic (ETOH : 100%) were obtained on mixing 100g of vegetable matter with 2 × 500 ml of each solution. The mixture is shaken up during 72 hours, then filtered. The filtrate obtained dried concentrated with a rotary evaporator is kept in a cool place (+4 °C) awaiting for its analysis.

Chemical Screening and Qualitative Analysis: Three (03) extracts, Water (100%), EtOH-H_2O (50:50 v/v) and ETOH (100%) were screened for their classes of bioactive compounds using standard procedures [**10-15]**. The extracts were tested qualitatively for the presence of chemical constituents such as tannins, terpenes, saponins, flavonoids, polyphenols, anthocyanins, cardiac glycosides, alkaloids and reducing sugar. The test for these chemical families were carried out according to the methods described by Békro et al.[**10]**. This survey is followed by a thin layer chromatography analysis to identify phenolic compounds using Neu reagents and Uv-visible lamp at 366 nm.

Measurement of Total Polyphenols: The reagent of Folin-Ciocalteu was used for the evaluation of total phenols of aqueous, hydroethanolic and ethanolic extracts. Folin-Ciocalteu is a mixture of phosphotungsten acid ($H_3PW_{12}O_{40}$) and phosphomolybdenum ($H_3PMo_{12}O_{40}$) of yellow color. The method is based on the oxidation of the phenolic compounds by this reagent. This oxidation draws the formation of new complex molybdenum tungsten of blue color that absorbs to 725 nm. The evaluation of Total Polyphenols is done by comparison of the optic density (D.O) observed to the one issued from a stallion of known acid Gallic concentration. The total phenol compounds are measured as follows: 0.1ml of the extract hydroethanolic is introduced in an Eppendorff tube of 2 ml, the extract is diluted with 0.9 ml of distilled water. 0.9ml of the reagent of Folin-Ciocalteu (1N) is immediately added after addition of 0.2 ml of Na_2CO_3 (20%) solution. The mixture is hatched to the ambient temperature during 40 minutes safe from light. The absorbance is measured with the spectrophotometer at 725 nm against a solution of ethanol used like white (control). A right of standardization achieved previously with the Gallic acid in the same conditions like the samples to analyze, permitted to calculate the total phenols contain. The results are expressed in mg equivalent to Gallic acid by gram of dry matter (mg EGA/gMS).

Measurement of Total Flavonoids (FVT): The colorless solutions of sodium nitrite ($NaNO_2$, 5%) and aluminum chloride ($AlCl_3$, 10 %) have been used for the evaluation of total flavonoids in aqueous, hydroethanolic and ethanolic extracts. The method is based on the oxidation of the flavonoids by these reagents; oxidation that draws the formation of a brownish complex which absorbed at 510 nm. The comparison of the optic density (D.O) observed to the one deriving from a stallion of known concentration Catechin permits to value the total content in flavonoids by colorimetric effect. In a ball of 10 ml are introduced 250 µl of extract and 1 ml of distilled water successively. At the initial time, 75 µl are added to a NaNO2 (5%) solution. After 5 min, 75µl of $AlCl_3$ (10%) are added; 6 minutes later, 500µl of NaOH (1N) and 2.5 ml of distilled water are added successively to the mixture. A curve of standardization is elaborated with solutions standards of catechin prepared at different concentrations. The results are expressed in mg equivalent to catechin by gram of dry matter (mg EC/gMS).

Determination of the Radical Scavenging Activity: The qualitative analysis of the scavenging activity has been evaluated on pulverizing the solution of 1.1-diphenyl-2-picrylhydrazyle (DPPH) at 2 mg/mL on TLC plate of silica gel. The migration solvent was ethyl acetate / formic acid / water (8/1/1). The appearance of pale yellow stains on a purple background shows the scavenging activity. Then, the quantitative analysis of the scavenging activity has been evaluated on mixing 10 mL of the solution of 1,1-diphenyl-2-picrylhydrazyle (DPPH) at 10 mg in 250 ml of ethanol and 100 µL of extract or the fractions at the concentrations of 100 to 3.12 µg/mL. After that, the activity has been measured at 517 nm in the shelter of the light after 30 minutes of incubation to darkness using a UV-visible spectrophotometer. The percentage of inhibition was calculated using the following relation: [(*A*517 white - *A*517 of the sample) / *A*517 white] × 100. *A*517: Absorbance at 517 nm.

RESULTS AND DISCUSSION

Chemical Screening: Table 1 presents the results of the phytochemical analysis of the three extracts from the leaves of *Dischistocalyx hirsutus*.

Table-1: Results of chemical screening of extracts from leaves of *Dischistocalyx hirsutus*

Chemical families	Extracts		
	Aqueous (AQ)	hydroethanolic (HE)	Ethanolic (ET)
Alkaloids	-	-	-
Anthocyanins	+++	+++	+++
Flavonoids	+++	+++	+++
Cardiotonic heterosides	++	+++	+
Saponosides	+	-	-
Tannins	+++	+++	+++
Terpenes	+/-	+/-	+/-

Legend: ++++ = very abundant; +++ = abundant; + = medium; +/- = traces - = absent

The phytochemical analysis of the three extracts from the leaves of *Dischistocalyx hirsutus* revealed the presence of several chemical families. We note the strong presence of anthocyanins, flavonoids, tannins and traces of terpenes in the three extracts. Cardiotonic heterosides are more marked in the hydroethanolic extract than in the other two extracts. However, saponosides are only present in the aqueous extract. We also note the absence of alkaloids in the three extracts. We observe a strong presence of polyphenolic compounds. In fact, previous studies have shown that anthocyanins, flavonoids, terpenes, tannins, saponosides and cardiotonic heterosides have important pharmacological properties, namely: anti-inflammatory, diuretics, antiseptics and scarring, antiviral, antitumor, antiallergic, anti-parasitic, anticancer, surfactants, antioxidants and cardiotonics [**12-14]**. The presence of these different chemical families in the plant could justify the use of this plant in the treatment of several pathologies. These results agree with those of Andzi et al. [**15]** on Dischistocalyx sp, a species of the same genus used in Gabon for the same therapies.

Qualitative Analysis: The identification of phenolic compounds in thin layer chromatography (TLC) of the hydro-ethanolic, ethanolic and aqueous extracts (Figure-1) shows a series of spots of different colors obtained after spraying the plate with Neu reagent and viewing with a ultra-violet lamp at 366 nm.

It appears

• yellow fluorescence spots with Rf = 0.2; 0.3 and 0.5, materializing the presence of compounds with flavonoid structure in the three extracts, with some differences for the compound with Rf = 0.2 which is absent in the ethanolic extract;

• light green fluorescence spots with Rf = 0.6 and 0.7, highlighting the presence of hydroxycinnaques derivatives in the plant;

• pink fluorescence spots with Rf = 0.8 and 0.9, signaling the presence of Anthocyanic compounds.

Indeed, according to Wagner and Bladt [**16]**, the orange-yellow fluorescence's after exposure of the plate to the lamp uv-366 nm are due to the quercetol derivatives and are subject to an ortho-di hydroxylated substitution in position 3' and 4' on cycle B of the flavonoid (Figure-2 and 3) and the light green fluorescence's and light blue fluorescence's would be assimilated to the hydroxycinnamic derivatives respectively to the chlorogenic and caffeic acid derivatives (Figure-4).

Table-2 summarizes the Rf of the 7 compounds revealed during the TLC of the three (03) extracts analyzed. However, the absence of compounds 1 and 6 is noted for the ethanolic extract and compound 7 for the aqueous extract.

Figure-1: Qualitative analysis of the three (03) extracts of *Dischistocalyx Hirsutus*

Table-2. Frontal Retentions of *Dischistocalyx hirsutus* extracts

Compounds	R_f CCM (x100) of Extracts			UV fluorescence at 366 nm
	HE	ET	AQ	
1	20		20	Yellow
2	30	30	30	Yellow
3	50	50	50	Yellow
4	60	60	60	Green-light
5	70	70	70	Green-light
6	80		80	Pink
7	90	90		Pink

Hydro-ethanolic extract (HE), Ethanolic extract (ET) and Aqueous extract (AQ)

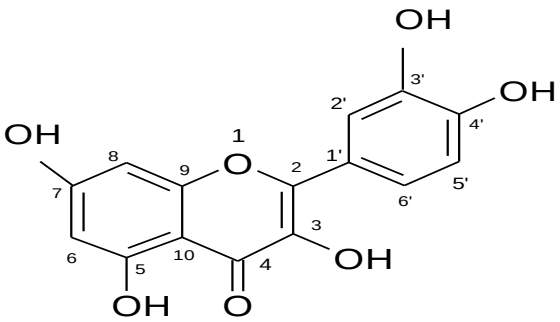
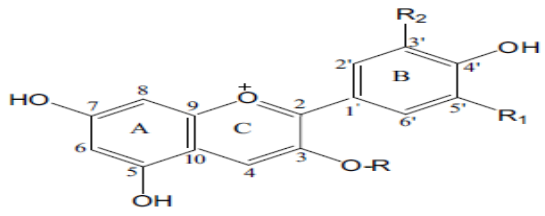


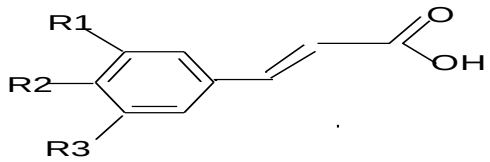
Figure-2 : **Structure of 3' 4', 5, 7-trihydroxyisoflavonol : quercetol**



For the glucosylated form R = glucose

Anthocyanidin (R = H)	R_1	R_2
Milvidin	OCH_3	OCH_3
Peonidin	OCH_3	H
Delphinidin	OH	OH
Petunidin	OCH_3	OH
Cyanidin	OH	H

Figure-3 : Structure of some Anthocyanic compounds



R1, R2, R3 = H, t-cinnamic acid; R1, R3 = H, R2 = OH : p-coumaric acid
R1= H, R2, R3 =OH : caffeic acid; R1=H, R2 =OH, R3 =OMe : ferrulic acid
R1=H, R2 =OH, R3 =OMe : isoferrulic acid

Figure-4: **Some examples of C_6-C_3 Acid phenol structures**

Quantitative Analysis: The calibration curve of gallic acid (Y = 1.8826X - 0.0095) was used for the determination of total polyphenols (PPT) and catechin (Y = 2.4057X + 0.0317) for the dosage of total Flavonoids (FVT). The results are expressed in mg gallic acid equivalent per gram of dry matter (mgEGa / gDM) for polyphenols and in mg catechin equivalent per gram of dry matter (mgEC/g DM) for flavonoids. The calibration lines were obtained with correlation coefficients of R^2 = 0.9974 for gallic acid and 0.9999 for catechin. The results of the quantitative spectrophotometric analyzes of the extracts of D. hirsutus are shown in Figure-5. Quantitative analysis of flavonoid compounds gives concentrations of 5.5 ± 0.1; 4.5 ± 0.1 and 3.3 ± 0.1 mg EC/gMS, respectively for hydro-ethanolic (1/1), ethanolic and aqueous extracts. Those in polyphenolic compounds give concentrations of 76.5 ± 0.2; 66.5 ± 0.1 and 55.3 ± 0.1 mg EGA/g MS, respectively for hydro-ethanolic, ethanolic and aqueous extracts. It appears that our plants are richer in polyphenolic compounds than in flavonoids. It should also be noted that the amounts are decreasing from the hydroethanolic extract to the aqueous extract. This shows that the Water-ethanol alcohol mixture would be a better solvent for extracting polyphenols and flavonoids from our plant. These results confirm those obtained by the qualitative analysis, which highlighted the presence of bands of yellow-orange fluorescence at the front retentions 0.2; 0.3 and 0.5 and light green at the front retentions 0.6 and 0.7, characteristic of the strong presence of polyphenolic compounds in the plant [**16]**.

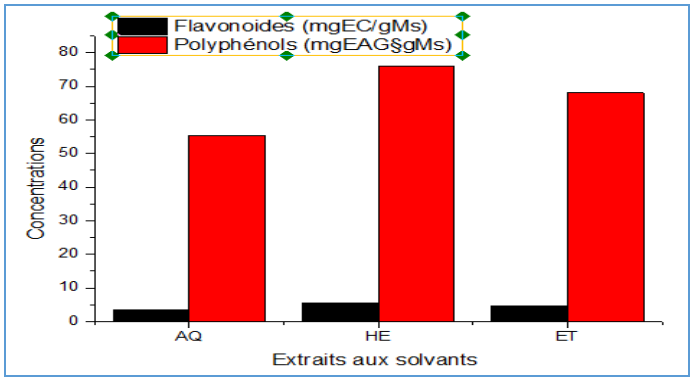


Figure-5: **Content of total polyphenols and flavonoids in *Dischistocalyx hirsutus* extracts**

These preliminary results may justify the use of this plant in the traditional African pharmacopoeia in general and the Congolese in particular. Indeed, polyphenols and flavonoids are recognized for their cardiovascular, antimicrobial, anti-inflammatory, Antioxidant and many others [**17-20]**. These results corroborate Andzi et al [**15]** with the leaves of Dischistocalyx sp, a species of the same family, used in Gabon in the treatment of anemia. The latter obtained higher polyphenol contents, the order of 290.33 ± 0.011 mgEAG/g MS.

Antioxidant Activity: The anti-radical effect of the aqueous, ethanolic and hydroethanolic extracts of D. hirsutus against the DPPH radical is measured using a spectrophotometer at 517 nm. This activity is characterized by a reduction of DPPH which changes from the purple color (DPPH.) to the yellow color (DPPH-H). This reduction capacity is characterized by a decrease in the absorbance induced by the secondary metabolites responsible for the antioxidant activity [**21, 22]**. The results of the quantitative evaluation of the anti-radical activity of the ethanolic, hydro-ethanolic and aqueous extracts are presented in Figure-6.

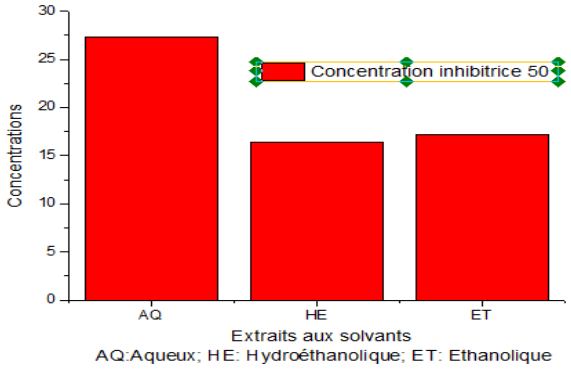


Figure-6 : IC_{50} of *Dischistocalyx hirsutus* extracts

It appears (Figure-6) that the hydro-ethanol extract has the lowest inhibitory concentration than the other two. IC_{50} are ranging from 160.21 ± 0.01; 270.30 ± 0.01 and 170.22 ± 0.13 µg/mL respectively for the hydroethanolic, ethanolic and aqueous extract. Thus, it can be suggested that the water-ethanol mixture would be conducive to better extraction of the anti-radical compounds in the leaves of D. hirsutus, since this extract has strong capacities for reducing free radicals at low concentrations. This activity confirms the results got during the determination of total polyphenols, because there are high concentrations of polyphenols in hydroethanolic extracts compared to ethanolic and aqueous extracts. These results agree with previous works of several authors which all show the impact of phenolic compounds on the reduction of free radicals in general and on various pathologies resulting from these radicals [**15,19, 21-25]**. The high free radical reduction capacity of extracts justifies the traditional use of *Dischistocalyx hirsutus*.

CONCLUSION

This present study focused on the chemical analysis of the leaves of Dischistocalyx hirsutus. It comes out after discussion that Dischistocalyx hirsutus leaves are rich in polyphenolic compounds, particularly in flavonoids, anthocyanins, tannins, terpenes and saponosides. These properties can be viewed as better antioxidant activity of its hydroethanolic extract compared to aqueous and ethanolic extracts. Furthermore, it transpires that these results could justify the traditional use of this plant in the treatment of certain diseases of anemic origin. Finally, we can conclude that this plant has significant anti-free radical potential.

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