International Journal of Science Academic Research
Vol. 03, Issue 02, pp.3492-3495, February, 2022
Available online at http://www.scienceijsar.com

ISSN: 2582-6425

Research Article

PHYTOCHEMICAL STUDY AND EVALUATION OF THE ANTIOXIDANT ACTIVITY OF FRACTION TO THE EXTRACT OF *LECANIODISCUS CUPANIOIDES (SAPINDACEAE)*

***Kossi Jean Marie D. Tokoudagba and Fernand A. Gbaguidi**

Laboratoire de Chimie Pharmaceutique Organique, UFR Pharmacie, Faculté des Sciences de la Santé, Université d'Abomey-Calavi, Campus du Champ de Foire 01BP 188 Cotonou Bénin

Received 12th December 2021; **Accepted** 18th January 2022; **Published online** 28th February 2022

Abstract

Lecaniodiscus cupanioides is a medicinal plant of the Sapindaceae family, widely used in traditional medicine in Benin. In the present work we prepared the hydro ethanolic extract from the dried leaves of this plant with an extract yield of around 23%. The quantitative estimation of flavonoids and total phenols by the colorimetric method and the Folin Ciocalteux method showed that the hydro ethanolic extract is very rich in these compounds. We did the liquid-liquid extraction from the solvent of increasing polarity and obtained apolar (cyclohexane and dichloromethane) and polar (ethyl acetate and methanol) fractions. The evaluation of the antioxidant power of ethyl acetate and methanolic fractions carried out using the method of scavenging the free radical DPPH and that of the reduction of iron of FRAP revealed that the fractions of ethyl acetate and methanolic of the hydro ethanolic extract of the leaves of *Lecaniodiscus cupanioides* has a higher antioxidant power than that of acorbic acid.

Keywords: Lecaniodiscus cupanioides, Flavonoids, Oxidative activity, Polyphenols.

INTRODUCTION

True health capital natural antioxidant molecules protect our organism from premature aging by opposing the action of free radicals. Polyphenols are natural compounds widely distributed in the plant kingdom. They appear to play an important role in both cancer and cardiovascular disease protection. The protection active against cancer would be explained by a mechanism quite similarto that of prebiotics by their ability to select a particular type of microbiotia in particular for cancers of the digestive system (stomach, colon etc..). Medicinals plants constitute an inexhaustible source of molecules with varied biological and pharmacological activity and alternation for the use of synthetic antioxidants products. It's for this reason that we studied *Lecaniodiscus cupanioides* a plant with antoxidant potential among the medicinal plants midely used in traditional medicine. *Lecaniodiscus cupanioides* is a species of plant in the family Salpindaceae and genus *Lecaniodiscus* It's a trie 9 meters high with strong woody branches with flowers in fascicles in axillary cluster of purplish green color and fragrant with slender petioles. The species is present in tropical Africa from Sierra leone to Sudan also in Angola to the South of the Democratic Republic of Congo and in Uganda. The species has multiple uses in traditional medicine in the from in inhalation The bark is used to freat headaches sinusitis otitis eye or hearing complaints. In decoction it's applied to relieve pains in the chiest bronchatis pleurisy kidned pain. Pruritus is treated with frictions of crushed dark. TQhe leaves are reputed to be antibacterial and rubefacient. They are applied to boils, bruises but are liable to cause burns if left in place for too long. The fruits are considered abthelmintic

Corresponding Author:** ***Kossi Jean Marie D. Tokoudagba
Laboratoire de Chimie Pharmaceutique Organique, UFR Pharmacie, Faculté des Sciences de la Santé, Université d'Abomey-Calavi, Campus du Champ de Foire 01BP 188 Cotonou Bénin.

MATERIALS AND METHODS

Plant material: The plant material consists of dried leaves of *Lecaniodiscus cupanioides* collected in December 2020 in Abomey-Calavi. The leaves of plant were identified at the National Herbarium of the University of Abomey Calavi. The leaves of the plant were washed and 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 hydro ethanolic mixture (40V/60V respectively) for 48 hours. After respective filtration on Whatman paper N°1 the filtrate obtained were evaporated using a rotary evaporator at 40°C. The residues of this filtrate were dried in the oven for 48 hours at 40°C to obtain the dry extract.

Liquid-liquid extraction method: The liquid-liquid extraction is carried out by the intimate contact of the solvent with the solution in a separating funnel. The separation of the phases is obtained by gravimetric or centrifugal decantation after stirring of the whole. The solution consists of the crude hydroethanolic extract dissolved in 50 mL of distilled water. We used successively during the extraction 500 mL of cyclohexane, dichloromethane, ethyl acetate and butanol. The different fractions (phase from the operation containing the extracted solutes) collected are evaporated with a rotavapor.

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.

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% $ALCL_3$ solution was added. To the mixture 0.2 ml of 1M $NaNO_2$ solution and 0, 25 ml of distilled water were added after a 5min rest. The whole 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 50 uL of extract at a given concentration are added 950µL of the DPPH solution. 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/L); 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:

$PI = 100(A_0 - A_1) / A_0$ $A_0 =$ DPPH absorbance A_1 sample absorbance

The IC_{50} (concentration of the sample required to neutralize 50% of the free radicals) was obtained using the software Statgraphics Plus 5/0.

FRAP test: The reducing power of the extracts is determined by FRAP method (Bassène 2012). Thus, 0.4mL 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 to it, then the tubes were centrifuged at 3000 rpm for 10 minutes. Then, 1mL of the supernatant from each tube was mixed with 0.2mL of 0.1% FeCl3 solution and kept in the dark for 30 minutes before measuring the 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: $RP = 100(A_a - A_b) / A_a$ A_a: Absorbance of the extract A_b: Absorbance of the blank

Statistical analysis: Significance tests are performed by Fisher's test through the software Stat View. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

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

Table 1. Phytochemical Screnning of Extract

Class	*Lecaniodiscus cupanioides* (extract hydro éthanolic)
Flavonoids	+
Tannins	+
Gycosylated couramines	+
Triterpenes	+
Alkaloids	+
Anthracene derivatives	+
Cardiotonic glycosides	-
Saponosides	-
Lignans	+

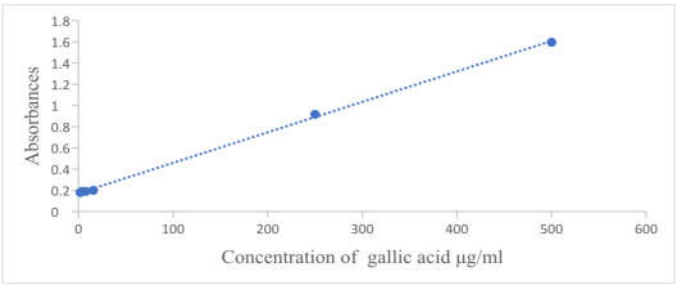
Fractionation: The yields obtained during the fractionation of the crude extract are given by the following table

Plant Materiel	Extract	Mass	Yield
Crude Extract (20g)	n Hexane Extract	0,25g	1,25%
	CH_2CL_2 Extract	0,29g	1,45%
	AcOEt Extract	3,58g	17,9%
	MEOH Extract	5,01g	25,05%
	Aqueous Residu	9,25g	46,2%

Total polyphenols content: The determination of the total polyphenols content in the hydroethanolic extract and the polar fractions was done by the Folin Ciocalteux method. The content was reported in mg gallic acid equivalent /g of dry plant material. The polar fractions are richer in total polyphenols compoud to the apolar extract. This is confirmed by phytochemical Screenig which reveals the presence of tannins flavonoids and saponosides in the hydrethanolic extract

Table 2. Total Polyphénols content

N°	Plant	Content of polyphénols µg Eq AG/mg of extract
1	Hydro éthanolic Extract	99,02 ± 3,7
	n Hexane Extract	12,9 ± 23,9
	CH_2CL_2 Extract	52,2 ± 13,9
	AcOEt Extract	305,6 ± 11,9
	MeOH Extract	542,2 ± 3,3
	Aqueous Residu	135 ± 15,9



Picture 1 : Gallic and calibration line

Determination of Flavonoids: The flavonoid content determined by the aluminum trichloride method for each extract was reported in mg equivalent of catechin/g of dry plant material. The results reveal that the polar fractions have a high content of flavonoid

Table 3. Yield and contents of total polyphenols and flavonoids

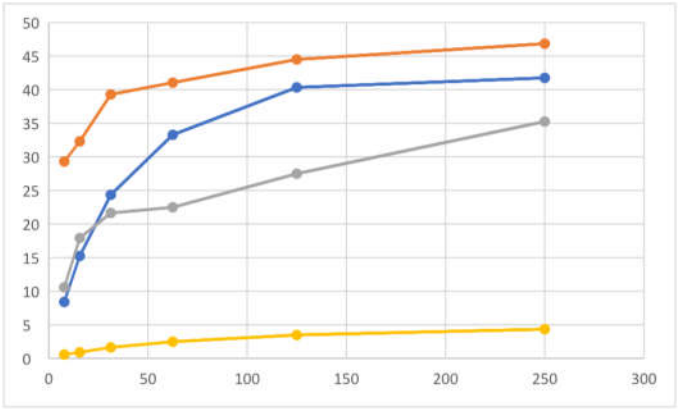
Extracts	Yield (%)	Content of total polyphenols µg Eq AG/mg d'extract	Content of Flavonoid mg QE
Hydro ethanolic Extract	20g	99,02 ± 3,7	389,62±6,8
n Hexane Extract	1,25%	12,9 ± 23,9	42,23 ± 6,2
CH_2CL_2 Extract	1,45%	52,2 ± 13,9	60,12 ± 5 ,4
AcOEt Extract	17,9%	305,6 ± 11,9	391,76 ±3,4
MeOH Extract	25,05%	342,2 ± 3,3	402 ,2 ± 4,1
Aqueous Residu	46,2%	135 ± 15,9	201,01± 1,2

Antioxidant Activity: The antioxidant activity was studied on the polar fractions and the hydro ethanolic extract of the dried leaves of the plant

DPPH Test: The antioxydant activity of the hydro ethanolic extract of the leaves of the plant and the polar toward the DPPH radical was evaluated at using a spectrophotometer by following the reduction of this radical which is accompanied by its passage from the violet color (DPPH°) to the yellow color (DPP-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 fractions tested and of the extract showed that the percentage inhibition of the ethyl acetate and methanol fractions is greated than 80% for concentrations of the order of 125ug/mL at 250ug/mL. The EC 50 values determined in mg/mL expressing the effective concentration of the antioxidant extract necessary for the trapping and the reduction of 50% of moles of DPPH dissolved in methanol (Table 4).

According to the results recorded the two fractions have an antioxydant power their respective EC 50 is 24,14± 9,73 and 35,02±15,08 significantly stronger than of ascorbic acid the value of which is order of 0,235mg/Ml. The polyphenols contained in the fractions are responsible for the antioxidant activity of the extracts

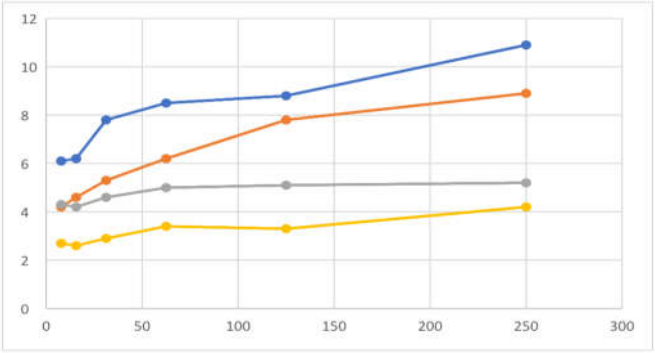


Picture 2. Percentage of DPPH inhibition according to different concentrations of the extract ; polar fraction and ascorbic acid

Table 4 . Value of IC $_{50}$ of the extract and of the polar fractions and of ascorbic acid

Extracts/Standard	IC50 ± Ecart type
Crude Extract	19,15 ± 8,75
AcOEt Extract	24,14 ± 9,73
MeOH Extract	35,02 ± 15,08
Ascorbic acid	0,235 ± 8,01

FRAP Test: The antioxidant activity of the polar fractions and of the hydro ethanolic extract was evaluated by the method of iron reduction FRAP. The presence of reducing agents in the polar fractions and of the plant extract causes the reduction of Fe3+ ferricyanide complex in ferrous from. Therefore Fe2+ can be evaluated by measuring the increase in the density of the blue color in the reaction medium at 700nm. The reducing power of the polar fractions and of the hydroethanolic plant extract is dose dependant (concentration – dependent) The evaluation of the reducing power of the fractions and the hydro ethanolic extract showed a better activity of the methanolic fraction of the extract compared to that the ethyl acetate fraction of the hydroethanolic extract of *Lecaniodiscus cupanioides* as show in Picture 3. Indeed the methanolic fraction of hydro ethanolic extract at concentration of 7,8-15,62-31,25-62,5-125-250µg/Ml gave respective reducing powers of 6,1%, 6,2%, 6,8%, 8,5%, 8,8%, 8,9%. At the same concentrations respective reducing powers 5,2%, 5,3%, 5,6%, 6,2%, 6,8%, 6,9% were observed for the ethyl acetate fraction of the hydro ethanolic extract of *Lecaniodiscus cupanioides*



Picture 3. Reducing power of the crude extract ; polar fractions and ascorbic acid according to different concentrations

Conclusion

The study of the antioxidant activity of the polar fractions of the hydroethanolic extract of the species of LC according to the method of iron reduction and that of the trapping of the free radical DPPH showed that the two fractions of the extract hydroethanolic have proven antioxidant activity These fractions could therefore constitute an alternative to certain synthetic antioxydant additives Further research is needed to identify isolate and purify these fractions

REFERENCES

Banerjee A., Dasgupa N., De B. In vitro study of antioxidant activity of *Syzygium cumini* fruit. *Food Chemistry,* 2005 90(4), 727-733.

Bhupendra K.K., Mahesh G.T., Yogendra S. Free radical scavenging effect of various extracts of leaves of *Balanites aegyptiaca* (L) Delile by DPPH method. *Asian Journal of Plant Science and Research,* 2012 2(3), 323-329.

Bougandoura N., Bendimerad N. Evaluation de l'activité antioxydante des extraits aqueux et méthanolique de Satureja calamintha ssp. Nepeta (L) Briq. <<Nature & Technologie<< B-Sciences Agronomiques et Biologiques, 2013 09(13) 14-19.

Boxin OU, Dejian H., Maureen AF, Elizabeth KD. Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) anf ferric reducing antioxidant power (FRAP) assays: a comparative study. *J Agric Food Chem.,* 2002 ;5 :223-8

Chinaka ON, Julius 00, Motunrayo GA. In vitro antioxydant potentials of some herbal plants from Southern. *Nigeria J Med Sci.,* 2013 ;13 :56-61

Huang D., Ou, B., Prior, R. L. The chemistry behind antioxydant capacity assays. *Journal of Agricultural and Food Chemistry*, 2005 55, 1841-1856.

Idiko B., Maria -Loredana S., Dominica R., Simona et Codruta C. HPTLC quantification of some flavonoids in extractsn of Satureja hortensis L. obtained by use of differents techniques. *Journal of PLanar Chromatography-Modern TLC* 2009 22(1),25-28.

Kone M, Toure A, Ouattara K, Coulibaly A, Phytochemical composition, antioxydant and antibacterial activities of root of *Uvaria chamae* P. Beauv. (Annonaceae) used in the treatement of dysentery in North of Cote d'ivoire. *Int J Pharmacogn Phtochem Res*., 2015 ;7 :1047-53

Majhenic L., kerget M.S., et Knez Z. Antioxidant and antimicrobial activity of guarana seed extracts. *Food Chemistry,* 2007 104, 1254-1268

Marc Fr., Davin A., Deglène-Benbrahim L., et Fernand C. Methodes d'évaluation du potentiel antioxydant dans les aliments Erudit, M/S : médecine sciences. 2004 20(4), 458-463.

Morteza Semnani K. Saeedi M, Shahnavaz B. Comparaison of antioxydant activity of extract from roots of liquorice (*Glycyrrhiza glabra L*.) to commercial antioxidants in 2% hydroquinone cream. *J Cosmet Sci.,* 2003; 54: 551-8

Rezaeizadeh A., Zuki A.B.Z., Abdollahi M., Goh Y.M., Noordin M.M., et al Determination of antioxidant activity in methanolic and chloroformic extracts of *Momordica charantia*. *African Journal of Biotechnology*, 2011 10(24), 4932-4940.

Roberto G., Baratta M.T., Deans S.G., Doman H.J.D. Antioxidant and antimicrobial activity of *Foeniculum vulgare* and *Crithmum maritimum* essentiel oils. *Planta.Med*., 2000 66, 687-693.

Sam SKG, Senthil KB., Ramachandran S., Saravanan M., Sridhar SK. Antioxydant and wound healing properties of *Glycyrrhiza glabra* root extract. *Indian Drugs,* 2001; 38: 355-7

Yadav SB., Tripathi V., Singh RK, Pandey HP. Flavonoid glycosides from *Cuscuta reflexa* stems and their antioxydant activity. *Indian Drugs,* 2001; 38 :95-6

****