UNIVERSITE D'ABOMEY–CALAVI (UAC)

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INSCRIPTION SUR LA LISTE D'APTITUDE AUX FONCTIONS DE MAITRE DE CONFERENCES (LAFMC) CAMES

PUBLICATION N°1

45e SESSION

CTS SCIENCES ET TECHNIQUES DES ACTIVITES PHYSIQUES ET SPORTIVES – JEUNESSE ET LOISIRS (STAPS-JL)

SPÉCIALITÉ : PHYSIOLOGIE DE L'EFFORT

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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH IN BIOLOGICAL SCIENCES

(ISSN:2348-8069)

(www.ijarbs.com)

(A Peer Reviewed, Indexed, Referred and Open Access Journal)

IMPACT FACTOR: 5.205(2022), NAAS SCORE: 3.33 (2020)

Index Copernicus Value: 83.08 (2016),

Coden : IJARQG(USA),

DOI: 10.22192/ijarbs (Crossref, USA),

IJARBS is a peer reviewed journal for publishing work of high standards as stated in UGC notification.
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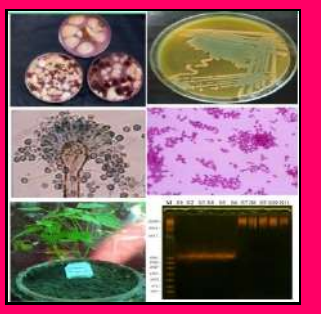

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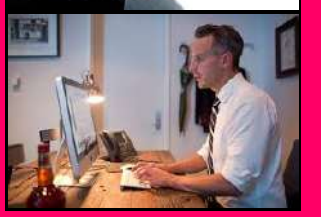

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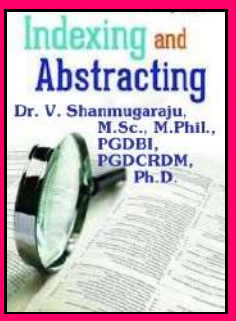

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Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.09.012

Phytochemistry, total polyphenol content and antiradical activity of *Rauvolfia vomitoria* (Apocynaceae) a plant used against asthma in Southern Benin

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Abstract

In Benin, as many African countries, people use traditional remedies to treat respiratory diseases. This work aims to contribute to the treatment of asthma by determining the chemical composition and antioxidant efficacy of *Rauvolfia vomitoria*, a plant species used in primary asthma care in Benin. Phytochemical analysis showed that the leaves contain tannins, terpenes, steroids, mucilages, flavonoids and alkaloids. The quantitative determination of total phenols in the aqueous extract of *Rauvolfia vomitoria* leaves gives a value of 17.04±0.20 mgEq of gallic acid per g of extract. The decoction of the leaves showed a very marked anti-radical potential with an IC50 = 5.01 ± 0.02 mg/ml but less than that of ascorbic acid (IC50 = 3.97 ± 0.01 mg/ml) used as reference antioxidant.

Keywords: Asthma, *R. vomitoria*, phytochemical anahysis, antiradical activity, Benin.

Introduction

Chronic respiratory diseases, including bronchial asthma and chronic obstructive pulmonary disease, are an underestimated and largely forgotten problem in sub-Saharan Africa. This problem is underestimated because their frequency and severity are much higher than those generally accepted. Several asthma prevalence surveys give figures of 4-14%, with higher trends in urban settings. This problem is neglected because in most developing countries, effective care for people suffering from these diseases is mostly absent or very defective (Ku et al.; 2020).

The determinants of asthma and its clinical expression are multiple, and both genetic and environmental factors play a role (Dumas et al; 2012; Laprise and Bouzignon; 2011, Lemanske et al.; 2010). The prevalence of asthma has increased sharply in industrialized countries since the second half of the twentieth century, and is beginning to increase in developing countries [Bousquet et al. ;2005, Eder et al.; 2006], which highlighted the importance of environmental or lifestyle factors in the etiology of the disease. Asthma is now the leading occupational respiratory disease in industrialized countries (Jaakkola and Jaakkola; 2012). Its treatmentis considered complex in clinical practice (Dumas et al; 2012; Burge; 2010; Vandenplas et al.; 2011). Like many chronic diseases, respiratory diseases, including asthma, are marked by social disparities (Temam; 2017). Worldwide, 339 million people suffer from

asthma. It is the most common chronic childhood disease, affecting 14% of children worldwide and increasing in number. It is among the top twenty causes of disability in children worldwide (Ahmed et al., 2017).

Most asthma deaths occur in low- and middleincome countries. They are favored by underscreening, insufficient treatment, poorly controlled asthma and severe exacerbations (WHO., 2018).

In most developing countries, between 70 and 95% of the population rely on traditional medicine, which has always occupied a central place in the global health system, to cure myriad ailments (Lehmann, 2013; Moses et al.; 2015). In recent decades, the use of combinatorial chemical and biosynthetic technology has provided new derivatives of natural products, optimized on the basis of their biological activities in order to produce chemical agents of considerable efficiency (Yagoub and Moulfi, 2019).

In Benin, very few studies have been undertaken for the treatment of asthma using medicinal plants. It is therefore urgent to use translational medicine, an alternative to initiate or even contribute to the phytotherapeutic management of this pathology. This is how traditional healers in the department of Couffo, in southern Benin, frequently use *Rauvolfia vomitoria* Fzel for the management of asthma in this area. It is therefore important to provide evidence as to the use of the latter with a view to its valuation.

Materials and Methods

Plant material

The plant material was fresh leaves of *Rauvolfia vomitoria* Fzel harvested in the region of South Benin (couffo) in the municipality of klouekanmè and brought back to the laboratory the same day. The samples were spread out in a cold drying room (22°C) for about 14 days. Then they were reduced to powder using an electric grinder (*Retsch type SM 2000/1430/Upm/Smf*). The powder thus obtained is sieved with a sieve with a diameter of 710 µm.

Phytochemical analysis

The phytochemical screening was carried out in accordance with the classic method of differential

Fig.1: Aerial part of *R. vomitoria*

Methods

Table 1: Specific reagent and Reaction

Classes of metabolites	Specific reagent and Reaction
Alkaloids	- Dragendorff alkaloids (potassium iodobismuthate): orange precipitate -Mayer (potassium iodomercurate): yellowish precipitate
Tannins	-$FeCl_3$: dark blue color
Flavonoïds	-Shinoda (reaction to cyanidin): orange-red coloration
Anthocyanins	- Red color in acid medium and purplish blue in alkaline medium
Leucoanthocyanins	Hydrochloric alcohol (EtOH 50°/HClcc 2:1 v/v): cherry red color

coloring and precipitation reactions of the main groups of chemical compounds contained in the matrix (Houngbèmè et al., 2014; Ombouma et al., 2021). The various tests performed are summarized in table 1 below:

Preparation of the raw extract

The extraction of the total chemical principles was carried out using the decoction method in accordance with the traditional use of the plant. Based on the extraction techniques cited in the literature (Houngbeme et *al.*, 2014), 50 g of powder were dissolved in 500 mL of distilled water. The mixture was brought to a moderate boil for 30 min. After cooling, the mixture was filtered (3 times in a row) on absorbent cotton and the filtrate was transferred to a 1000 mL flask then subjected to evaporation at 40° C using a rotavapor (*HeidolphLaborota 4000 efficient*) coupled with a water chiller (*Julabo FL 300*). The dry residue obtained represents the decoction. The yield was calculated according to the expression:

Yield (%)=100× (mass of dry extract / initial mass of powder).

Evaluation of the antiradical activity

The anti-radical activity of the extract by the DPPH° (2,2-diphenyl-1picrylhydrazyl) radical method was evaluated using the procedure used successfully by Gandonou et *al.* (2018). This method was based on the reduction of the stable free radical DPPH° in the presence of an H° radical giving.

Principle

DPPH is a stable radical which in solution exhibits a characteristic absorption at 517 nm giving it a violet color. This color quickly disappears when the DPPH is reduced by a free radical scavenger (antioxidant) to give the reduced form DPPH-H with a yellow color in solution (Molineux et al., 2004).

Quinone derivatives	-Bornträger (reaction between quinone cycles in NH4OH medium): purplish red coloration
Saponosides	--Determination of the foam index (positive if IM>100)
Steroids and Terpenes	--Liebermann-Burchard (Acetic anhydrideH_2SO_4cc 50:1 v/v) :violet color -Kedde (dinitrobenzoic acid 1% in EtOH + NaOH 1N 1:1): purple red color (cardenolides)
Cyanogenic derivatives	-Guignard (Paper impregnated with picric acid): brown color

Preparation of the stock extract solution: 1mg of the extract was dissolved in 1mL of methanol (Cm=1mg/mL) then a 1/100 dilution was carried out.

Preparation of the DPPH radical solution and description of the method

We dissolved 4mg of DPPH° in 10mL of methanol to have a mass concentration of 0.4mg/mL. Then 1.5 mL of the extract (diluted solution) was mixed with 3 mL of the methanolic solution of DPPH°. The mixture was incubated for 15min at room temperature and the absorbance was read at 517 nm. The antiradical activity of the extract was determined using the calibration curve established with ascorbic acid (0-10mg/mL). The percentage of free radicals by trapping of DPPH is calculated according to the following relationship:

(%DPPH) = 100 X [White Abs – Sample Abs) /White Abs], where white Abs; the absorbance of the control (reaction mixture excluding test compounds) and Sample Abs; the absorbance of the test compounds.

The IC50 values, which was the concentration of plant drug extract which caused a neutralization of 50% of the DPPH radical, are calculated from the graph of the percentages of recovery against the concentration of DPPH. Each test is carried out in duplicate.

Dosage of total phenols

The quantitative determination of polyphenols was perfomed according to the method used by Kim et al. 2003; Maiga et al., in 2020. 125µL of sample at 1 mg/ml was taken, which was dissolved in 625µL of Folin-Ciocalteu reagent. After incubation for 5 min, 500 µL of sodium carbonate Na_2CO_3 at 75mg/mL are added. The mixture was vortexed and then incubated for 2 hours in the dark. The absorbance was read using a spectrophotometer (Genova type) at 760 nm.The content of polyphenols was deduced from the regression curve established with gallic acid (010mg/ml) and was expressed in mg equivalent of gallic acid per gram of dry extract according to the expression:

$$T(mg\acute{e}qAG/g) = \frac{C\ .Vr}{Vp\ .Cp}$$

T = Content of compounds; C = Concentration obtained from the calibration curve; Vr = Reaction volume; Vp = Volume of extract sample; Cp = Concentration of the solution of the extracted extract.

Results and Discussion

Yield of crude extract

The yield in mass percent is calculated as the average of the two tests with the standard deviation. The value found is: 10.98± 0.09%. This result shows that in the plant, there are compounds having an affinity with water, that is to say polar and heat-resistant molecules.

Chemical groups of the plant

The different metabolites identified in *R. vomitoria* powder are summarized in the table below:

Table 2: Chemical groups identified in leaves of *R.vomitoria*

Researched Chemical Groups	Results
Catechic tannins	++
Gallic tannins	-
Flavonoids	+
Anthocyanins	-
Leuco anthocyanins	–
Alkaloids	+
Reducing compounds	-
Mucilage	++
Saponoside	-
Cyanogenic derivatives	–
Triterpene	+
Steroid	+
Coumarin	_
Quinone derivatives	_
Free anthracenics	_
C–heteroside	-
O-heteroside	_
Cardiotonic derivatives	_

+: present; ++: highpresent; -: absent

Total phenol content of the extract curve established with gallic acid (figure 2) and is expressed in mg of gallic acid equivalent per After reading the absorbances, the concentration gram of dry extract. of polyphenols is deduced from the calibration

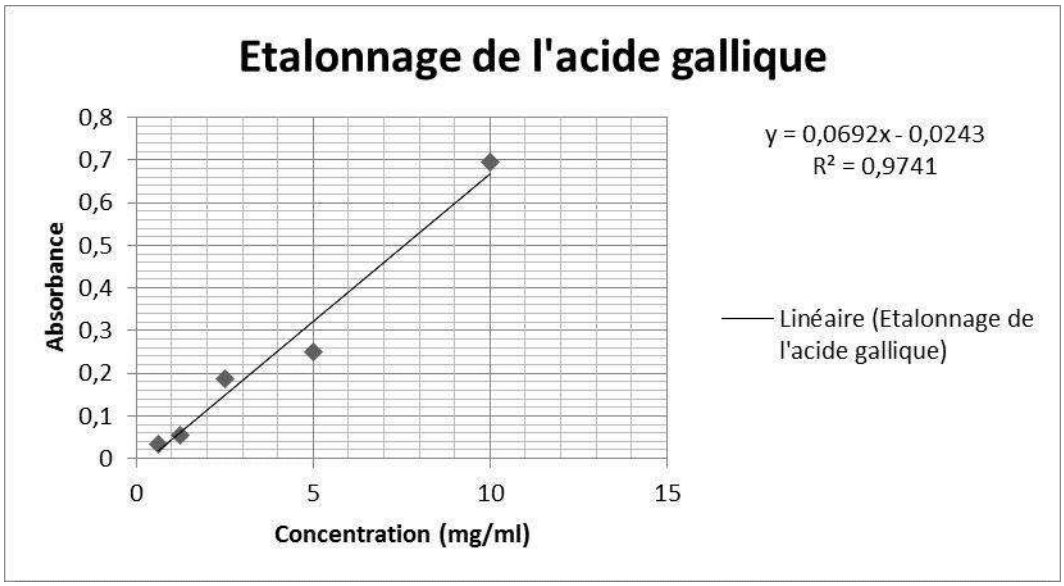

Figure 2: Gallic Acid Calibration Curve

The calculated total phenol content is summarized in Table 3 below:

Table 3: Total polyphenol content of *R. vomitoria* leavesaqueous extract

Chemical components	Quantity of metabolites	Curve equation	R_2
Total phenols	17,04±0,20mgEAG/g ES	Y = 0.0692x-0.0243	0.9741

The correlation coefficient (R^2) is substantially equal to 1, which means that there is a good correlation between the concentrations applied and the optical density read. These results reveal that the aqueous extract of *R. vomitoria* leaves is rich in total phenols. These results justify those of the phytochemical screening which reveals the presence of these compounds in the plant.

Antioxidant activity of the extract

The antioxidant capacity of the extract is measured in terms of radical scavenging capacity by following the reduction in absorbance of a methanolic solution of DPPH.After spectrophotometric measurement, the optical density values obtained made it possible to calculate the percentage of radicalinhibition the and to draw curves having a linear appearance with the presence of a stationary phase which signifies the almost total reduction of the DPPH in its non-radical form. From these curves we determined the value of the IC_{50} (EC_{50}) for the extract. The lower the IC_{50} value, the better the extract has antioxidant activity (Hounkanrin, 2018). The activity is considered as a decrease in the absorbance of the sample compared to the standard solution of DPPH. The regression lines expressing the percentages of inhibition of the radical according to the concentrations of ascorbic acid and of the extract of *Rovolfia vomitoria* Fzel are presented in figures 3 and 4 below:

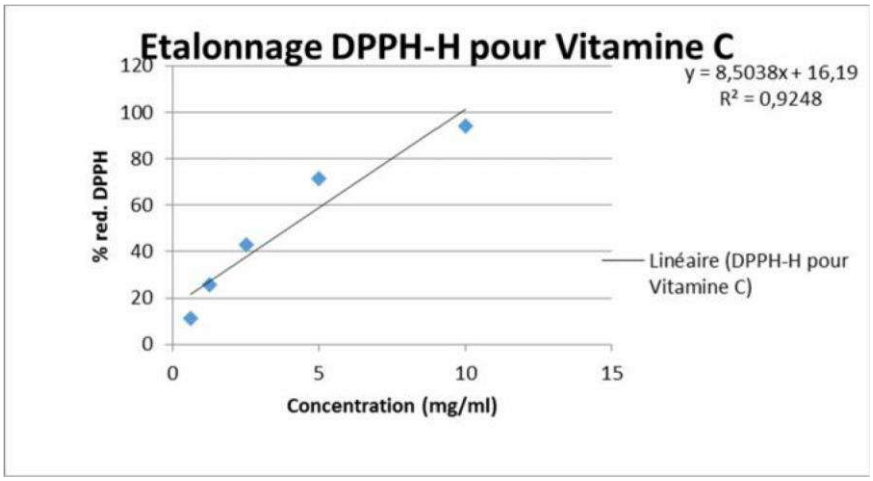

Figure 3: Regression curve of the antiradical activity of vitamin C (ascorbic acid).

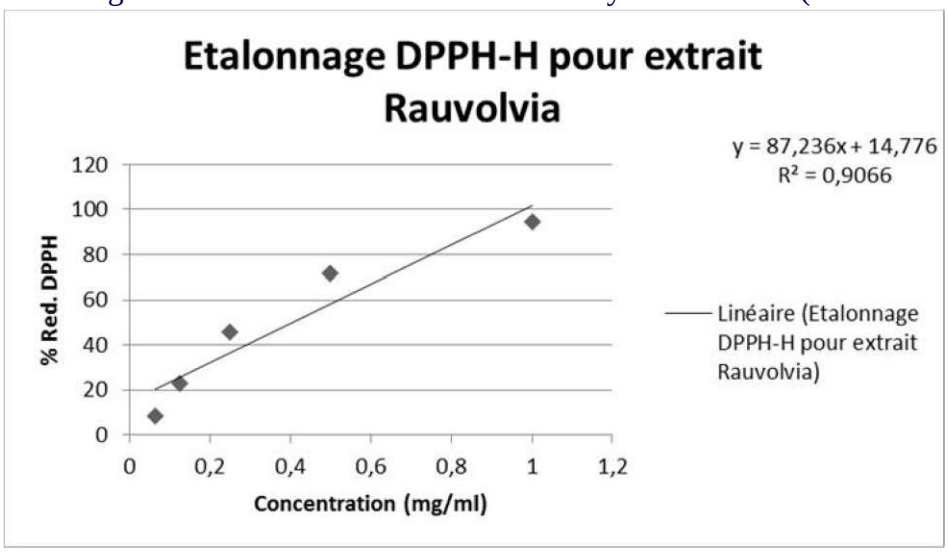

Figure 4: Regression curve of the antiradical activity of the *Rauvolfia vomitoria* extract

The results show that the percentage of reduction is proportional to the concentration of the substrate and this, for the extract and for the vitamin C. More precisely the increase in concentration of the sample causes an increase in percentage of reduction of the free radical and hence a strong antioxidant activity is exhibited. Table 4: IC_{50} values of extract and vitamin C

Dry extract	IC_{50} (mg/ m L)
R. vomitoria	5.01±0.02
Vitamin C	3.97±0.01

The antioxidant activity of the extract is expressed in IC50, the values of which are expressed in the following table:

R. vomitoria extract has a very high antioxidant potential compared to the standard antioxidant (vitamin C). The leaves of*R. vomitoria* are a source of antioxidant molecules that can be used to fight against oxidative stress diseases.

The antioxidant activity of the extract studied could be explained by the presence of the majority compounds which are mainly total flavonoids and tannins recognized as antioxidant (Yang et al., 2000; Marfak, 2003; Boukhobza, 2014).

Conclusion

This work made it possible to show that this plant is rich in chemical groups which justify a priori its antiasthmatic potential. These results provide evidence that the leaves of *Rauvolfia vomitoria* are useful for the treatment of various diseases caused by free radicals. The plant is said to be useful as a free radical scavenger and thus aid in the treatment of many diseases. This preliminary study contributes to the valorization of the Beninese flora and deserves to be deepened by evaluating the antiasthmatic power of this extract.

Acknowledgments

We address our thanks to Dr. Houngbeme G. Alban for the various manipulations.

Conflict of interest

The authors declare that there is no conflict of interest.

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How to cite this article:
AGBODJOGBÉ Kpèdétin Wilfrid Dieu-Donné, HOUNGBEME Gouton Alban; OUSSOUAMI Simplice Innocent, TCHIKEZO Mathias, MESSAN Foly. (2022). Phytochemistry, total polyphenol content and antiradical activity of *Rauvolfia vomitoria* (Apocynaceae) a plant used against asthma in Southern Benin. Int. J. Adv. Res. Biol. Sci. 9(9): 127-136. DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.09.012