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Phytochemical constituents and evaluation of total polyphenols and total flavonoids content of the ethanolic extract of *Harungana madagascariensis* trunk bark from Gabon.

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Abstract:
Harungana madagascariensis is a plant used in the traditional medicine in Africa. Many studies show that it can be used for the treatment of many deseases : diarrhea, diabete,malaria, grounds, hypertension,…etc. The objective of this studiy consists to release phyotochemical screening and evaluation of polyphenols and flavonoids content of the ethanolic extract of his trunk bark. Phytochemical analysis revealed the abundance of alkaloids, polyphenols, flavonoids and tannins. Saponins, terpenoids and reducing compounds are present. However, sterols and coumarins are absent. The total polyphenols (Folin-Ciocalteu method) and total flavonoids ($AlCl_3$ method) content was respectively 584.07±6.89 mg GAE/100g of dry matter and 146±6.76mg QE/100g of dry matter. These results showed a hight content in phenolics compounds in the ethanolic extract of Harungana madagascariensis trunk bark.

Key Word*: Harungana madagascariensis; ethanolic extract; Phytochemical; total polyphenols; total flavonoids***.**

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I. Introduction

Medicinal plants are used by many people for the treatment of many deseases. The therapeutic properties of these plants can be scientifically explained by the presence of bioactive substances so that alkaloids, flavonoids, tannins, coumarins, reducing compounds, saponins, sterols and terpernoids.[1] The utilization of *Harungana madagascariensis* (hypericaceae) in traditional medicine can therefore be explain by the presence of these compounds. Many studies show that *Harungana madagascariensis* is used for the treatment of diarrhea, diabete, malaria, grounds, hypertension, …etc.[2-5] However, very few studies on this plant have been released in Gabon. The study described in this paper consists to release phyotochemical characterization and evaluation of polyphenols and flavonoids content of the ethanolic extract of the trunk bark of *Harungana madagascariensis*.

II. Material And Methods

Plant Material

Harungana madagascariensis trunk barks were harvested at 80 km of Libreville to the way to Kango in march 2021. Identification was confirmed in the National Herbarium of IPHAMETRA (Institut de Pharmacopée et de Médecine Traditionnelle) of Libreville in Gabon. The bark was chopped in the laboratory at the room temperature and out of the sunlight for three weeks. The dried material was then ground into the fine powder using an electrical grinder. The powder obtained was stored in airtight bottle at 4^0C.

Preparation of extract

To 10 g of powder of *Harungana madagascariensis* trunk barks, was added 150 mL of ethanol. The mixture was stirred at room temperature during 24h and filtered through the Whatmann No. 1 filter paper. The filtrate obtained was then stored at 4^0C until analyses.

Phytochemical analyses

Phytochemical constituents were determined using a qualitative methods based on staining or precipitation reactions.[6,7]

To 2 mL of filtrate was added few drops of Dragendorf reagent. The appearance of orange-red color indicated the presence of **alkaloids**.

3 mL of filtrate was mixed with 1 mL of 10% lead acetate solution. The presence of **tannins** was indicated by the formation of white precipitate.

The presence of **saponins** was revealed by the observation or the persistent foam 15 minutes after shaken vigorously the mixture composed of 5 mL of filtrate and 3 mL of distilled water.

The mixture of 2 mL of filtrate and 1 mL of Fehling reagent was heated in a water bath for 15 minutes. The appearance of the red precipitate indicated the presence of **reducing compounds**.

To 2 mL of filtrate was added 3 mL of 10% NaOH aqueous solution. Observation of yellow color after shaken indicated the presence of **coumarins**.

To 2 mL of filtrate was added 1 mL of Folin-Ciocalteu reagent and 1 mL of 20% Na_2CO_3 solution. The appearance of a dark-green color indicated the presence of **polyphenols**.

To the mixture of 5 mL of filtrate and 5 mL of 1% of NH_3 solution was added few drops of concentrated H_2SO_4 solution. The appearance of yellow color indicated the presence of **flavonoids**.

To 2 mL of filtrate was added few drops of HCl concentrated solution. Observation of dark-red color or green color indicated the presence of **terpenoids** and **sterols** respectively.

The intensity of coloration determines the abundance of the compound present.

Total polyphenols

Total polyphenols content was determined using the method of Folin-Ciocalteu with few modifications.[7] To 200µL of dry extract (1mg/L) was added 1mL of Folin-Ciocalteu reagent (0.2 N diluted in ethanol). The mixture was incubated in the dark at room temperature for 1h. A blank was then prepared with ethanol at the same conditions. Absorbances were measured at 765 nm using a GENESYS 10 UV spectrophotometer. Gallic acid (0-200 mg/L) was used as the reference. All tests were performed in triplicate and the results were expressed in mg of Gallic Acid Equivalent (GAE) per 100 g of dry extract according to the following formula:

C = (Cl x D x 10/m) x 100

C: concentration of the sample in µgGAE/100 mg of dry extract
Cl: concentration of the sample in µgGAE/100 mL of dry extract
D: dilution factor
m: mass of the sample (mg)

Total flavonoids

Total flavonoids content was determined using the method of $AlCl_3$ with few modifications.[8] To 1 mL of $AlCl_3$ solution in ethanol (2%) was added 1 mL of dry extract solution in ethanol (1mg/mL). The mixture was incubated in the dark at room temperature for 30 minutes. A blank was then prepared with ethanol at the same conditions. Absorbances were measured at 415 nm using a GENESYS 10 UV spectrophotometer. Quercetin (0-200 mg/L) was used as the reference. All tests were performed in triplicate and the results were expressed in mg of Gallic Acid Equivalent (QE) per 100 g of dry extract according to the following formula:

C = (Cl x D x 10/m) x 100

C: concentration of the sample in µgQE/100 mg of dry extract
Cl: concentration of the sample in µgQE/ mL of dry extract
D: dilution factor
m: mass of the sample (mg)

III. Results

Phytochemical screening of the ethanolic extract of *Harungana madagascariensis* trunk bark from Gabon showed the presence of **alkaloids**, **tannins**, **flavonoids**, **reduicing compounds**, **polyphenols**, **saponines** and **terpenoids** (Table no 1). However, **coumarins** and **sterols** are not present.

Table no 1: Results of phytochemical analyses of ethanolic extract of *Harungana madagacariensis* trunk bark.

Compounds	Result
Alkaloids	++
Tannins	++
Coumarins	-
Flavonoids	++
Reducing compounds	+
Polyphenols	++
Saponins	+
Sterols	-
Terpenoids	+

(++) abundance; (+) presence; (-) absence

The Table no 2 shows the results of total polyphenols and total flavonoids content of *Harungana madagascariensis* trunk bark. Total polyphenols content (584.07±6.89 mg GAE/100g of dry matter) was determined using the regression equation Y= 0.013X + 0.073 with R^2 = 0.9986. Total flavonoids content (146±6.76mg QE/100g of dry matter) was determined using the regression equation Y= 0.0258X + 0.043 with R^2 = 0.9973. Figure 1 and Figure 2 show the graph of standard solution for polyphenols and flavonoids content respectively.

Table no 2: Results of total polyphenols and total flavonoids content of ethanolic extract of trunk bark of *Harungana madagascariensis*

	Total polyphenols (mg GAE/100g)	**Total flavonoids (mg QE/100g)**
Results	**584±6.89**	**146±6.76**

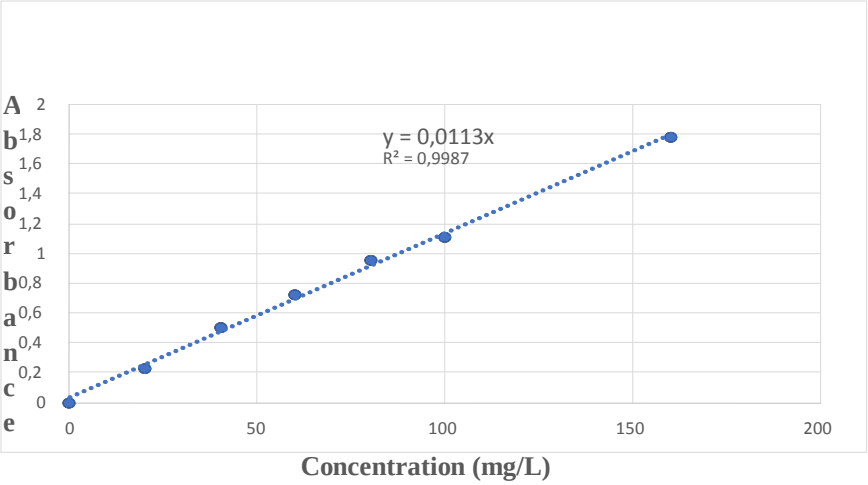

Figure no 1: Standard curve of different concentration of gallic acid.

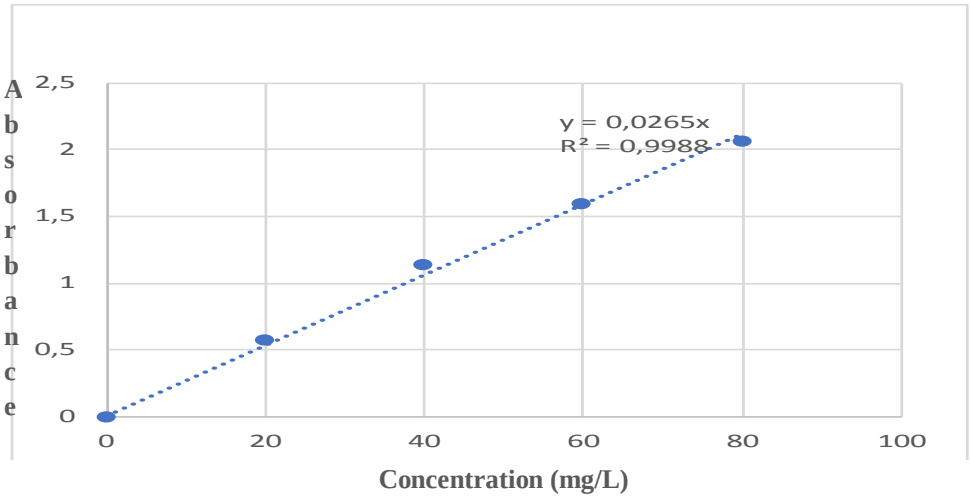

Figure no 2: Standard curve of different concentration of quercetin.

IV. Discussion

For the same groups of compounds, the results are identical as those found in Cameroon.[9] Indeed, the two studies present abundance of polyphenols, alkaloids, flavonoids and tannins. Many phytochemical constituents found in the ethanolic extract of *Harungana madagascariensis* could explain its uses in the traditional medicine.[2-4] These uses can be confirmed by many studies. Indeed, *Harungana madagascariensis* has anti-bacterial, antidiabetic, antiinflamatory, analgesic, antioxidant, antityphoid and antihypertensive activities.[5,10,11,12]The antimicrobial activity can be explained by the presence of alkaloids, polyphenols, tannins and flavonoids.[13] The presence of alkaloids, tannins, polypenols and reducing compounds could justify the antidiabetic property of *Harungana madagascariensis*.[1,13] The antiinflamatory activity could be attributed to the presence of flavonoids, tannins and saponins.[12,13] The antioxidant activity could be related to the presence of tannins, polyphenols and flavonoids.[1,10,13] Alkaloids, tannins, flavonoids and polyphenols could justify the utilization of *Harungana madagascariensis* against diarrhea, hypertension, malaria and cancers.[14]

Quantitative analysis of total phenols and total flavonoids gave 584.07±6.89 mg GAE/100g of dry matter and 146±6.76 mg QE/100g of dry matter respectively. In the literature, the methanolic extract of *Harungana madagascariensis* steam bark from Nigeria revealed 132.24±0.61 mg GAE/g of total polyphenols and 259.05±2.85 mgQE/g of total flavonoids.[15] Comparison between the two studies shows that *Harungana madagascariensis* from Gabon is very rich in phenolic compounds.

V. Conclusion

Qualitative and quantitative studies of the ethanolic extract of *Harungana madagascariensis* trunk bark from Gabon were released. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, polyphenols, saponins, terpenopids, and reducing compounds. However, coumarins and sterols are absent. Total polyphenols (584.07±6.89 mg GAE/100g of dry matter) and total flavonoids (146±6.76 mg QE/100g of dry matter) content shows that this extract is very rich in phenolic compounds. The results obtained could justify the utilization of *Harungana madagascariensis* in the traditional medicine.

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