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CHEMICAL SCREENING AND QUANTITATIVE DETERMINATION OF THE TOTAL POLYPHENOLS, TOTAL FLAVONOIDS AND ANTIOXIDANTS OF EXTRACTS AND FRACTIONS OF TWO CENTRAL AFRICAN MEDICINAL PLANTS WITH ANTIDIABETIC PROPERTIES: CASE OF PAULLINIA PINNATA L. AND OCIMUM GRATISSIMUM L.

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SUMMARY

Diabetes is a real public health problem in the world. It results in an abnormally high sugar level, measured in the blood several months apart. It is a chronic metabolic disease that occurs when the pancreas does not secrete insulin: insulin-dependent diabetes (type I) that usually affects the young person before age 30 or when the pancreas does not produce enough insulin and the secretion of it is deficient; form of diabetes found in adults and obese: non-insulin-dependent diabetes (type II). Indeed, faced with the dissatisfaction of modern remedies, the traditional phytotherapeutic approaches seem to reinforce an interesting potential, whose process of development, from the plant to the phytomedicine, through adequate scientific processes, could offer a credible alternative, for the communities.

KEYWORDS: Chemical screening, total polyphenols, total flavonoids, medicinal plants, antidiabetic agents.

1- INTRODUCTION

Today, endemic diseases such as onchocerciasis, hepatitis, malaria, diabetes and AIDS are among the scourges against which Third World countries in general and African countries in particular have to contend with. The consequences that result are, amongothers: the cost of certain drugs that are not accessible to the majority of populations often far from health centers; the manifestation, for socio-cultural reasons, of a certain mistrust of the living people, especially in rural areas with regard to modern medicine, preferring to turn to traditional healers who do not very often master notions like the dosage of remedies to administer to their patients.

The corollary of all this is increasing morbidity and mortality which slow down the development of the countries concerned and thereby increase the poverty of their populations.

To solve these specific public health problems in the Central African Republic, in particular, one of the ways seems to us to be the use and the valorization of the medicinal plants which our forests abound in abundance and which have already proven their effectiveness.

As part of our research project to obtain the Ph.D. Ph.D. and to make our modest contribution to solving public health problems, we have focused our attention on one of these scourges, diabetes, which has been recognized by the World Health Organization (WHO) as an urgent national and international priority.[1,2]

Indeed, the prevalence rate of diabetes in the world, according to forecasts made by experts[3], was 4% or 135 million people in 1995, this rate would reach 5.4% or 300 million people in 2025 The strongest progressions of this insidious and creeping disease will be observed in developing countries: there will indeed be an increase of 17%, ie an increase of 84 to 228 million patients between 1995 and 2025. During the same period During the Central African Republic, the increase in the prevalence rate of diabetes will be among the highest, rising from 72,000 to 210000 diabetics.[4]

In the Central African Republic, a good number of diabetics are currently between 40 and 65 years old[5], which has the effect of calling into question our development policy, since this category of patients are workers who play an important role. in the building of the nation. They will rather, during the many years they are expected to produce, face chronic complications of their disease, which will involve the use of resources for

their constant medical monitoring, very often expensive for the state and their families.[6,7] Finally, it should be emphasized that our objective is to provide African populations with Traditional Enhanced Medicines (MTAs) based on paullinia pinnata L. and ocimum gratissimum L. in order to solve the crucial problem of medicines, and gradually create the necessary conditions. the establishment of the future African pharmaceutical industry.

II- MATERIALS AND METHODS

- Plant material

In general, two (2) medicinal species have been identified and inventoried in Bangui and its.

Scientific name (Family)	Organs used	Vernacular names (Issongo)	Medical use
Paullinia pinnata L. (Sapindaceae)	leaves	Gagambolo	Leaves mixed with slightly toasted sesame are eaten by the patient three times a day
Ocimum gratissimum L. (Lamiaceae)	Leaves	Ngbanda	Diabetic disease will consume macerated leaves as a drink

The plant material consists of the leaves harvested in the region of Bangui and its surroundings. These plants are also used traditionally for the treatment of several diseases. Their botanical identification was carried out at the Faculty of Sciences in the Department of Matter Sciences of the University of Bangui. The samples are dried at the ordinary temperature of the laboratory away from the sun and then crushed and extracted.

II- Methods

The following methodology is adopted:

• Ethnobotanical survey:

- the identification of traditional healers;
- The collection of botanical samples of plants designated by traditional healers by study team;
- Documentary research on the pharmacological properties of these harvested plants and their different uses in traditional medicine in the African pharmacopoeia (Congolese, Cameroonian, Senegalese and Central African available to us).

• Phytochemical study

Several solvents are used for the extraction of plant material. The different parts of the plant substance will be chosen from the therapeutic uses of traditional healers according to the solvent used. The extraction was done cold (maceration) or hot using an ultrasonic. Subsequently, these different extractions are performed at the Chemistry laboratory; a chemical screen was carried out on the crude extracts of the various samples in order to highlight the family of alkaloids, triterpenes, flavonoids, sterols, tannins, saponosides, etc., according to the method described by Abayomi (1996).[8] Finally, the total polyphenols, total flavonoids and antioxidant activities were measured on the extracts and fractions of these two plants.

III- RESULTS AND DISCUSSION

- Identification by reaction in tubes

The results of the chemical screening carried out on the leaves of Paullinia pinnata L and Ocimum gratissimum L. are summarized in Table II below.

Chemical families / Plant (Organ)	Saponosides	Flavonoïdes	Alcaloïdes	Stéroïdes et terpénoïdes	Tanins	Leucoanthocyanes	Quinones libres	Phlobatanins	Acides Aminés	Mucilages	Oses et Holosides	Composés réducteurs	Anthraquinones	Heterosides cardiotoniques	Tétrahydrocannabiols	Coumarines	Caroténoïdes
Paullinia pinnata Linn. (L)	++ (150)	++	++	-	++	-	-	-	t	-	-	-	-	t	-	++	-
Ocimum gratissimum Linn. (L)	++ (200)	++	++	++	++	++	+++	++	-	-	t	-	-	t	-	++	-

Leaves (L) : barks; (): foam index; +++: abundant; ++: medium; +: weak; t: traces; -: absent

-Determination of total polyphenols

(H3PW12O40) and phosphomolybdenum (H3PMo12O40) yellow.

The principle of the method is based on the oxidation of phenolic compounds by this reagent, it leads to the formation of a new blue-colored molybdenum-tungsten complex which absorbs at 725 nm, the PPT assay is

carried out by comparison of the Optical Density observed at that obtained by a gallic acid standard of known concentration.

The total phenol compounds are determined in the following manner, 0.1 ml of the plant extract is introduced into a 2 ml Eppendorff tube, the extract is then diluted with 0.9 ml of distilled water, followed by 0.9 ml of water. 1 ml of the Folin-Ciocalteu reagent (1N) is added and immediately afterwards 0.2 ml of a solution of Na2CO3 (20%) is added. The resulting mixture is incubated at room temperature for about 40 minutes in the dark. The absorbance is then measured spectrophotometer at 725 nm against a solution of methanol used as white. Note that a calibration line is previously performed before the analysis with gallic acid under the same conditions as the samples to be analyzed. The results obtained are expressed in mg gallic acid equivalent per gram of dry matter (E GA / g Ms).

- Calibration curve for the determination of total polyphenols (PPT)

This curve is established using gallic acid as a reference and the results are expressed in mg gallic acid equivalent per gram of dry matter (mgEGa / gMs). The calibration curve is established with a correlation coefficient R2 = 0.987 (FIG. 1).

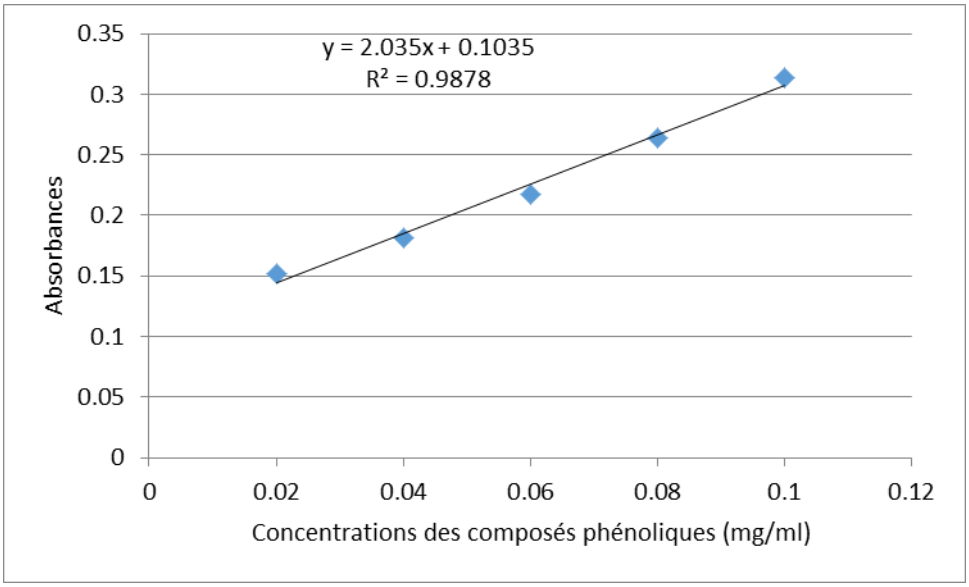


Figure 1: Calibration curve for the determination of total polyphenols.

III.1.2.2. Determination of total flavonoids

The reagents used are: colorless solutions of sodium nitrite (NaNO 2, 5%) and aluminum chloride (AlCl 3, 10%). The principle of the oxidation of flavonoids by these reagents, it causes the formation of a brownish complex which absorbs at 510 nm. The comparison of the observed OD with that obtained by a Rutin standard of known concentration makes it possible to evaluate the total content of flavonoids.

The total flavonoids are evaluated by colorimetry, in a 10 ml flask are successively introduced 250.mu.l of the extract and 1 ml of distilled water. At the initial time (0 minutes) are added 75 µl of a solution of NaNO 2 (5%), after 5 minutes 75 µl of AlCl 3 (10%) are added. And at 6 minutes, 500 µl of NaOH (1N) are added and 2.5 ml of distilled water are added successively to the mixture.

The second method used indicates that the flavonoid contents were measured by a suitable method of[9,10], using aluminum trichloride (AlCl3) as a reagent. The presence of a free space in AlCl3 forms a dative bond with the oxygen free doublets of the flavonoid OH groups, producing a yellow-colored complex, whose maximum absorbance is recorded at 430 nm.

A calibration curve (Figure 2) is developed with standard rutin solutions prepared at different concentrations. The absorbance of the mixture obtained is directly measured with the UV-visible spectrophotometer at 510 nm and the results are expressed in milligram equivalent rutin / gram of dry matter (mgERt / g Ms).

Another calibration curve (Figure 3) is developed with standard catechin solutions prepared at different concentrations. The absorbance of the mixture obtained is directly measured with a UV-visible spectrophotometer at 430 nm and the results are expressed in milligram equivalent catechin / gram of dry matter (mgECt / g Ms).

- Calibration curve for total flavonoid assay (FVT)

The reference compound used to establish this curve is rutin. The curve is established with a correlation coefficient R2 = 0.993 (FIG. 2). The results obtained are expressed in milligram equivalent Rutin per gram of dry matter (mgERt / g Ms).method is based on the.

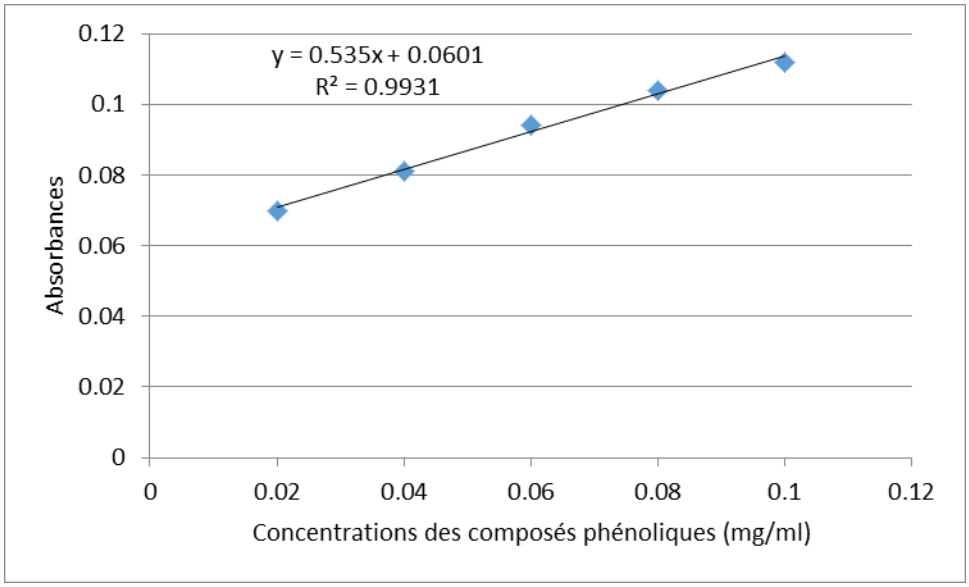


Figure 2: Calibration curve for total flavonoid assay (510 nm).

Another reference compound used for establishing another curve is catechin. The curve is established with a correlation coefficient R2 = 0.999 (FIG. 3). The results obtained are expressed in milligram equivalent Catechin per gram of dry matter (mgECt / g Ms).

3: Calibration curve for the total flavonoid assay (430 nm)

- Analysis of extracts

• Leaves extracts of paullinia pinnata L.

¬ The results of the UV-visible spectrophotometer quantitative analyzes of the alcoholic and hydroalcoholic extracts studied are shown below (Figure 4). In this composition we find that the alcoholic and hydroethanolic extracts of the leaves of paullinia pinnata Linn. are quantitatively richer in total flavonoids compared to total polyphenol contents.

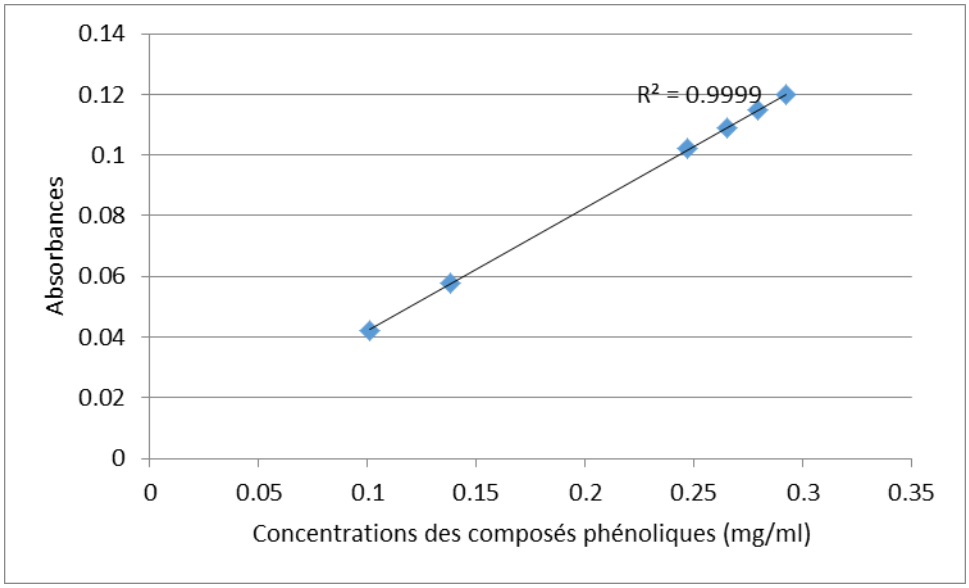


Fig 3

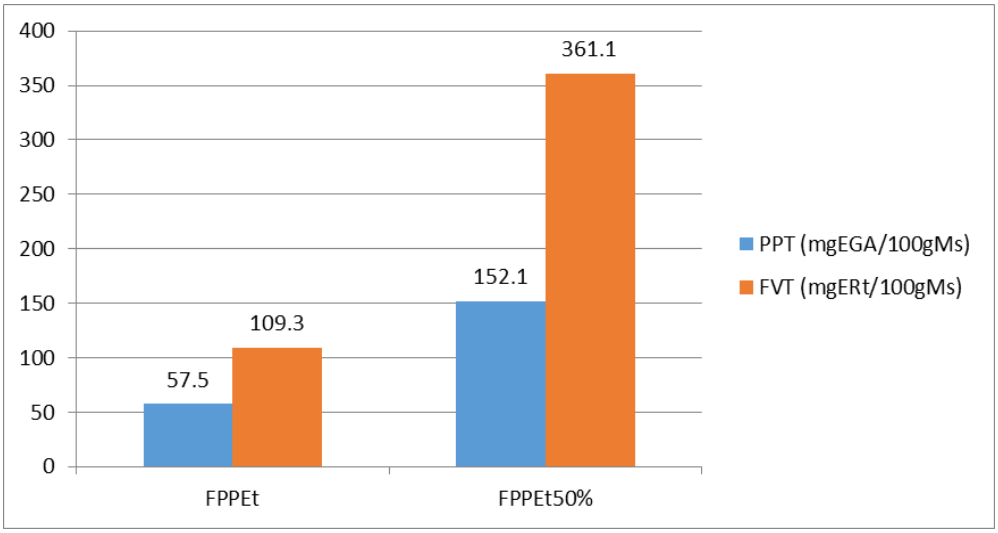


Figure 4: Phenolic composition of leaf extracts of paullinia pinnata.

FPPEt: ethanolic extracts of leaves of paullinia pinnata; FPPEt50%: Hydroethanolic extracts of leaves of paullinia pinnata; PPT: total polyphenols; FVT: total flavonoids.

FIG. 5 shows the results of the composition of total flavonoid compounds of the alcoholic and hydroalcoholic extracts of different percentages of Paulina pinnata Linn analyzed by UV-visible spectroscopy. In analyzing these results, we note that:
80% alcoholic and hydroalcoholic extracts prepared at US70°C. are rich in flavonoids with respect to the other extracts;
Subsequently, the 70% and 60% hydroalcoholic extracts have a greater activity in flavonoids than in the extracts obtained by maceration;
- Thus, the 50% hydroethanol extract is richer in flavonoids in that obtained by reflux;
Finally, in the 40% hydroethanolic extract, it is the extract obtained at US70°C. which contains more flavonoids compared to the others.
Finally: the extract ETOH 70% obtained by maceration = the extract ETOH50% obtained by reflux> extract ETOH80% obtained by US70°C> extract ETOH70% obtained by US30°C.

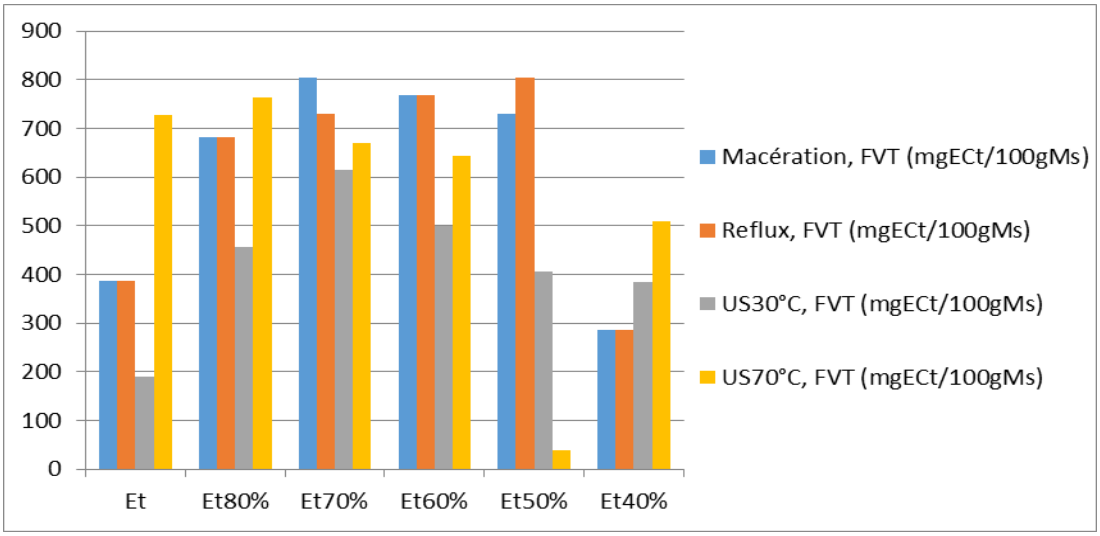
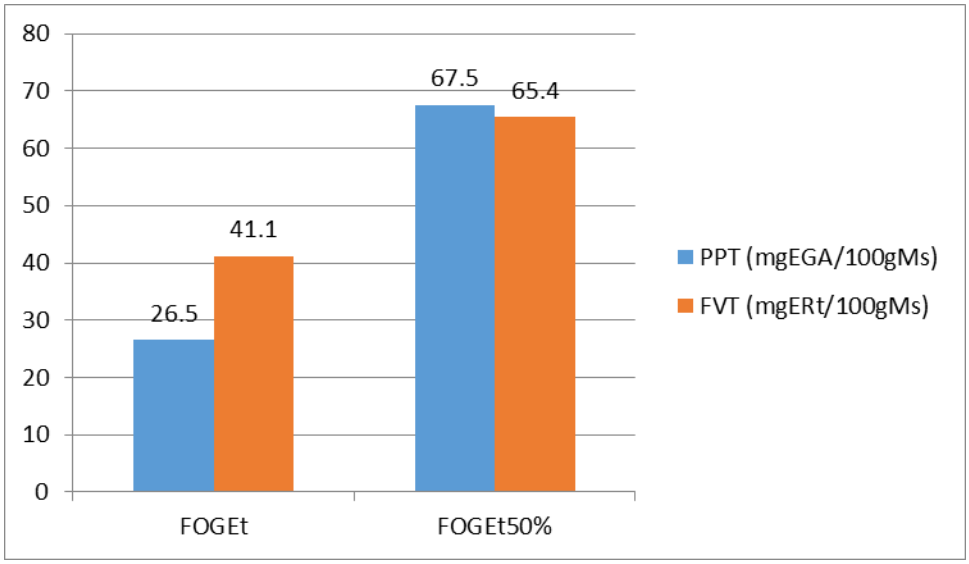


Figure 5: Flavonoid composition of extracts of leaves of paullinia pinnata.

US30°C: Ultrasound at 30°C; US70°C: Ultrasound at 70°C; And: pure ethanol; Et80%: 80% Hydroethanol; Et70%: 70% Hydroethanolic; And 60%: 70% ethanol; And 60%: Hydroethanol 60%; Et50%: 50% Hydroethanol and 40% Ethanol: 40% Hydroethanol.

• Extracts from the leaves of ocimum gratissimum L.
The results of the quantitative analyzes in phenolic compounds of the extracts of ocimum gratissimum leaves are reported in FIG. 6. These results indicate that the hydroethanolic extracts of the ocimum gratissimum leaves studied mainly consist of flavonoids and total polyphenols. Its composition in ethanolic extracts is low (PPTEt50%> FVTEt50%> FVTEt> PPTEt).



IV- CONCLUSION

This work constitutes our modest contribution to the valorization of the traditional Central African and African Pharmacopoeia. It allowed to have a better knowledge of the plants used in the Central African Republic.

Two (02) plants were chosen for the experimental study: Paullinia pinnata Linn. and Ocimum gratissimum Linn.

These results allow us to know the chemical profile of the various plant extracts selected by tube reactions.

The place to work of chromatography techniques.

The quantitative analysis allows us to know the content of total polyphenols, total flavonoids and total alkaloids in the various extracts of selected plants.

The set of analytical results and biological tests have confirmed the traditional use of both plants in the treatment of diabetes.

Nevertheless, in the use of traditional healers, it is nice to note that the use of these plants is generally done in association with other plant species.

This is the essential treatment of the Chemical and Chemical Analysis. Plant species that contain these bioactive substances can be formulated galenically.

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