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Correlation Between The Antioxidant Activity And The Total Polyphenol Content Of The Solvent Extracts Of Rhizomes Of Curcuma Mangga Valeton And Zigp From The Congo Cataracts Plateau.

By

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Abstract
Curcuma mangga Val is a zingiberaceae whose leaves are used as aromas and rhizomes to relieve various ailments. This use suggests an impact on the biological action of the different organs of this plant. The samples collected at four different sites are dried and then ground. The determination of total polyphenols is carried out with the Folin-Ciocalteu reagent and the antioxidant activity with the reducing agent DPPH. The amount of total polyphenols is greater by methanol compared to the two other solvents used: ethyl acetate and chloroform. Methanol extracts have good antioxidant activity with an IC50 of 415.18 µg / ml compared to vitamin C of 296.42 µg / ml. There is a positive correlation between the content of total polyphenols and the anti-radical activity.

Keywords: Correlation, antioxidant activity, polyphenol and Curcuma mangga.

Introduction

Free radicals are the basis of many diseases in the human body. Antioxidant activity defines the ability of a chemical compound to protect an organism from free radicals. Most of the antioxidants used are of synthetic origin, which leads man to seek, in his natural environment, a means of protection without drawbacks. In many foods drawn from the Congolese flora, there are a large number of phenolic molecules which can be used as alternative sources of synthetic compounds in the fight against several cardiovascular and inflammatory diseases but also against Cancer. Among these plants rich in phenolic compounds we have a range of pharmacological plants recognized in the Republic of Congo, including plants of the zingiberaceae family. The zingiberaceae

family includes a large number of species, most of which are used as spices in food. In this family, we have the genus Turmeric which counts between (40) to (110) species, originally answered in tropical regions:

Asia, Africa and Northern Australia; seasonal precipitation.

Curcuma mangga Valeton and Zigp is an embryo, spermaphytes, phylum, angiosperms, Class, monocotsmonoaperturées and monocotylées; of the Subclass: advanced or commelinideae, of the Order of zingiberal, of the Tribe of zingibéracéae; the Tribe: hedychieaes, of the Genus Curcuma. Some work carried out on the rhizomes of Curcuma manga Val. We have revealed a pharmacological activity of this plant on cancer cells [1]. Similarly, plants such as Curcuma mangga; Curcuma amada, Curcuma caesu, Curcuma langa, Curcuma purpurasus, Curcuma xanthorrhiza and Curcuma zodouria, inhibit the proliferation of cancer cells. In this work, we will limit ourselves to the study of the content of total polyphenols in the solvent extracts of the rhizomes of Curcuma mangga Val. From Congo and to the evaluation of the antioxidant activity in order to establish a correlation.

Materials and methods

2.1. Material: Plant material

The rhizomes of Curcuma mangga Val were harvested in the Republic of Congo, in four (04) different localities, notably Brazzaville, Loukoko, Mindouli and Loulombo (figure 1). They are dried out of the sun, under laboratory conditions, for one week . These rhizomes were ground using a ProBlend 6 type mixer, then the powder obtained was used for various tests.

2.2. Methods

2.2.1. Preparation of extracts

50 g of powder of Rhizomes of Curcuma manga Val are immersed in 500 ml of methanol and left under stirring for 72 hours. The maceration obtained is filtered, then the filtrate is evaporated or concentrated using a rotary pyre-type evaporator.

Figure 1: *location map of sample collection sites*

2.2.2. Total polyphenol content

We weigh 0.1g of the dry extract of Rhizomes from the powder of Curcuma mangga val. diluted in 100 mL of distilled water. A standard range is produced in an aqueous medium with 20 points of concentration from 0 to 34 µg / ml with a reference polyphenol which is gallic acid. To carry out the assay, 1500 µl of Folin-Ciocalteu reagent at 2M, diluted 10 times in distilled water, or add 300µL of diluted extract to the point of range. Then 1200 µL of sodium decarbonate (7.5 g / L) is added. The reaction mixtures are left to incubate for 1 hour after being homogenized. The reaction blank does not contain the extract, it is considered to be the 0µg / mL point of

the range. The abundances are read at 735nm using a UV-Visible spectrophotometer. A calibration curve is plotted against each concentration for the points in the range. The average concentration of polyphenols present in the plant extract is determined in µg equivalent of gallic acid / mL. [2 and 3].

2.2.3. Anti radical activity

The method used is the trapping of the stable free radical DPPH. The purple DPPH solution has a maximum absorption at 517nm. The antioxidant power of the methanol extract is evaluated by comparison with a reference antioxidant, vitamin C (ascorbic acid). However, three measurements are made respectively at the extract concentrations used. For this, the solutions of the extracts are prepared in a following concentration range 400, 600, 800, 1000, 1200, 1400 µg / ml of methanol. A stock solution of DPPH of 0.004% concentration is prepared. The absorption is read for each concentration for 60 min at an interval of 5 min. The antioxidant activity (AA%) is calculated according to the following formula:

$$AA\% = \frac{(\text{Control absorbance}) - (\text{sample absorbance})}{\text{control absorbance}} \times 100$$

Results and discussions

3.1. Gallic acid calibration curve

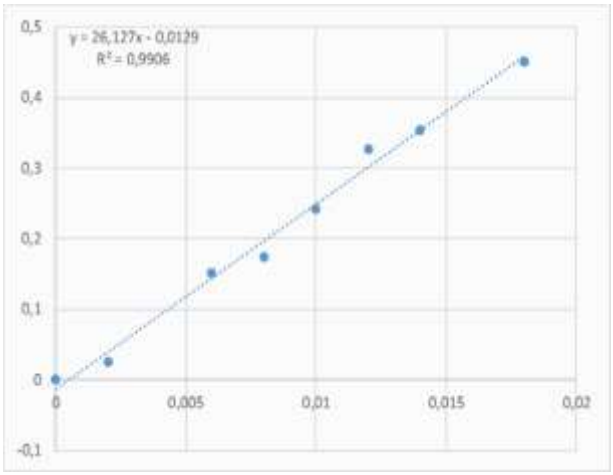


Figure 2: *Gallic acid calibration curve.*

We measured the total polyphenol content of the extracts. Estimated in mg equivalent of gallic acid per gram of dry matter. It is obtained by the calibration curve established with the concentrations. The calibration curve which is a linear straight line of the form Y = aX + b makes it possible to determine the quantity of total polyphenols. The polyphenol content is unknown from our relationship, which is determined by reference to folin-ciocalteu The content of total polyphenols differs from plant to plant, the nature of the extract and the part of the plant. Three types of extract were dosed: extract with methanols, ethyl acetate and chloroform. Each measurement is performed three times. Figure 2: From the curve, we see that the regression coefficient R^2 = 0.9906 close to 1. We can therefore use it for the determination of the total polyphenol content of our extracts

3.2. Amount of total polyphenols

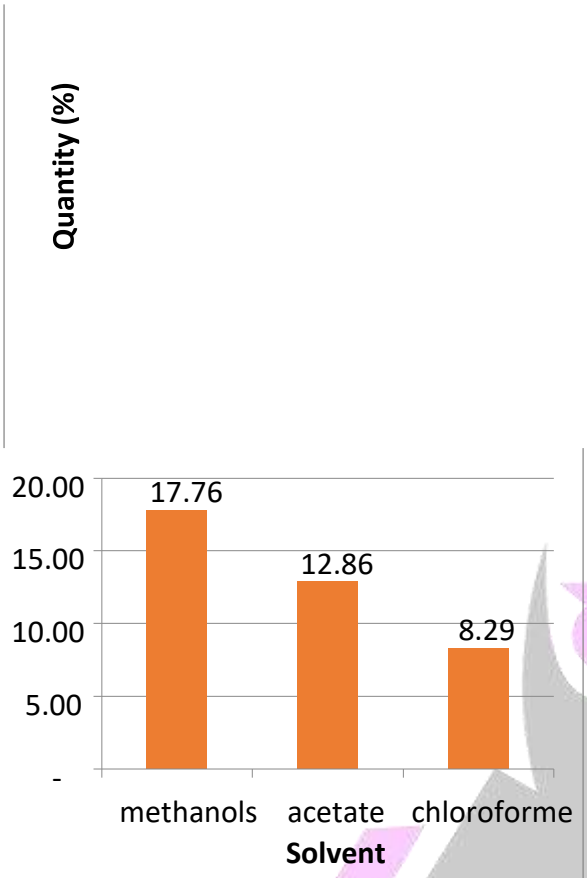


Figure 3: *quantity of total polyphenols by extract.*

The methanol extracts have a high content which is much higher than the others. The amount in the chloroform extract is just a few traces. It is twelve times larger than that of ethyl acetate. However, the content of the ethyl acetate extract is about three times that of the chloroform extract (Figure 3). The polyphenol content of the methanol extracts is 46.66 ±

3.37 mg equivalent of gallic acid / gram of dry matter, that of the ethyl acetate extracts is 4.01 ± 0.97 mg equivalent of gallic acid / gram of dry matter and that of the chloroform extract represents practically the traces 1.86 ± 0.21 mg equivalent of gallic acid / gram of dry matter. In fact, the difference in polarities between the solvents allows better extraction of the polyphenols [4]. A significant variation in the contents of total polyphenols has been shown depending on the extraction solvent. For this, methanol gives extracts more concentrated in total polyphenols in the rhizomes of Curcuma mangga Val. (Figure 3).

3.3 Kinetic curves of solvent extracts

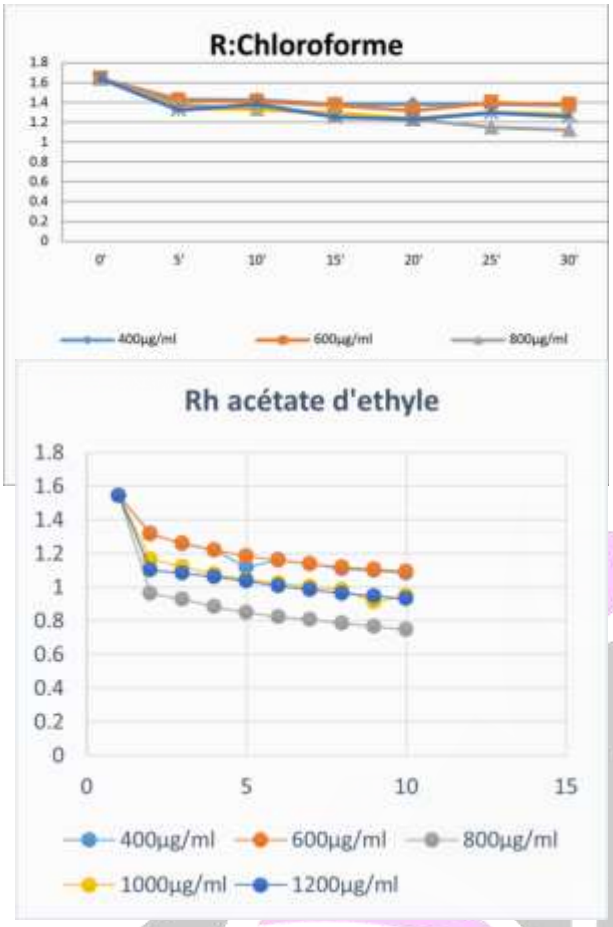


Figure 4*: kinetic curves of the reduction of DPPH.*

The chloroform, ethyl acetate and methanol extracts show kinetic curves which give the amount of DPPH reduction. The chloroform extract gives a kinetics little differentiated by the curves. The curves have three parts. The first is from zero to two minutes. This part is characterized by a partial decreasing. The second is from two (2) to six (6) minutes, it decreases sharply and the last one, which is characterized by a stage until the end. Which shows the end of the reaction. For each concentration, the kinetics do not make a big difference. However, at the highest concentrations after 800 µg / ml, we have great kinetics. At concentrations below 800 µg / ml, all the curves give a level of 2 at the end. The corresponds to an activity. (Figure 4). The kinetic curves of methanol are in practically the same states as that of vitamin C. (Figure 5)

3.4. Inhibition of extracts with solvents.

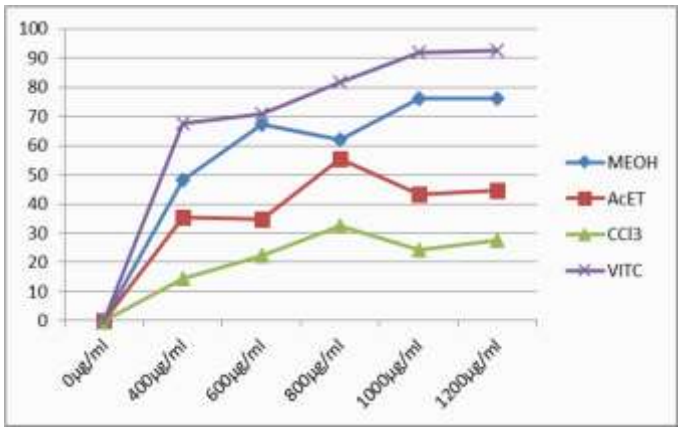


Figure 5*inhibition curves.*

The inhibition curves of the extracts give the appearanc according to their activities. For this purpose, the i is compared with vitamin C which is the reference that the curve of the methanolic extract is compar of ascorbic acid. This is followed by the curve of the acetate extracts. The results show that the antioxidant activity of the extracts varies with the extraction so comparing the inhibition of each extract, we find that the inhibition of the methanol and ethyl acetate extracts is clearly above the inhibition of the chloroform extract. activity is linked to the family of total polyphenols

nature of these kinetics shows the absence of activity. The curves are confusing, The ethyl acetate extract, the kinetic curve shows two parts. It decreases from zero to two (2) minutes. Two (2) minutes, at the end of the reaction, we have a plateau. The extract activity with this solvent is proportional to the concentration. It is also a function of time. (Figure 4).

Comparing the benchmark kinetics of vitamin C to that of ethyl acetate extract, there is a small activity but less important compared to ascorbic acid. But the kinetic curves show a small activity by the presence of two phases: The active phase and the end of reaction phase. Like other kinetics, that of the methanol extract has two parts. The first is that which decreases from zero to five (5) minutes. During this

period, there is a reaction in the environment. The second gives a five-minute timeframe until the end. Which shows the end of the reaction. The activity of this extract is proportional to the concentration. Each concentration

the extract. The inhibition percentages are for the three extracts respectively 76.12%; 55.43%; 32.57% and 91.80% for vitamin c.

3.5. IC50 diagram.

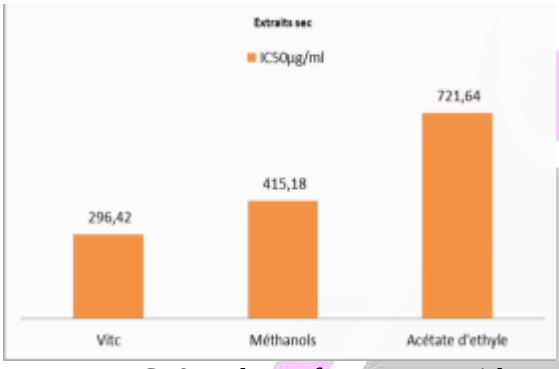
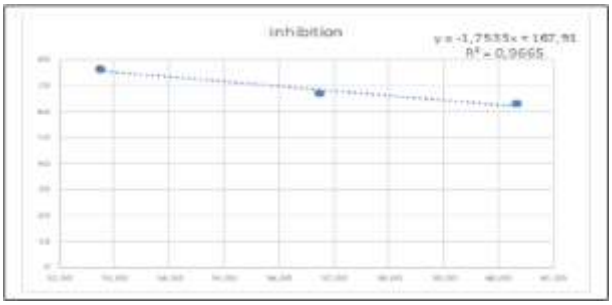


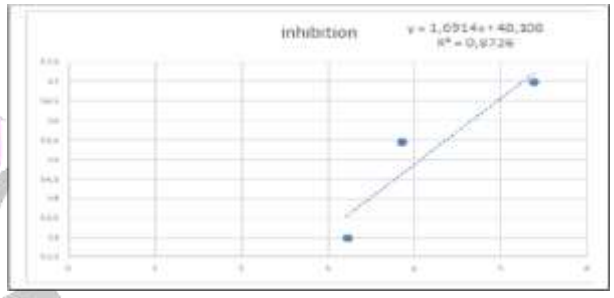
***Figure 6** : IC50 value of extracts with solvents*

The methanol extract has an IC50 of 415.178 µg / ml and the ethyl acetate extract has an IC50 of 721.64 µg / ml and the ascorbic acid has an IC50 of 296.42 µg / ml. The work of Wahyu Widowati et al. [5 and 6] shows that the extract of Turmeric mangga Val. had an IC50 of 277.79 µg / ml. This activity was more important than that of ascorbic acid (296.42 µg / ml). However, the chloroform extract gives no IC50 value. These results show that ascorbic acid remains the most effective antioxidant than those of the methanol extracts of the rhizomes of Curcuma mangga Val., Followed by those of the ethyl acetate extracts. (Figure 6). This parameter is defined as the antioxidant concentration required to decrease the initial concentration by 50 %, it is inversely related to the antioxidant capacity [7 and 8].

3.6. Correlation between antioxidant activity and total polyphenol content of the extracts.



(a): Methanol extract



(b): Ethyl acetate extract

***Figure 7:** correlation between the level of total polyphenols and antioxidant activity.*

A very important positive correlation is observed between the antioxidant activity and the total polyphenol content of the methanol extracts. This correlation is shown by the correlation coefficient between the polyphenol content in the extract and the percentage of inhibition. The correlation coefficient is R^2 = 0.97 (Figure a). This correlation shows that the trapping of the free radical is due to the presence of total polyphenols in large quantities in these extracts. For ethyl acetate extracts, the correlation coefficient is R^2 = 0.87, the content of total polyphenols and the antioxidant power are less effective. This correlation shows that the amount of total polyphenols is less than in the methanol extract. These results show that, the methanol and ethyl acetate extracts of the Curcuma manga Valeton and Zigp

rhizomes contain a good amount of total polyphenols, which allows these extracts to have significant antioxidant activity. Polyphenols are recognized as antioxidants. The weak antioxidant activity found in the chloroform extract shows that the amount of total polyphenols is low. The best solvent for extracting total polyphenols from the Curcuma manga Valeton and Zigp rhizomes is methanol, followed by ethyl acetate. The correlation coefficients found in the three cases show the correlation that exists between the amount of total polyphenols and the activity [9].

Conclusion.

These results give a trend in the total polyphenol content of the extracts in different extraction solvents. The two extracts, ethyl acetate and methanol, contain polyphenols respectively 4.01 mgeq of gallic acid per gram of dry matter and 46.66 mg eq of gallic acid per gram of dry matter. Methanol is the best solvent for the determination of polyphenols. The methanol extract gives good antioxidant activity. The IC50 value is 415.17 µg / ml and that of ethyl acetate extract is 731.64 µg / ml. The antioxidant activity is improved in the methanol extracts. There is a positive correlation between the quantity of polyphenols and the antioxidant activity of the extracts. The correlation coefficient is R^2 = 0.966 around 1 for the methanol extract and R^2 = 0.872 for the ethyl acetate extract.

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