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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING, TOTAL POLYPHENOLS AND FLAVONOIDS CONTENT AND ANTIRADICAL ACTIVITY OF METHANOLIC EXTRACT OF *ANNICKIA CHLORANTHA* FROM GABON

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Abstract

In traditional medicine, *Annickia chlorantha* (Annonaceae) is used in many parts of Africa to treat several pathologies. It is used for the management of stomach problems, for the treatment of jaundice, tuberculosis, urinary tract infections, malaria, hepatitis and ulcers forms, for gallstones or even diarrhea. The phytochemical analysis and determination of the antiradical activity of the methanolic extract of the trunk bark from *Annickia chlorantha* from Gabon was the subject of this study. Phytochemical screening revealed the abundance of alkaloids, polyphenols and catechic tannins. The total polyphenol content released by the Folin-Ciocalteu method was 309.17 ± 38.05 g of gallic acid equivalent per 100 mg of dry matter and the total flavonoids content performed with $AlCl_3$ was 155.16 ± 21.06 g of quercetin equivalent per 100 mg of dry matter. The antiradical activity was limited to $81.58\% \pm 1.25$ without disappearance of the $ABTS^{+}$ radical after 6 minutes of incubation. These results confirm the correlation between the polyphenol content and the antiradical activity.

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Introduction:-

In tropical Africa, difficult access to modern medicine, associated with the high cost of drugs, leads nearly 70% of the population to rely heavily on plants for treatment (NgonoNgane et al., 2011). In 2007, Newman and Cragg demonstrated that two thirds of current drugs have a natural origin (Newman and Cragg, 2007). This means that 60% of current drugs are made from herbal remedies. Indeed, medicinal plants represent an immense source of phenolic compounds (phenolic acids, flavonoids, tannins) and others (alkaloids, coumarins...). Isolation of chemical species from medicinal plants can be a prolific resource for new drugs (Karmakar et al., 2011). In order to help and protect populations, it is necessary to study the properties of commonly used medicinal plants. It is with this in mind that our work has focused on the chemical screening and evaluation of the antiradical activity of the methanolic extract of *Annickia chlorantha* (Annonaceae). This plant is used for its therapeutic properties, in particular in the treatment of malaria, fever growth, typhoid fever and hepatic infections as some forms of ulcers

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(Olamide and Mathew, 2013; Olivier et al., 2015). However, despite the use of this plant in traditional medicine, no study has been carried out on the Gabonese plant.

Material Et Methods:-

Plant material:

The trunk bark of *Annickia chlorantha* was collected in august 2018 between Oyane 4 and Four Place in Estuaire Provincial. The fresh trunk bark was dried for about fifteen days protected from light in an air-conditioned room. It was powdered using an electric grinder (Flour Mills Nigeria, El MOTOR N°1827). The resulting powder was stored at room temperature in a clear labeled packaging bag. The plant has been identified in the National Herbarium of Gabon.

Preparation of methanolic extract:

200 ml of methanol were added to 10 g of powder contained in an Erlenmeyer flask with a magnetic bar. After hermetically closing the Erlenmeyer flask, the mixture is left under magnetic stirring for 24 hours and then filtered using Whatman N°1 filter paper. The filtrate was stored at 4°C until analysis.

Qualitative Phytochemical analysis:

Phytochemical screening of dry extracts was achieved through simple methods as described in Ciulei, 1964 and Harborne, 1998.

Determination of Total polyphenols content:

The evaluation of the total polyphenols was carried out by the Folin-Ciocalteu method described by Singleton et al. (1999) and modified by Mefouet Abessolo et al. (2021) on methanolic extract. Gallic acid was used as a standard to establish the standard range (0-150 mg/l). The results are expressed in mg of Gallic Acid Equivalent (QE) per 100 g of dry extract.

Determination of Total flavonoids content:

The total flavonoids are determined according to the method of Arvouet-Grant (1994) with some modifications. Quercetin was used as a standard to establish the standard range (0-50 mg/l). Results are expressed in mg of quercetin equivalent (QE) per 100 g of dry extract.

Evaluation of antiradical activity:

Antiradical activity was determined by the ABTS method (Re et al., 1999). The antiradical activity of methanolic extract is deduced from its ability to inhibit $ABTS^{*+}$ compared to a reference antioxidant (gallic acid). The $ABTS^{*+}$ radical ion is obtained by reacting the ABTS molecule (7 mM) with potassium persulfate (2.45 mM) in distilled water for 16 hours at room temperature and in sunlight. The $ABTS^{*+}$ solution obtained is diluted with sodium phosphate buffer (5 mM, pH = 7.4), in order to obtain a stock solution having an initial absorbance value of between 0.65 and 0.70 at 734 nm (UV spectrophotometer).

The inhibitory effect of ABTS was calculated according to the following formula:

$$\text{Anti-free radical activity (\%)} = [1 - Ar/Ai] \times 100$$

Ar = Absorbance remaining of $ABTS^{*+}$

Ai = Initial Absorbance of $ABTS^{*+}$

Results And Discussion:-

Phytochemical screening:

The phytochemical tests carried out on methanolic extract of trunk bark of *Annickia chlorantha* show the abundance presence of alkaloids, catechics tannins and polyphenols. There's also reducing compounds, flavonoids (flavones), sterols and coumarins (Table 1).

Some of these groups of compounds identified as alkaloids have several pharmacological activities, namely antimalarial (Uzor, 2020; Bribe, 2018), antibacterial (Kakpo et al., 2019), hepatoprotective (Jan et al., 2017). Some countries such as Cameroon have isolated aporphinic alkaloids (Wafo et al., 1999) and protoberberine alkaloids (Nyongbela et al., 2019) from *Annickia chlorantha*. Protoberberine alkaloids (palmatin, jatrorrhizine, columbamine)

isolated from the stem bark have been combined to make a phytodrug (HEPAZOR®) for the treatment of viral hepatitis (Nyongbela et al., 2019).

According tannins catechics, studies reveal antibacterial and antiviral activities with capacities to reduce blood pressure (Bele et al., 2010; Coșarcă et al., 2019). The presence of these important bioactive compounds indicates the medicinal value of *Annickia chlorantha* and could be at the origin of various activities revealed by the population and traditional healers.

Table 1:- Phytochemical composition of methanolic extract of the trunk bark of *Annickia chlorantha*.

phytochemical compounds		Extract
Alkaloids		++
Tannins	Gallic	-
	Catechic	++
Saponosides		-
Reducing compounds		+
Flavonoids	Flavones	+
	Flavanones	-
	Flavonols	-
Sterols et triterpenoides	Sterols	+
	Terpenes	-
Coumarins		+
Polyphenols		++

(++):Abundance; (+):presence; (-): absence

Total Phenolic and Flavonoid contents:

The total phenol content was determined in comparison with a standard which is gallic acid. The Results (Table 2) were expressed in milligrams of gallic acid per 100 grams of dry matter (mg GAE/100 g DM), using the linear equation extract: $Y = 0,011X + 0,0291$ (with $R^2 = 0,9972$). Thus, total phenolic content is $309,18 \pm 38,06$ mg GAE/100 g DM.

The total flavonoid content was determined in comparison with quercetin. Results were expressed in milligram equivalent of quercetin per 100 grams of dry matter (mg QE/100g DM. using the linear equation $Y = 0.0258X + 0.043$ (with $R^2 = 0.9973$). Thus, total flavonoids content is $155,16 \pm 21,06$ mg QE/100 g DM.

Table 2:- Total phenolic and flavonoids contents in methanolic extract of trunk bark of *Annickia chlorantha*.

	Total phenolic content (mg GAE/100 g DM)	Flavonoids content (mg QE/100 g DM)
Extract	$309.18 \pm 38,06$	$155.16 \pm 21,06$

In Nigeria, Olanlokun and Akomolafe (2013) showed that the methanolic extract has a high content of total polyphenols (838.03 ± 34.40 mg GAE/g of extract) and an appreciable content of total flavonoids (140 ± 0.008 mg QE/g of extract). In Cameroon, Djimeli et al. (2017) obtained a total polyphenol content of 690.53 ± 23.597 mg GAE/g of extract. In view of these results, the stem bark of *Annickia chlorantha* from Nigeria and Cameroon are more rich on total polyphenol than the trunk bark of *Annickia chlorantha* from Gabon. However, the trunk bark of the Gabonese plant contains more total flavonoids.

Antiradical activity:

The antiradical activities of gallic acid and methanolic extract from the trunk bark of *Annickia chlorantha* were reported in Figures 1 and 2.

For the gallic acid (Figure 1), the percentage of the antiradical activity increases with the concentration after 6 minutes of incubation. The antiradical activity reaches 100% after 6 minutes of incubation for concentrations greater than or equal to $0.84 \mu\text{g/ml}$.

However, regarding the methanolic extract, the anti-radical activity was limited to $81.58\% \pm 1.25$ without disappearance of the $ABTS^{++}$ radical after 6 minutes of incubation (Figure 2).

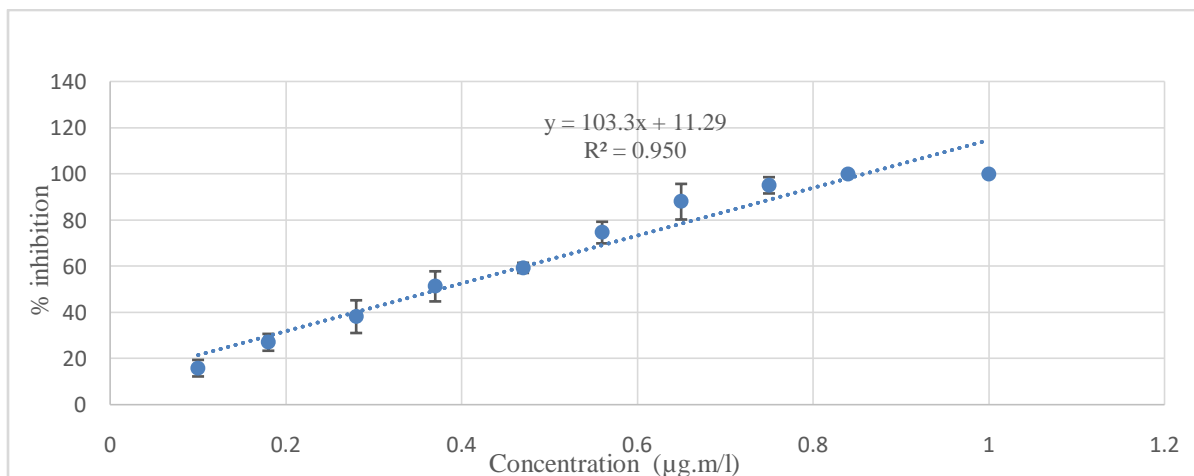


Figure 1:- Percentage of Gallic acid inhibition curve at different concentrations.

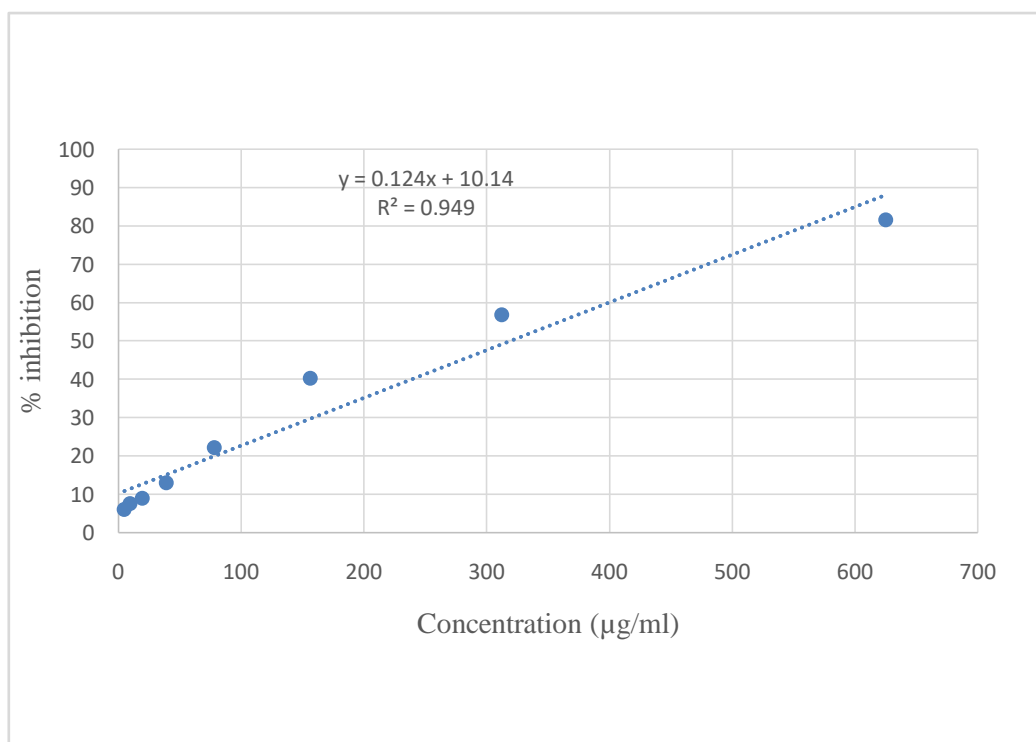


Figure 2:- Percentage of methanolic extract inhibition curve at different concentrations.

According to the absence of disappearance of ABTS, we can deduce that the methanolic extract of the bark of *Annickia chlorantha* has no antioxidant activity or that it's very weak. This result could be explained by the low contents of polyphenols and total flavonoids obtained.

Conclusion:-

This study is the first carried out on *Annickia chlorantha* from Gabon. Through this study, the phytochemical composition of the methanolic extract of the trunk bark revealed the presence of alkaloids, catechins, tannins, reducing compounds, flavonoids (flavones), sterols and coumarins. These compounds can explain the use of this

plant for the treatment of many pathologies. Furthermore, by the ABTS method, this methanolic extract showed weak anti-free radical activity. A result which could be explained by the low content of polyphenols.

Knowlegment:-

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