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Phytochemical study and antioxidant activity of *Prosopis africana* leaf extracts

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ABSTRACT: *Introduction:* The species we have chosen is *Prosopis africana* (Guill. & Perr.) of the Fabaceae family is a medium-sized shrub that can reach about 30 m in height and is widespread in tropical Africa, locally known in the Central African pharmacopoeia. It is a species widely used in traditional medicine for its therapeutic properties.
Objectives: As part of the valorization of medicinal plants used in the Central African pharmacopoeia, the interest of this work is focused on ethnobotanical investigations, chemical screening and antioxidant activity of *Prosopis africana.*
Methods: Four solvents of different polarities were used (cyclohexane, dichloromethane, acetone and methanol) for extraction. Qualitative analyses were based on staining reactions, followed by quantification by the 96-well plate reader to determine polyphenols, tannins, flavonoids and anthocyanins.
Results: The extraction results showed that the methanol extract has the best yield of 14.45%. Qualitative analyzes revealed the presence of tannins, flavonoids, sterols, triterpenes and saponosides. The polyphenol content was better than the other families of compounds quantified (Table 2). It varies between 42,90 and 247.89 mg of gallic acid equivalent (EqAG) /g.
The methanol and acetone extracts have strong antioxidant activity. They have respectively the percentages of inhibition of DPPH the values 98.47±00.70 and 98.55±00.10% (Figure 5).
Principal component analysis (PCA) was performed using the «FactoMineR» and «factoextra» packages of the R software circle of variable correlation.
The best Person (r) correlation coefficient obtained was 0.95 between polyphenols and DPPH inhibition.
Conclusion: The phytochemical study revealed the presence of certain molecules such as polyphenols and alkaloids, which can give *Prosopis africana* (Guill. & Perr.) its therapeutic properties.

KEYWORDS: *Prosopis africana,* extraction, phytochemical and antioxidant activity.

1 INTRODUCTION GÉNÉRALE

For centuries, traditional medicine has been a source of reliable, acceptable and affordable health care for the people of Africa. Until today. According to the WHO (2022), 80% of the continent's population depends on traditional medicine to meet their basic health needs. Medicinal plants occupy an important place in the pharmacopoeia thanks to their richness in secondary metabolisms.

Plants are reservoirs of a wide variety of secondary metabolites: alkaloids, phenolic compounds and terpenoids Zerargui et al [1].

Pharmacological studies have shown that different extracts of Prosopis africana (Guill. & Perr.) have antibacterial and antioxidant properties Yanda et al [2]. Sasquiterpenes (Prosoterpene) flavonoids, alkaloids, phenolic acids were isolated from this plant [3]. Although many studies have been carried out on medicinal flora, very little of the data in the literature on

phytochemical and pharmacological analyses of the Central African Republic are available. Polyoxygenated steroles and triterpenes have selective cytotoxicity and antitumor properties [4].

The interest of this study which concerns the valorization of medicinal plants in order to rationalize their use as phytomedicines or food supplements. This work will consist of phytochemical characterization to determine chemical family groups of pharmacological interest. Then to carry out the biological activities of the extracts obtained.

With regard to the beneficial effects of polyphenols and their derivatives, alkaloids and other natural compounds, our research is oriented towards the class of chemical molecules endowed with biological activities. *Prosopis africana* (*Guill. & Perr*.) of the *Fabaceae* family, with the vernacular name Ngbangre (in Banda), was selected from among the medicinal plants used in the Central African pharmacopoeia.

2 MATERIAL AND METHODS

2.1 ETHNOBOTANICAL STUDY AREA AND SAMPLE COLLECTION

Fig. 1. Ethnobotanical study site and sample collection

The town of Ndélé is located 552 km from Bangui and has 10,850 inhabitants. The geographical coordinates of Ndélé 8° 44' 34" North, 20° 53' 28" East with Latitude 8.74287, and Longitude 20.8911. The ethnobotanical surveys carried out, followed by a review of the bibliography, made it possible to select the plant. The leaves of *Prosopis africana* (Guill. & Perr.) were collected in Ndélé at the city of friendship (Ndah site). The plant (figure 2) was identified at the Center for Studies and Research in Pharmacopoeia and Traditional African Medicine (CERPHAMETA) by Doctor Olivia SEMBOLI. The harvested parts were dried in the open air at the Laboratory of Analysis, Architecture and Natural Substance Reactivity (LAARSN) for two weeks and reduced to powder.

Fig. 2. leaves of Prosopis africana

2.2 PHYTOCHEMICAL STUDY

2.2.1 PREPARATION OF EXTRACTS

The method of Worowounga et al [5] then of Keuete Kamdoum et al [6] was used with modification. Four solvents of different polarities were used and in ascending order of polarity. A quantity of 100g of powder from the leaves of *Prosopis africana* was put in 400 mL of cyclohexane, stirred for 4 hours of the time at room temperature. After filtration, the extract is evaporated using a rotavapor at 35 ° C and the residues are taken up with the following solvent.

2.2.2 QUALITATIVE ANALYZES

To search for the major families of secondary metabolism, the classical methods based on tube color reactions were used. The tannins by the $FeCl_3$ test, the anthocyanins by the ammonia reaction, the saponins by the foam test and the alkaloids were identified by the Mayer tests. On the other hand, for triterpenes and steroids, the Liebermann-Burchard test was used [4].

2.2.3 DOSAGE OF POLYPHENOLS

The method of Worowounga et al [7] was used with modification for the determination of polyphenols. A volume of 20 µL of extract was added to 100 µL of Folin's reagent solution (0.2 N). The mixture is stirred for 30 seconds followed by incubation for 5 minutes in the dark. After incubation, a volume of 80 µL of Na_2CO_3 was added. The mixture is again stirred for 30 seconds with 15 minutes of incubation at room temperature. The absorbance is read at 620 nm. The reference used was gallic acid.

2.2.4 DOSAGE OF CONDENSED TANNINS

The vanillin solution is prepared at 1% in H_2SO_4 (7 M). A volume of 150 µL of this solution is added to 50 µL of extract. After incubation for 15 minutes at 25°C, the absorbance is measured at 500 nm [8]. The results are expressed in mg catechin equivalent/g dry matter (DM) and the catechin is the reference used. Different concentrations between 0 and 1000 µg/ml prepared from a stock solution of catechin, made it possible to plot the calibration curve.

2.2.5 ANTHOCYANIN ASSAYS

The differential pH spectrophotometric method allows rapid and accurate measurement of total anthocyanins even in the presence of degraded polymerized pigments and other interfering compounds [9]. It is based on the determination of the absorbance of extractive solutions diluted with buffer solutions of pH = 1 and pH = 4.5. Anthocyanins are reversibly transformed under the influence of pH. The structural change associated with the modification of the chromophores determines the different color of the solutions of the anthocyanins according to the pH. The colored form (oxonium) predominates at pH=1 and the colorless form (hemi acetal) at pH=4.5. Two solutions of different pH were prepared. We have prepared two solutions of different pH are prepared. The solution at pH=1 contains KCl (0.2 M) and HCl (0.2 M). That at pH=4.5 is a mixture of CH_3COOH

(0.2 M), CH_3COONa (0.2 M) and H_2O. A volume of 100 µL of extract was added to 100 µL of the pH=1 solution in four wells, the next four wells contain 100 µL of extract and 100 µL of the pH=4.5 solution. The absorbance of the extract is measured at two wavelengths, 450 and 620 nm after incubation for 15 minutes at 25°C [10]. The results are expressed in mg cyanidin-3-glucoside equivalent/g plant material.

2.2.6 DOSAGE OF FLAVONOIDS

The quantification of flavonoids was carried out by a method based on the formation of a very stable complex between aluminum chloride and the oxygen atoms present on carbons 4 and 5 of the flavonoids. The protocol used is based on that described by [10] with some modifications. The solution of $AlCl_3$ (0.02 g/mL) in methanol was prepared. A volume of 100 µL of this solution is added to 100 µL of extract (3 mg/mL). For the control blank, 100 µL of methanol is added to 100 µL of solvent used to dissolve the extract. The measurement is made after 15 minutes of incubation at room temperature, the absorbance of the extract is measured at 415 nm.An aqueous solution of quercetin was prepared. Daughter solutions prepared from the stock solution at different concentrations between 0 and 1000 µg/ml will allow the calibration curve to be plotted.

2.3 ANTIOXIDANT ACTIVITY

A solution of DPPH (0.35 M) prepared in MeOH is diluted 10 times. From the Prosopis africana extract solution (Guill. & Perr.) (3mg/mL), a range of 16/20 dilutions; 1/2 and 1/5 was prepared. In the well, 180 µL of DPPH are added to 20 µL of the diluted solution of Prosopis africana extract (Guill. & Perr.). The mixture is subjected to stirring for 30 seconds and followed by incubation for 25 min. The absorbance is estimated at 450nm [10]. Ascorbic acid is used as a reference (0.25 mg/mL). The percentage inhibition is calculated from the following formula:

$$\%Inhibition = [(Ab - Ae)/Ab] \, x \, 100$$

Ab: Absorbance white
Ae: extract absorbance

2.4 STATISTICAL ANALYZES

Principal component analysis (PCA) was performed using the "FactoMineR" and "factoextra" packages of the R software circle of variable correlation.

3 RESULTS AND DISCUSSION

3.1 RESULT OF ETHNOBOTANICAL SURVEYS

In the Central African Republic the plant is used to treat fever, stomach aches and funeral rites. Edible caterpillars feed on leaves of *Prosopis africana* in the Central African Republic.

3.2 EXTRACTION YIELD

The extraction yield of *Prosopis africana* leaf powders obtained varies between 2.4 and 14.45% (Figure 3). The methanol extract has the best yield of 14.45%. It is observed that the extraction yield evolves proportionally to the polarities of the solvents used.

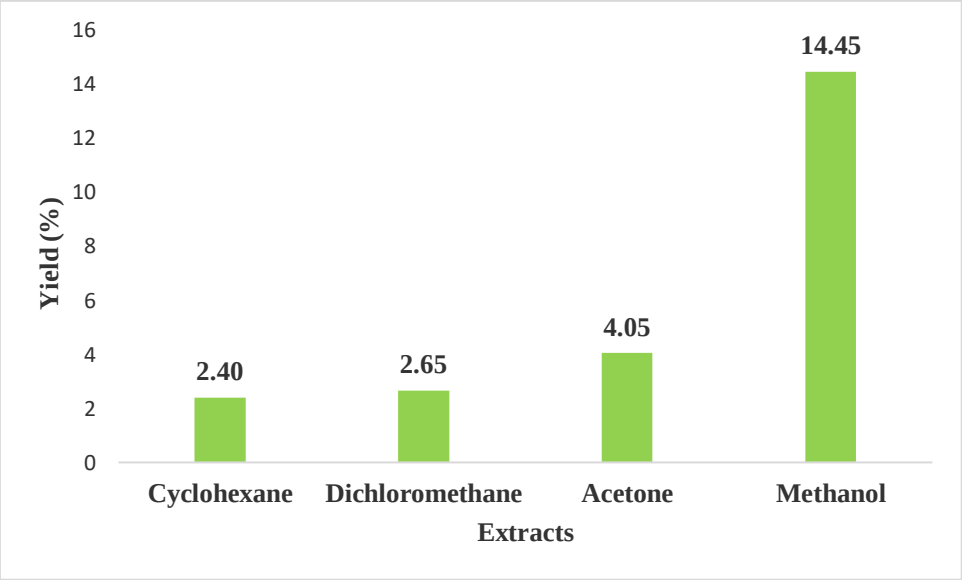

Fig. 3. Yield of Prosopis africana extracts.

3.3 QUALITATIVE ANALYSIS

Qualitative analyses carried out on extracts of *Prosopis africana* leaves revealed the presence of secondary metabolisms: tannins, flavonoids, sterols and triterpenes as well as saponifies (foam index which is 48.75). However, we noted the absence of alkaloids and anthocyanins (Table 1).

Table 1. Qualitative analysis of Prosopis africana leaf extracts

Plant	Tannins	Flavonoids	Anthocyanins	Sterol and triterpene	Alkaloids	Saponosides	
						Presence	foam index
Prosopis africana	+++	+	-	+	-	++	48.75

The results of the qualitative analyzes that we obtained are in agreement with the work of HAIDARA Mahahmat et al [10]. on the leaves of *Prosopis africana,* which highlighted the presence of tannins, flavonoids, alkaloids, sterols, triterpenes as well as saponoside with foam index 250.

3.4 CHEMICAL COMPOSITION IN POLYPHENOLS, TANNINS, FLAVONOIDS AND ANTHOCYANINS

The quantitative analysis made it possible to have the contents of total polyphenols of the various extracts which vary between 11.73±00.93 and 247.89±18.91mg of gallic acid equivalent (mg EqAG/g). The methanol extract has the best polyphenol level 247.89±18.91 mg EqAG/g, followed by the acetone extract 182.40±24.31 mg EqAG/g. They are followed by anthocyanins which vary between 3.26±01.75 and 4.92±03.13mg EqCya/g. We noted however that the flavonoid and tannin contents were very low compared to the polyphenols of the different extracts (Table 2).

Table 2. Content of polyphenols, flavonoids, tannins and total anthocyanins

Extracts	Polyphenols (mg EAG/g)	Flavonoids (mg EQ/g)	Tannins (mg EC3G/g)	Anthocyanins (mg EqCya/g)
Cyclohexane	42,90±00.90	ND	0.08±0.01	3.26±01.75
Dichloromethane	44.47±20.61	ND	0.07±0.00	4.92±03.13
Acetone	182.40±24.31	0.09±00	0.14±01.00	ND
Methanol	247.89±18.91	ND	0.14±02.00	ND

3.5 ANTIOXIDANT ACTIVITY

The four extracts of *Prosopis africa* (0.3mg/mL) tested on DPPH are all active. The percentage of inhibition varies between 52.98 and 98.55%. The acetone and methanol extracts similarly inhibited DPPH with the percentages of 98.55±0.10 and 98.47±0.70% respectively.

Ascorbic acid (3.33ug/mL) was used as reference. Consequently, the four extracts tested contain antioxidant molecules.

The results of work by Lambert Yanda et al [2]. on the antioxidant activity (DPPH) of the methanolic extract of *Prosopis africana* leaves gave IC50=0.093mg/mL. Their results are very close to our results, IC_{50}=0.02 mg/mL (Tableau 3).

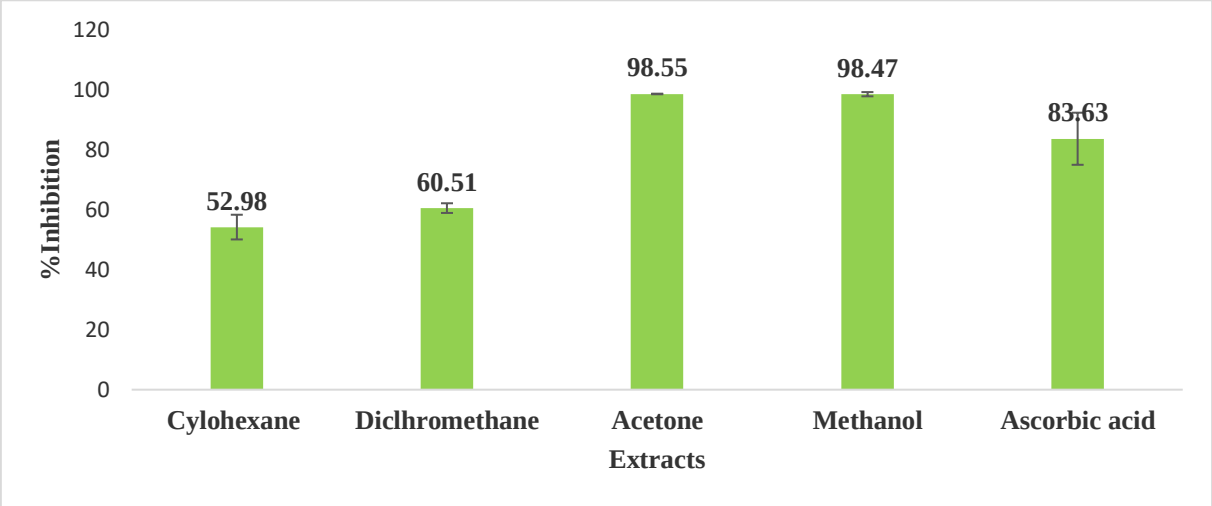

Fig. 4. Antioxidant activity of extracts Prosopis africana

PRINCIPAL COMPONENTS ANALYSIS

The results of the principal component analysis (PCA) carried out using the "FactoMineR" and "factoextra" software of the R software circle of correlation of variables are in Figure 6. The correlated variables are grouped together. There is a correlation positive between polyphenols and tannins. Variables near the center of the graph are less important for the first components. Principal component analysis (PCA) was performed to understand how TPC, TFC and TTC contribute to the biological activity (anti-DPPH) of plant extracts. Principal components (PC), PC1 and PC2 showed 95.5 and 3.3% of the total data variance respectively.

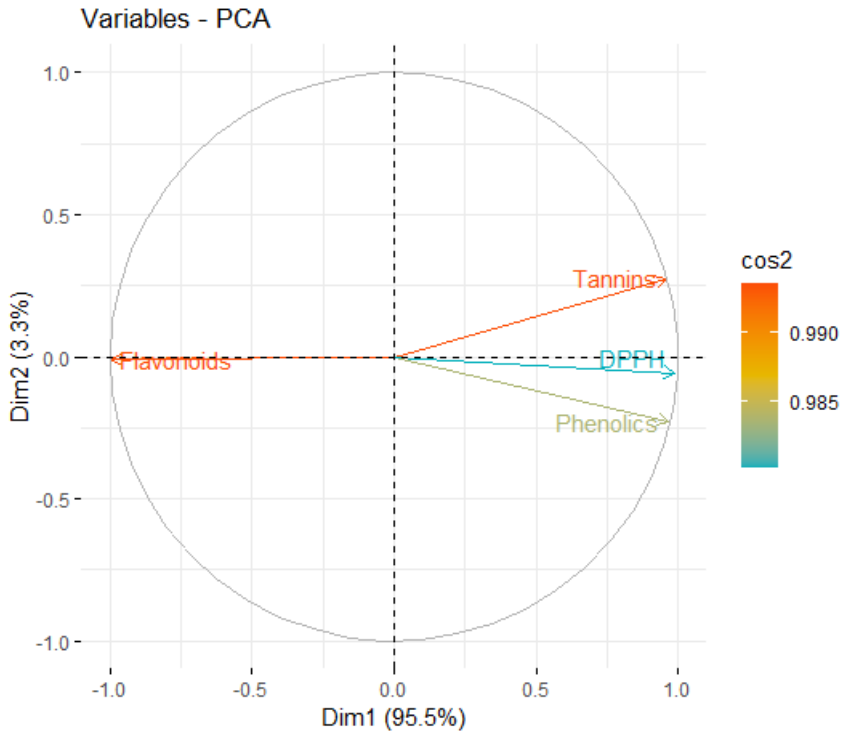

Fig. 5. Analysis of the principal components of "FactoMineR" and "factoextra" packages of antioxidant properties (Total polyphenols; Total flavonoids; Total tannins; DPPH: antioxidant activity) PS.

PEARSON CORRELATION MATRIX

It shows the correlation values (degree of linear relationship between each compensation variable). If both variables tend to increase and decrease at the same time, the value of the correlation is positive. When one variable increases while the other decreases, the value of the correlation is negative (Table 3).

Overall, there was a strong positive correlation between DPPH and polyphenol levels. Polyphenol and tannin contents contributed positively to an increase in potential inhibition against DPPH with Pearson correlation coefficients (r) equal to 0.95 and 0.92 respectively. However, there is a weak negative correlation between flavonoid level and DPPH inhibition with a Pearson (r) value of -0.99 (Table 3).

This indicates that the families of compounds, polyphenols and tannins contributed to the mentioned DPPH inhibition.

Table 3. Pearson correlation matrix (Pearson (n)), n=12

	Polyphenols	Flavonoids	Tannins	DPPH
Polyphenols	1.00	-0.95	0.87	0.95
Flavonoids	-0.95	1.00	-0.95	-0.99
Tannins	0.87	-0.95	1.00	0.92
DPPH	0.95	-0.99	0.92	1.00

4 CONCLUSION

The results of the qualitative analyzes of the leaves of *Prosopis africana* powders (*Guill. & Perr*) revealed the presence of tannins, flavonoids, saponosides, sterols and triterpenes. The quantitative analyzes gave a high rate of polyphenolics than the other families of the compounds quantified. The results of the antioxidant activity of the different extracts of this plant were better compared to the reference used (ascorbic acid).

Overall, there was a strong positive correlation between DPPH and polyphenol levels. Polyphenol and tannin contents contributed positively to an increase in potential inhibition against DPPH with Pearson correlation coefficients (r). However, there is a weak negative correlation between flavonraoid level and DPPH inhibition.

In perspective, we plan to carry out additional biological analyzes and activity such as antibacterial activity, to identify bioactive compounds.

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REFERENCES

[1] F. Zerargui, S. Boumerfeg, N. Charef, A. Baghiani, M. Djarmouni, S. Khennouf, L. Arrar, M. Abu Zarga, M. Mubarak, «Antioxidant Potentials and Xanthine Oxidase Inhibitory Effect of Two Furanocoumarins Isolated from *Tamus communis L»,* Medicinal Chemistry, vol. 11, no. 5, pp. 506-513, 2015.

[2] Yanda L, Tatsimo SJN, Tamokou JDD, Matsuete-Takongmo G, Meffo-Dongmo SC, Lannang AM, and Sewald N, Antibacterial and Antioxidant Activities of Isolated Compounds from *Prosopis africana* Leaves. International Journal of Analytical Chemistry, 2022: 1-10.

[3] Raza A, Kayode Muritala S, Muhammad A, Humera J, Priya T, Rimsha I, Show A. A new sesquiterpene (Prosoterpene, 1) and eleven reported compounds (2-12) of several classes, such as flavonoids, alkaloids, phenolic acids, and long-chain alcohols, were isolated from the BuOH extract of Prosopis africana (Guill. & Perr.) Taub. Natural Product Research, 9, 2022: 1-8.

[4] Bang Luu B, Ourisson G. Stérols et triterpènes polyoxygénés: une famille de produits à larges pectres d'activités biologiques. Médecine/sciences, 1989, no. 5, pp. 403-407.

[5] Worowounga X, Rahmani R, Namkona AF, Cazaux S, Syssa-Magalé JL, Matondo H, And Bouajila J. metabolites profiling of *Manilkara mabokeensis* Aubr´ev bark and Investigation of Biological Activities. International Journal of Analytical Chemistry. 2022: 1-14.

[6] Keuete Kamdoum E, Fomen Youmbi MB and Tsopmbeng Noumbo GR. Antifungal Potential of Three Plant Extracts in the Control of Some Major Fungi Associated with Cashew Seeds (*Anacardium occidentale* L.) Journal of Experimental Agriculture International. *2022 44 (9): 168-179.*

[7] Worowounga X, Namkona AF, Semboli O, Issa-Madongo M, Koueni-Ouakounda HK, Foto BAE, Syssa-magalé JL. Quantification of Total Phenolics, Flavonoids, Tannins, Anthocyannins and Antioxidant Activities of *Cola urceolata K. Schum.* International Journal of Innovation and Applied Studies. 2022, 36: 670-677.

[8] Nguerependе OS, Zinga I, Kamba Mebourou E, Semballa S, Worouwonga X, Namkona AF, Nguerekossi B, and Robert Ndjouenkeu. Study of the Nutritional Quality and Therapeutic Virtues of Cassava Leaves Infected by the Mosaic Virus and Healthy in Central African Republic. Journal of Experimental Agriculture International. 2022, 44 (10): 150-162.

[9] Giusti MM and Wrolstad RE. Anthocyanins: characterization and measurement with UV-visible spectroscopy Current protocols in food analytical chemistry.New York: John Wiley & Sons: Unit. 2001.

[10] A. F. Namkona, O.S.F. Bolevane, F. Moustapha, X. Worowounga, P. Ngaissona, J.N. Koane, JL. Syssa-Magalé, «Biological activities and phytochemical analysis of extracts *Afrostyrax lepidophyllus* Mildbr. Seeds», *The Journal of Phytopharmacology, vol.* 6, no. 2, pp. 102-106, 2017.

[11] Haidara M, Denou A, Diarra M, Tembely AD, Sanogo R. Etude pharmacognosique de Prosopis africana (Guill. & Perr.) Taub. et Tamarindus indica L., deux plantes utilisées dans la prise en charge de la dysfonction érectile au Mali Revue RAMReS – Série Pharm. Méd. Trad. Afr. 2021, 20 (2): 89-96.