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Determination of chemical composition, polyphenol contents, and evaluation of the antioxidant activity of three plants used in the management of malnutrition

Amadou Ibrahim Halilou*[1], Rabiou Abdou Mamane Ousseini[2], Bakasso Sahabi[2], Sabo Haoua[3], Mahamane Idi Issa Abdoulahi[2], Ikhiri Khalid[2], Hassimi Sadou[3]

[1]*Department of Chemistry and Biology, Faculty of Science and Technology, University of Zinder, BP: 656 Zinder, Niger*

[2]*Laboratory of Natural Substances and Synthesis Organic, Abdou Moumouni University, Faculty of Science and Technology, Department of Chemistry, BP 10662 Niamey, Niger*

[3]*Nutrition and promotion of agro-resources laboratory, Abdou Moumouni University, Faculty of Science and Technology, Department of Chemistry, BP 10662 Niamey, Niger*

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Abstract

Guiera senegalensis (Combretaceae), *Commiphora africana* (Burseraceae), *Schwenckia americana* (Solanaceae) are plants used in traditional medicine against malnutrition. The objective of this study is to contribute to the valorisation of these three species used in the management of malnutrition in children aged 0 to 5 years in Niger. The chemical composition of the extracts of these plants was determined using standard methods. The contents of total polyphenols, total tannins and antioxidant activity were determined by the spectrophotometric method. The determination of total polyphenols on the extract of the bark of *C. africana* gave the highest content of 86.62±6.31 mg EAG/g. For total tannins, high levels were observed with the bark extract of *C. africana* (16.14±0.95 g/L). The determination of the reducing power showed the highest activity with the leaf extract of *G. senegalensis* (2±0.04 mmol EAA/g). The analysis of the overall chemical composition showed that the extract of *G. senegalensis* leaves is richer in proteins (11.38%) and lipids (4.02%). The highest carbohydrate content (72.47%) is observed in the bark extract of *C. africana*. For the overall ash content, the whole plant extract of *S. americana* has the highest ash content at 11.04%. Extract from the leaves of *G. senegalensis* gave the highest dry matter content (92.42%). These results could contribute to the formulation of fortified foods used to improve the nutritional status of children in particular and populations in general.

* **Corresponding Author:** Amadou Ibrahim Halilou ✉ halilou2002@yahoo.fr

Introduction

For many years now, humanity has been using natural resources, mainly of plant origin, for various necessities for food and health care. Niger is a Sahelian country with a population estimated today at more than 20 million inhabitants, and all nutritional indicators are in the red. Nutrition can be defined as the set of metabolic reactions by which our organism transforms and uses everything it needs to function properly and stay alive (UNICEF/WHO, 2019). Inadequate nutrition can lead to the onset of diseases such as malnutrition with reduced immune defenses, delayed motor development, reduced learning, and cognitive abilities, and all the risk consequences of increased mortality; In Niger, malnutrition remains a public health problem and varies with the lean season (a period when old crops are exhausted and new ones not yet available) (Amadou and Lawali, 2022). Moreover, studies have shown an increase in the rate of child mortality linked to malnutrition in Niger. Over the last ten years, global acute malnutrition rates (12.3%) have exceeded the 10% alert threshold in Niger, with one child in five dying before his or her fifth birthday (Amadou *et al.,* 2021). Faced with this situation, the population is resorting to local plants to improve their nutritional status (Sahabi *et al.,* 2021). In Niger, much of the work has focused on medicinal plants, some of which have been used to highlight certain biological activities such as antioxidant and antimicrobial activities (Abdoulahi *et al.,* 2022). Ainsi la plante *Guiera senegalensis* J.F. Gmel est reconnue être anti-hypertension artérielle (Dirar and Devkota, 2021), antitussif, anti-inflammatoire, antiparasitaire (Pousset, 1998), anticholérique, antimicrobienne, antivirale (Oguntibeju, 2018), anti-dysentérique et anti-diarrhéique (Dan Lamso *et al.,* 2015). La plante *Commiphora africana* (A. Rich.) possède des propriétés médicinales et surtout nutritionnelles avec une appétence remarquable dans l'alimentation (Thiombiano *et al.,* 2012). Et enfin la plante *Schwenckia americana D. Royen ex L* a des nombreuses vertus médicinales dont notamment antirhumatismale, anti-inflammatoire (Eriyamremu and Iorliam, 2018), antimicrobienne et antiparasitaire (Chukwuma *et al.,* 2018). However, there is little or no phytochemical data on plants used in the management of malnutrition in children. It is within this framework that this study was conducted, the objective being to determine the chemical composition and evaluate the antioxidant activity of three plants *Guiera senegalensis* J.F. Gmel, *Commiphora africana* (A. Rich.) Engl and *Schwenckia americana* D. Royen ex L, commonly used in Niger in the management of malnutrition.

Material and methods

Plant material

The samples used are the leaves of *G. senegalensis*, the bark of *C. africana*, and the whole plant of *S. americana*. These samples were collected, dried, and crushed. The powders obtained from each sample were used for extractions.

Extraction: aqueous maceration at room temperature

The extraction method used is an aqueous maceration, so twenty grams (20g) of each sample was weighed and brought into contact with 200 ml. The mixture is left at room temperature for 48 hours with stirring. After filtration, the macerate obtained is concentrated into the dry extract. The research was carried out on dry residues. The yield in the dry extract was calculated by the following equation: $r = (\frac{m}{M})100$.

With r: yield; m: mass of evaporated extract and M: mass of plant matter.

Determination of protein content

The protein content of the samples was determined according to the Kjeldahl method. Each numbered crucible was filled with 0.2 g of the sample, plus one Kjeldahlck catalyst tablet (compressed Kjeldahl), plus 10 mL of concentrated sulphuric acid. The mixture was then mineralised on a heating block at a temperature of 420°C for 30 minutes. Mineralisation is completed when the solution turns light green. The product obtained after mineralisation is the mineralised product. This was first cooled and then placed in the distillation-titration unit. Once the

analysis cycle was started, the distillation followed by titration took place automatically in the presence of the 10 N NaOH solution (neutralizer), 0.2 N sulphuric acid (titrant), distilled water, the coloured indicator (40g of boric acid dissolved in 1L of distilled water plus 15 mL of bromocresol red). At the end of the process, the total protein content was automatically given as a percentage by the apparatus according to the burette drop observed during the titration (AOAC, 1990).

Determination of fat content

Fats are compounds soluble in organic solvents (petroleum ether, hexane). The fat content has been determined according to the Soxhlet extraction method using hexane as a refluxing solvent (AOAC, 1990). The flask is first washed and dried. The tare weight of the flask (Po) is recorded. Five grams (Pe) of the sample are placed in the extraction cartridges, which are plugged with cotton and placed in the Soxhlet. The flask was filled with 150 ml of hexane before being connected to the Soxhlet. The Soxhlet is connected to a refrigeration system and is connected to a cryostat to condense the solvent vapours to remove the lipids. The extraction process lasted 4 hours. The flask was then dried in an oven at 105°C before being cooled in a desiccator for 30 minutes and then weighed. The total lipid content was obtained according to the following relationship:

$$Total\ lipid = \left(\frac{Pf - Po}{Pe}\right)100$$

Po = weight of the empty balloon in grams;
Pf = final weight of the balloon containing the lipids in grams;
Pe = test sample.

Determination of moisture and dry matter content

The moisture content is determined according to the official method (AOAC, 1990). In crucibles previously dried in an oven at 103°C for 30 minutes, cooled in a desiccator and then weighed (Po), 5 g (Pe) of the sample are placed in the crucibles. These crucibles are placed in the oven at 103°C for three hours. They are weighed again to obtain a final weight. The moisture content is then determined by the following formula:

$$H(\%) = \left(\frac{Pe - (Pf - Po)}{Pe}\right)100$$

H %= moisture content
Pe= Test sample (5 g);
Po = Crucible tare weight;
Pf = Final weight (crucible + MS).

Determination of the mineral material

Incineration is carried out in such a way as to obtain all the mineral salts in the form of ashes (AOAC, 1990). Crucibles are dried in an oven at 103°C for 30 minutes, cooled in a desiccator and then weighed (Po). Then 5g (Pe) of the sample is placed in these crucibles and incinerated in an oven at 550°C for 4 hours. At the end of incineration, the crucibles are removed and cooled in a desiccator for 30 minutes before being weighed (Pf). The percentage of ashes is given by the following relation:

$$Ash(\%) = \left(\frac{Pf - Po}{Pe}\right)100$$

Pe: test sample;
Pf: final weight (crucible + ashes);
Po: empty weight of the crucibles.

Determination of carbohydrate content

The carbohydrate content has been determined using the differential method, which considers dry matter as the sum of carbohydrates, lipids, proteins and minerals. Dry matter = Protein + Fat + Carbohydrates + Ash.

Carbohydrates = Dry matter - Protein - Ash - Fat.

Spectrophotometric dosage

Dosage of total polyphenols

The determination of total phenolics was carried out according to the procedure described by Waterhouse, (Waterhouse, 2002). A standard curve was first produced using gallic acid (200 mg/l) as the reference substance. Thus, to each test tube, according to the solutions obtained after dilution, 0.125 ml of the sample solution was determined and 0.625 ml of the RCF solution (0.2 N) was added. After 5 minutes of incubation, 0.5 ml of sodium carbonate solution (75g/L) was added. After stirring, the different solutions were left to stand, and protected from light

for 2 hours. The readings were taken at 760 nm, with a spectrophotometer (Thermospectronic, type Helios Alpha V4.6), against a blank consisting of a mixture of 0.5 ml of RCF and 1 ml of sodium carbonate. Three readings were taken for each concentration and the results were expressed in mg gallic acid equivalents per 100 mg dry extract. The following formula was used to calculate the total phenolic compound content of each extract. $c = \frac{C_1 \times V}{m}$

C = being the total polyphenol content expressed in mg EAG/g dry extract.

C_1 = the concentration of gallic acid established from the calibration curve in mg/L,

V =the volume of extract in L

m =the mass of the plant extract in g.

➢ Total tannin dosage :

The dosage of total tannins is based on the property of proanthocyanidins to be transformed by cleavage of the inter-flavan bond in an acid medium and at 100°C, into coloured anthocyanidins (yellow-green) absorbing mainly at 550 nm. This reaction is commonly known as the Bate-Smith reaction. To 2 mL of extract of each residue (1g/L) placed in a hydrolysis tube, 3 mL of hydrochloric acid (12N or 37%) is added. The tube is then closed with a Teflon-sealed cap and placed in a water bath at 100°C for 30 min. At the same time, a control tube containing the same solution is left at room temperature. After cooling the hydrolysed tube, the optical density is read at 550 nm. The total tannin contents are calculated according to the following equation:

C = 19,33 (Doh−Dot)

C being the total tannin content expressed in g /L,

Doh the optical density of the hydrolysed tube and

Dot the optical density of the test tube (Bate-Smith, 1965; Gayon and Street, 1966; Hamadou *et al.,* 2018).

Determination of antioxidant activity by the FRAP method

The FRAP (Ferric reducing antioxidant power) method is based on the ability of extracts to reduce ferric ion (Fe3+) to ferrous ion (Fe2+). The total antioxidant capacity of each plant extract was determined by the method used by Hinneburg *et al.* (2006). Thus 0.5mL of an aqueous solution of each extract (1g/L) was mixed with 1.25 mL of phosphate buffer (0.2 M; pH 6.6) and 1.25 mL of the aqueous solution (1%) of potassium hexacyanoferrate [$K_3Fe(CN)$ 6]. After 30 min. incubation at 50°C; 1.25 mL trichloroacetic acid (10%) was added. The mixture was then centrifuged at 2000 rpm for 10 min. 0.625 mL of the supernatant was then mixed with the same volume of water and 0.625 mL of a freshly prepared aqueous solution of $FeCl_3$ (0.1%) was added. Absorbances were read at 700 nm against a calibration curve obtained from ascorbic acid (0-200 mg/L). The reducing power was expressed in ascorbic acid equivalents (AAE) (mmol ascorbic acid/g dry extract) considering 1 mM equals FRAP of 1 mL of the dry extract according to the following formula:

$$c = \frac{c \times D}{M \times Ci}$$

C = concentration of reducing compounds in mmol EAA/g dry weight;

c = concentration of the sample read;

D = dilution factor of the stock solution;

Ci = concentration of the stock solution;

M = molar mass of ascorbic acid (176.1g);

Results and discussion

Protein content of the extracts

Fig. 1 shows that the protein contents vary from 11.38% to 4.70%. High content is observed in the leaf extract of *G. senegalensis* (11.38%). A low content is observed in the bark extract of *C. africana* (4.70%). Proteins are essential for the active metabolism of the body and have a role both intra- and extracellular. They perform several functions: Structural proteins which participate in the constitution of cell membranes and intracellular organs. Motor proteins are represented by actin and myosin, which allow muscles to contract. And regulatory proteins have various roles: enzymatic, hormonal, immune, transport for non-water-soluble lipids, and gene expression (transcription and translation) (Moussa *et al.,* 2016).

Table 1. Percentage of dry matter of extracts from the leaves of *G senegalensis*, the bark of *C. africana* and the whole plant of *S. americana*.

Plants	dry matter content in %
G. senegalensis	92,42±0,06[a]
C. africana	90,03±0,25[a]
S. americana	91,76±0,14[a]

According to medical experts and researchers, proteins are one of the most important nutrients not only for growth but also for the development of infants (Sidnell and Greenstreet, 2009).

Fat content of the extracts

The maximum and minimum fat contents in Figure 2 are 4.02% and 2.34%. Extracts from the leaves of *G. senegalensis* and the whole plant of *S. americana* have very similar maximum fat contents of 4.02% and 3.68% respectively. The minimum content is observed in the bark extract of *C. africana* (2.34%). Lipids are essential elements for the body. They have several roles in the organism, in particular, the energy reserve through the adipose tissue, and the structural role through the various phospholipids which participate in the formation of the membrane of each cell of the organism. Phospholipids and glycolipids by their position and composition play a role in the transduction of messages (receptor function); thus, plays the role of transporting fat-soluble vitamins (Sidnell and Greenstreet, 2009; Moussa *et al.,* 2016).

Table 2. Percentage in global ash of extracts from the leaves of *G senegalensis*, the bark of *C. africana* and the whole plant of *S. americana*.

Plants	total ash content in %
G. senegalensis	6,33±7,43[b]
C. africana	10,52±0,35[a]
S. americana	11,04±1,07[a]

Dry matter content of the extracts

Extracts from the leaves of *G. senegalensis* and the whole plant of *S. americana* have dry matter contents of 92.42% and 91.76% respectively. The extract from the bark of *C. africana* has a dry matter content of 90.03%. as shown in Table 1 there was significant difference ($p < 0.05$) among the samples of the three plants studied.

Total ash contents of the extracts

It can be seen from Table 2 that extracts from the whole plant of *S. americana* and the bark of *C. africana* showed the highest ash content (11.04% and 10.52% respectively) with a significant difference ($p < 0.05$) for the third plant. The extract of the latter had a low level of 6.33%.

The ash content of the extract from the bark of *C.africana* bark gave a rate of 10.52%. This result is close to that found by Houérou in 1980, which shows that the mineral matter has a rate of 9.39% in the young leaves and 7% in the fruits of *C. africana*. This could be due to the difference in the parts analysed (Sidnell and Greenstreet, 2009).

Table 3. Total polyphenol content of extracts from the leaves of *G senegalensis*, the bark of *C. africana* and the whole plant of *S. americana*.

Plants	Total polyphenol content in mg GAE/g
G. senegalensis	64,54±7,84[ab]
C. africana	86,62±6,31[a]
S. americana	33,95±10,22[b]

Carbohydrate contents of the extracts

In this Fig. 3, the high carbohydrate content is observed in the bark extract of *C. africana* (72.47%), followed by the leaf extract of *G. senegalensis* (70.69%) and the whole plant extract of *S. americana* (67.25%). The essential function of carbohydrates is to provide the body with energy from food, especially from plants. They have a structural role through heterosides, derived from complex molecules containing proteins, phosphorus, etc. associated with carbohydrate elements, which are found in connective tissue such as cartilage, certain membrane receptors, mucus (Moussa *et al.,* 2016).

Table 4. Total tannin content (g/L) of extracts from the leaves of *G. senegalensis*, the bark of *C. africana* and the whole plant of *S. americana*.

Plants	Total tannin content in g/L
G. senegalensis	3,75±0,41[b]
C. africana	16,14±0,95[a]
S. americana	1,43±0,47[b]

Total polyphenol content

The total phenolics were determined from a regression curve obtained using a gallic acid solution (200 mg/L stock solution) which gave the following equation: Y = 0.011x + 0.120; R^2= 0.984 (Fig. 4). The values recorded in this Table 3 are expressed in milligrams (mg) gallic acid equivalent (GEA) per gram (g). The values, which have no letters in common, are statistically different ($p < 0.05$). The extract from the bark of *C. africana* gave the highest content of phenolic compounds (86.62±6.31 mg GGE/g), the low content was observed in the extract from the whole plant of *S. americana* (33.95±10.22 mg GGE/g). However, a non-significant difference was recorded between the levels of phenolic compounds in the leaves of *G. senegalensis* and the bark of *C. africana*. This high value obtained in *C. africana* is lower than that obtained by Moussa *et al.,* 2016 with different solvents and organs of *C. africana*. Indeed, on a methanolic fraction of the leaves and stem of *C. africana*, they quantified total polyphenols with a content of 260.73 ± 4.50 mg EAG/g. This inferiority could be justified by the difference in the parts of the plants used.

Table 5. Reducing power of extracts from the leaves of *G senegalensis*, the bark of *C. africana* and the whole plant of *S. americana*.

Plants	Reducing power of extracts in mmol AAE/g
G. senegalensis	2,00±0,04[a]
C. africana	1,49±0,08[b]
S. americana	1,33±0,19[b]

Total tannin content of the extracts

Total tannin contents (g/L) are presented in Table 4. The maximum and minimum levels are 16.14±0.95 g/L to 1.43±0.47 g/L, respectively. High tannin contents are observed in the bark extract of *C. africana* (16.14±0.95 g/L). The contents, which do not have a letter in common, are statistically different ($p < 0.05$). Extracts from the leaves of *G. senegalensis* and the whole plant of S. americana showed a non-significant difference in total tannin contents. The tannins contained in plant extracts are used as astringents, against diarrhea, as diuretics, against stomach and duodenal tumors, and as anti-inflammatory, antiseptic, antioxidant and haemostatic (Abdoulahi *et al.,* 2020). They stop bleeding and fight infections. Plants rich in tannins are used to tighten soft tissues, as in the case of vancous veins, to drain excessive secretions, as in diarrhea, and to repair tissue damaged by burns (Abdoulahi *et al.,* 2020).

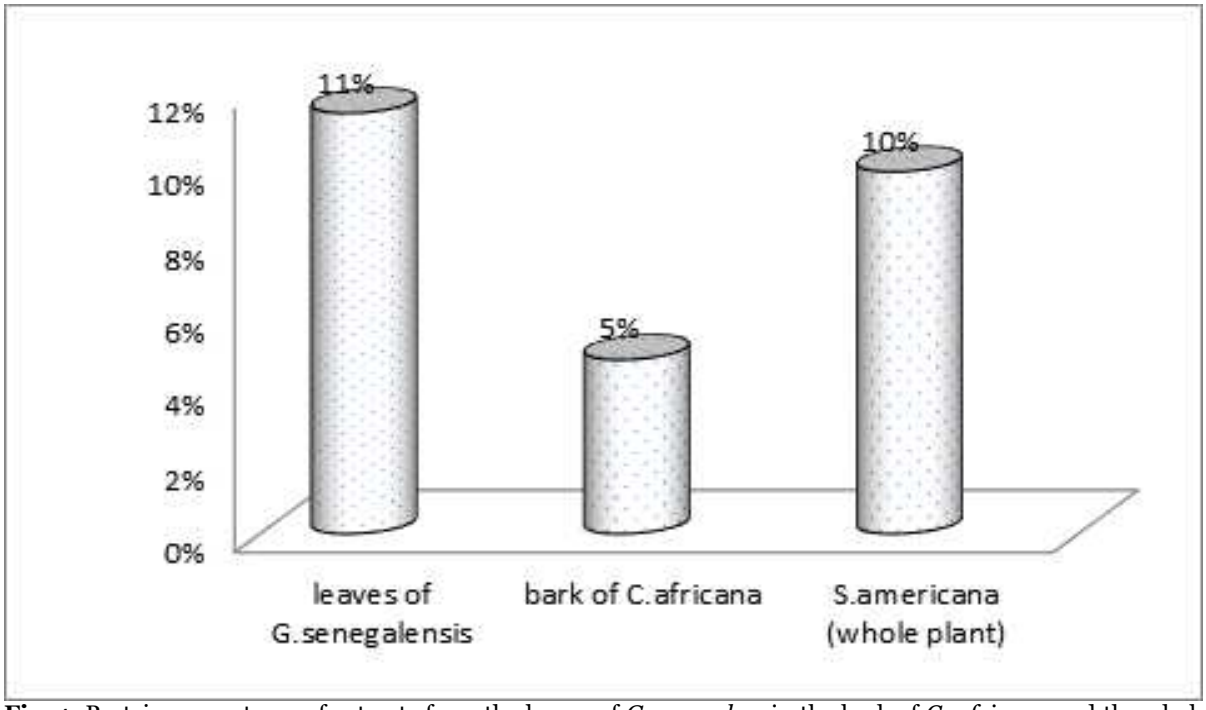

Fig. 1. Protein percentages of extracts from the leaves of *G senegalensis*, the bark of *C. africana* and the whole plant of *S. americana*.

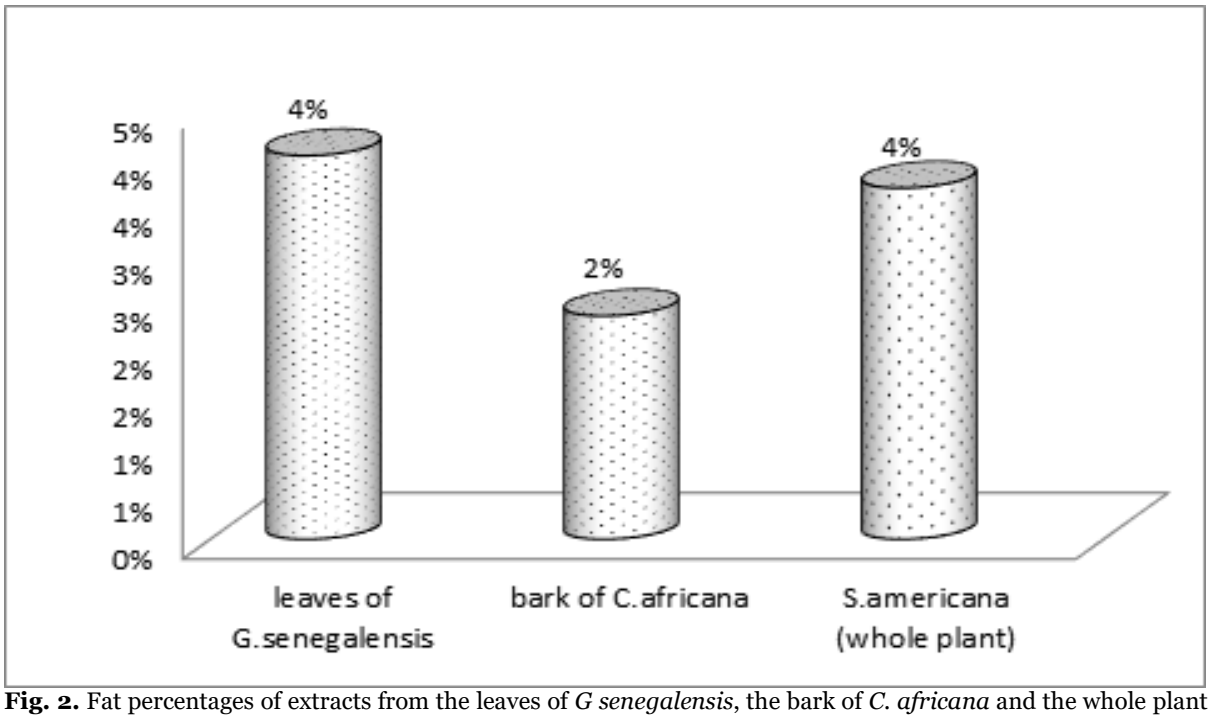

Fig. 2. Fat percentages of extracts from the leaves of *G senegalensis*, the bark of *C. africana* and the whole plant of *S. americana*.

Antioxidant activity by the FRAP method of the studied extracts

The concentrations were determined against a reference curve obtained with ascorbic acid (200 mg/L stock solution), with the regression curve: y = 0.007x + 0.122, R^2 = 0.978 (Fig. 5). The values obtained with extracts from the leaves of *G. senegalensis*, the bark of *C. africana* and the whole plant of *S. americana* are given in Table 5 above. The high activity is obtained with the leaf extract of *G. senegalensis* (2±0.04 mmol EAA/g) with a significant difference ($p < 0.05$) compared to the other extracts.

Extracts from the leaves of *G. senegalensis* and the bark of *C. africana* have strong reducing powers thanks to their richness in phenolic compounds. These results are confirmed by the positive correlation coefficient obtained between the phenolic compounds and the antioxidant activity r = 0.32. The determination of the reducing power by the FRAP method on the methanolic extract of the stems and leaves of *C. africana* gave 157.40 ± 4.67 mgAAE/g (Hinneburg *et al.*, 2006; Bakasso *et al.*, 2013).

These results are superior to our results. This could be explained by the use of different solvents with different capacities to extract secondary metabolites.

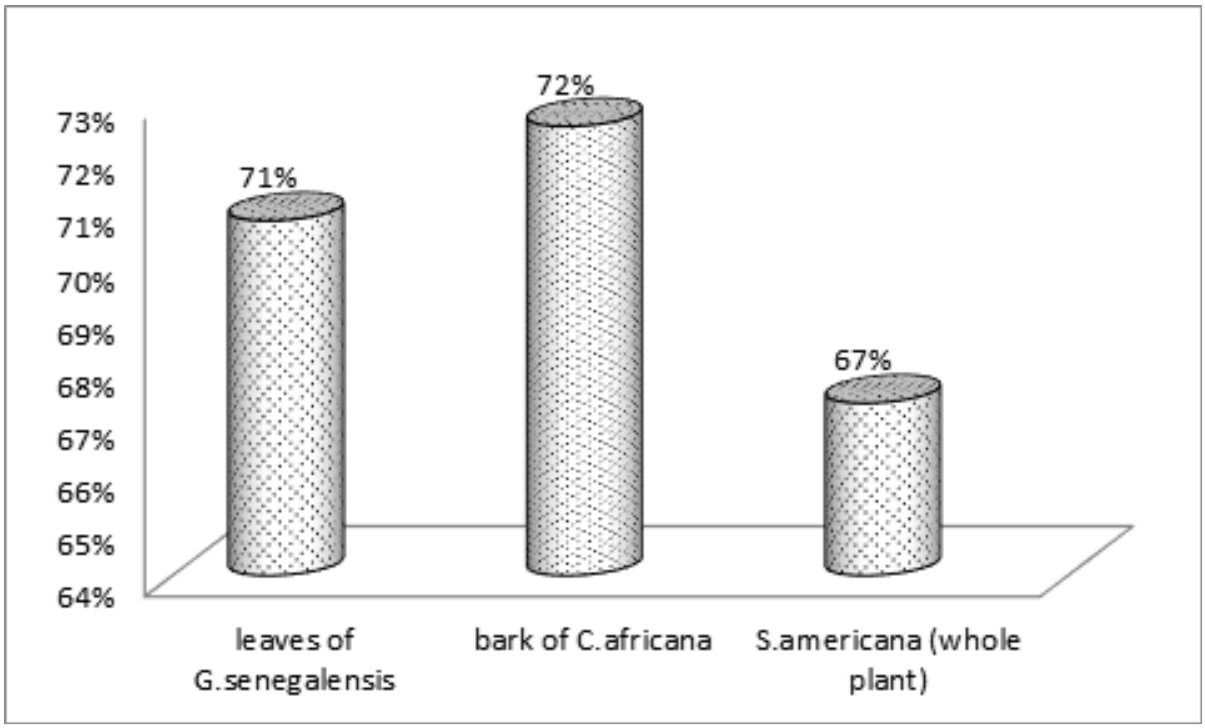

Fig. 3. Carbohydrate percentages of extracts from the leaves of *G senegalensis*, the bark of *C. africana* and the whole plant of *S. americana*.

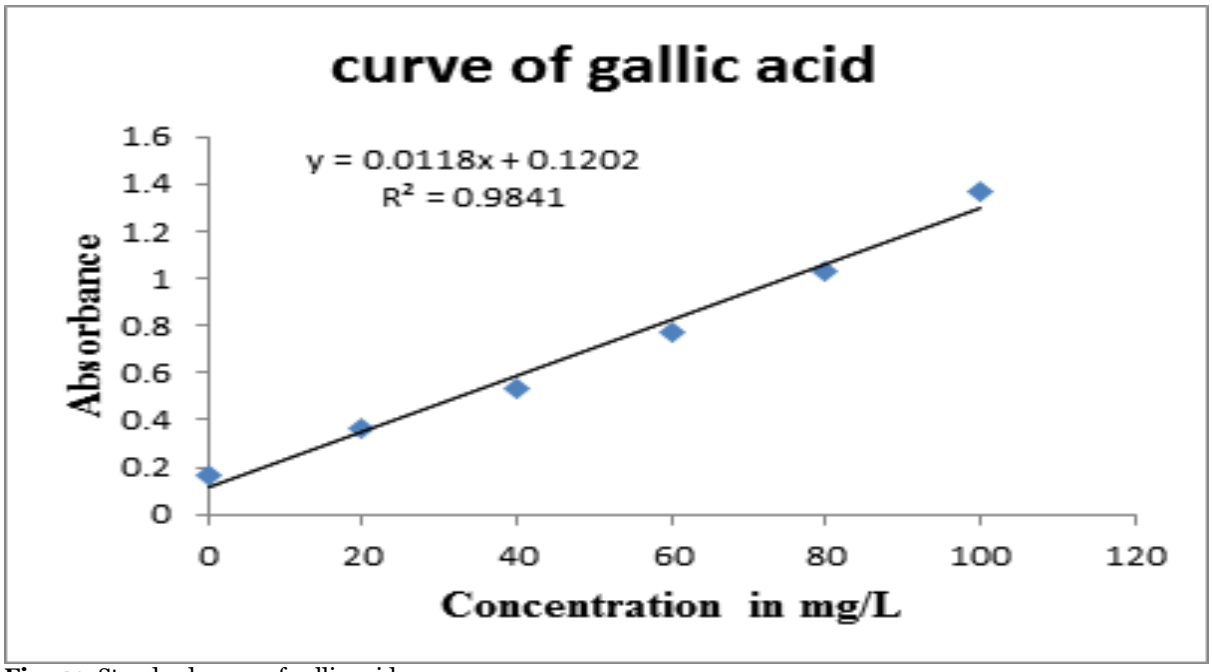

Fig. 41. Standard curve of gallic acid.

Correlation between total polyphenols, total tannins, and antioxidant activity by the FRAP method

Fig. 6 shows the correlation coefficients (R) and regression equations (Y) obtained between total polyphenols, total tannins, and antioxidant activity. The correlation coefficients (R) are low. Abdoulahi *et al.* (2020) found low correlation coefficients (R) for total tannins. The correlation coefficients obtained were 0.91 for total polyphenols, 0.84 for flavonoids and 0.27 for tannins. On the other hand, researchers found a strong correlation between antioxidant activation and total phenolic concentration in plant extracts (Moussa *et al.*, 2016).

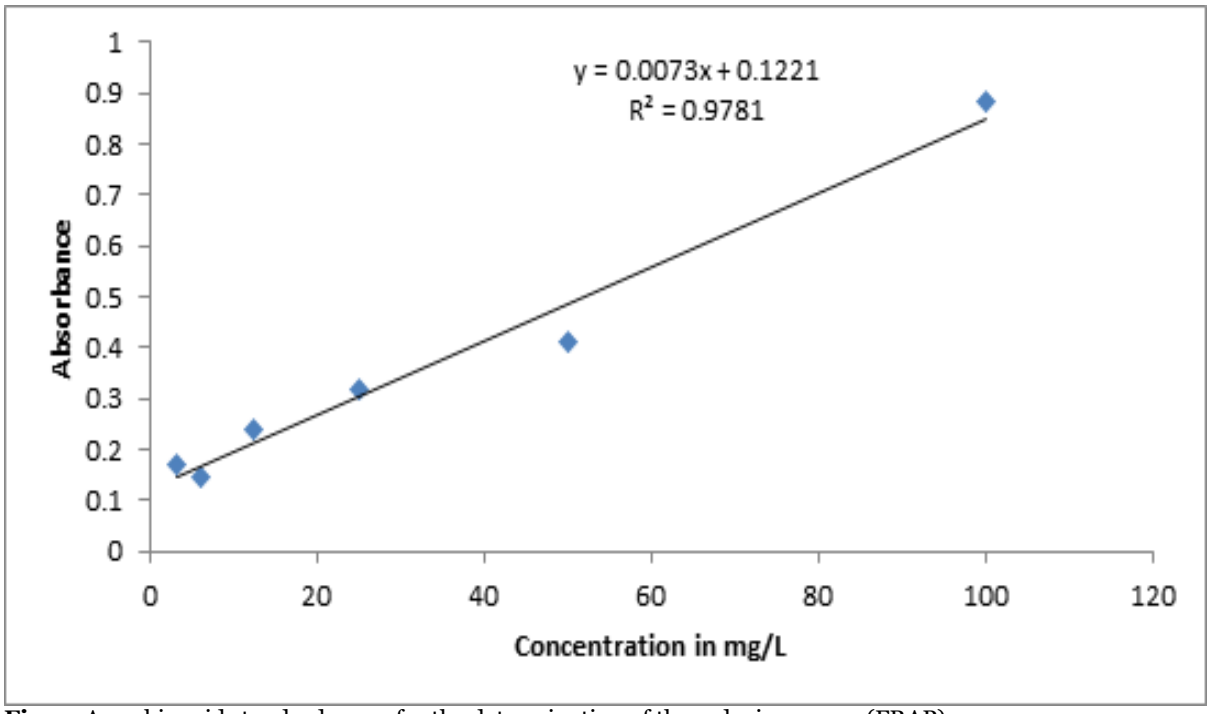

Fig. 5. Ascorbic acid standard curve for the determination of the reducing power (FRAP).

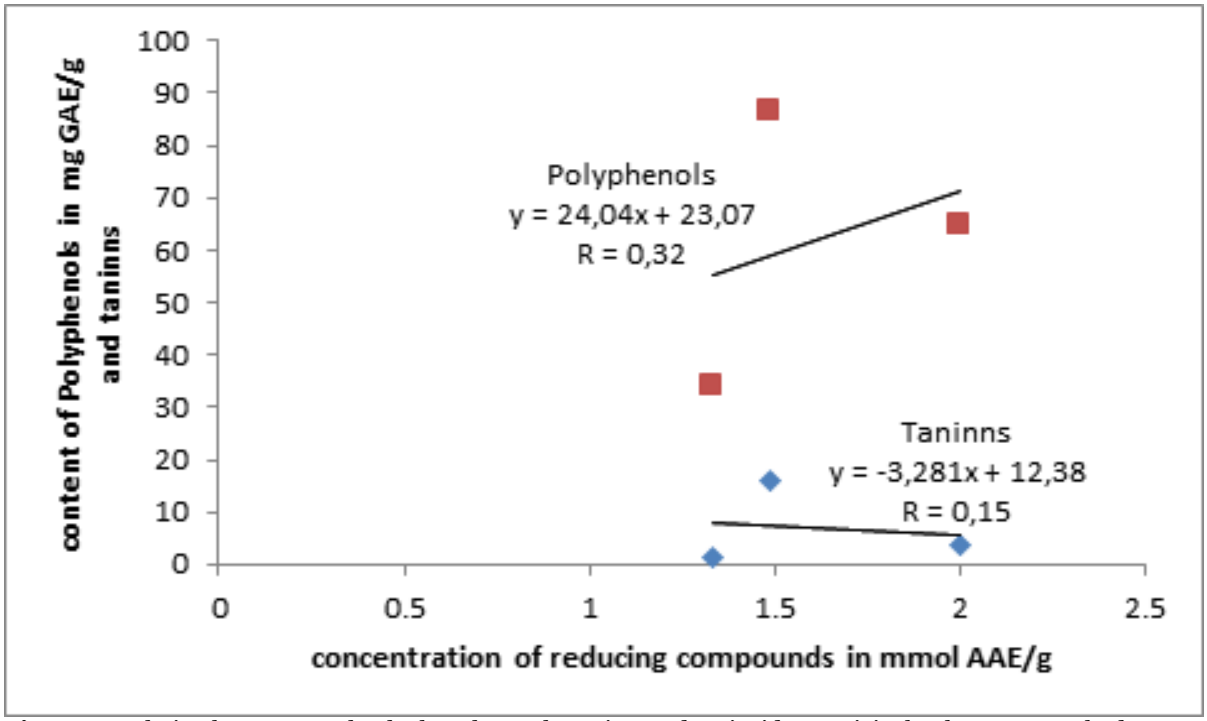

Fig. 6. Correlation between total polyphenols, total tannins, and antioxidant activity by the FRAP method.

Conclusion

This study examined the chemical composition of three plants (*Guiera senegalensis*, *Commiphoraafricana*, *Schwenckia americana*) used in traditional medicine in Niger. The parts of the plants used are the leaves of *G. senegalensis*, the bark of *C. africana* and the whole plant of *S. americana*. The polyphenol assay showed that all three plants had good contents of phenolic compounds. *C. africana* is a plant that contains more phenolic compounds and more tannins. The richness of the extracts of these plants in polyphenols would justify their strong reducing power. The different parts used in the extracts of *Guiera senegalensis*, *Commiphora africana*, *Schwenckia americana* showed a good content of organic and mineral matter. However, particularly high contents of mineral elements were found in the extracts studied (6-11% total ash). The results of the study on these plants justify their therapeutic use, especially in the treatment of malnutrition in children. Further studies are necessary to evaluate the toxicity and other biological activities of these plants for their best use.

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