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Antioxidant Properties Evaluation of Trunk's Barks of 10 Plants used in Traditional Medicine against Hepatic Pathologies

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Herbal remedies are known for their anti-inflammatory and antioxidant properties. The aim of this study was to evaluate the antioxidant activities of the aqueous extracts of the bark of the trunk of 10 medicinal plants used in traditional medicine for the management of hepatic pathologies. This is *Acacia nilotica* (L.) Willd. ex Delile (Fabaceae – Mimosoideae), *Adansonia digitata* L. (Bombacaceae), *Bombax costatum* Pellegr. & Vuill. (Bombacaceae), *Balanites aegyptiaca* (L.) Delile (Balanitaceae), *Cassia sieberiana* DC. (Fabaceae – Caesalpinioideae), *Piliostigma reticulatum* (DC.) Hochst. (Fabaceae – Caesalpinioideae) *Tamarindus indica* L. (Fabaceae – Caesalpinioideae), *Daniellia oliveri* (Rolfe) Hutch. & Dalziel (Fabaceae – Caesalpinioideae), *Khaya senegalensis* (Desr.) A.Juss. (Meliaceae) and *Gymnosporia senegalensis* (Lam.) Loes.) (Celastraceae)..

Methodology: In this study, the total phenolic and flavonoid content of the aqueous extracts of the trunk bark of 10 plants were determined and their antioxidant activities by the DPPH and FRAP methods were evaluated. The link between phenolic compounds and antioxidant activity was sought through a regression curve.

Results: *Acacia nilotica* had the best contents of total phenolics and flavonoids respectively with 21.28 ± 0.18 g ETA / 100 g DM and 0.207 ± 0.003 g EQ / 100 g DM. For the evaluation of antioxidant activity, *Acacia nilotica* also gave the best activities by the DPPH method with a percent inhibition of 1.08 ± 0.03 AAE and a reducing capacity of Fe^{3+} to Fe^{2+} of 0.107 ± 0.03 AAE. A strong correlation was found between FRAP and total phenolics (r2 = 0.9559).

Conclusion: This study shows that all the plants used for the treatment of liver pathologies had an interesting antioxidant capacity but among these 07 plants had the best activities. In-depth studies on the anti-inflammatory and even hepatoprotective activity of these extracts would justify their use in traditional medicine.

Keywords: Medicinal plants; total phenolics; flavonoids; DPPH; FRAP.

1. INTRODUCTION

Liver disease (acute and chronic) is of global concern [1] and epidemiological studies have estimated that 3.5% of the world's population are chronically infected with viral hepatitis [2]. Viral hepatitis B (HBV) has a prevalence in children in Africa of 3% [2]. In Burkina Faso, its prevalence had already exceeded 12% since 2013 according to Lazarus et al. [3]. “More and more, other types of hepatitis are developing around the world (drugs, alcoholics, toxicants, etc.) [4]. These types of hepatitis are at the origin of a strong production of free radicals which can be at the origin of other pathologies or aggravate the hepatic consequences. Medical treatments for these diseases are often difficult to achieve and may have limited effectiveness. These are traditionally treated with medicinal plants especially in rural areas where they are widely available” [1]. These plants are widely used for their hepatoprotective activity. Herbal remedies provide a natural source of phytochemicals with biological activity, such as antioxidant properties [5]. Among these phytoconstituents, phenolic compounds and flavonoids are the most well known for their antioxidant properties [6]. In its program to combat hepatic diseases, the Institute for Research in Health Sciences carried out a survey in the Center-West region of Burkina Faso in order to identify the plants and parts of plants used for treatment. of his pathologies. At the end of the survey, ten plants were selected according to the part used (trunk bark). The aim of this study was to evaluate the antioxidant activity as well as the total phenolic and flavonoid contents of the aqueous extracts of the trunk bark of the different plants selected.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents Used

1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid and quercetin were purchased from Sigma Chemical Co. (St. Louis, MO, USA); potassium hexacyanoferrate [$K_3Fe(CN)_6$], trichloroacetic acid, trichloroferrate [$FeCl_3$] for evaluation of antioxidant activity, and Follin-ciocalteu and sodium carbonate, $AlCl_3$, for phenolics compounds.

2.2 Plant Material and Extraction

The plant material consisted of the trunks bark of 10 plants collected in Ouagadougou in the

province of Kadiogo in Burkina Faso: *Acacia nilotica* (L.) Willd. ex Delile (Fabaceae – Mimosoideae), *Adansonia digitata* L. (Bombacaceae), *Bombax costatum* Pellegr. & Vuill. (Bombacaceae), *Balanites aegyptiaca* (L.) Delile (Balanitaceae), *Cassia sieberiana* DC. (Fabaceae – Caesalpinioideae), *Piliostigma reticulatum* (DC.) Hochst. (Fabaceae – Caesalpinioideae) *Tamarindus indica* L. (Fabaceae – Caesalpinioideae), *Daniellia oliveri* (Rolfe) Hutch. & Dalziel (Fabaceae – Caesalpinioideae), *Khaya senegalensis* (Desr.) A.Juss. (Meliaceae) and *Gymnosporia senegalensis* (Lam.) Loes.) (Celastraceae). The residual moisture content of the different powders was determined.

Maceration, which is the traditional form of use, was used at 10%, or 2.5 g of plant material in 25 ml of distilled water, for 24 hours at laboratory temperature. After 24 hours, the extract was first filtered with a nylon filter and then centrifuged at 3000 rpm for 10 min. The supernatant obtained was recovered and packaged in 4 Eppendorf tubes then placed in the freezer at -4 ° C.

2.3 Determination of Phenolic Compounds in Plant Extracts

2.3.1 Total phenolics content

"The total phenolic compounds were measured according to the Singleton method" [7]. "These compounds react with the Folin Ciocalteu reagent (FCR) in an alkaline medium. The reaction mixture consisted of 1 ml of extract (1 mg / ml), 1 ml of 2N FCR and 3 ml of a 20% sodium carbonate solution in a test tube. After incubation for 40 min at room temperature, the absorbance of the mixture was measured at 760 nm on the spectrophotometer (Agilent 8453) against a tannic acid standard curve (R2 = 0.999). In the blank control tube, the extract was replaced with distilled water. The tests were realized and the total phenol concentration of the extract, expressed in gram-equivalent tannic acid per 100 g dry matter (DM) (g ETA / 100 g DM), was calculated" [7].

2.3.2 Total flavonoids content

The flavonoid dosage was realized according to the Kumaran method [8] adapted by Abdel-Hameed [9]. "Two (2) ml of extract (1 mg / ml in methanol) were mixed with 2 ml of aluminium trichloride (2%). After 40 min of incubation at ambient temperature, the absorbance was measured at 415 nm using a spectrophotometer (Agilent 8453) against a quercetin standard curve of R2= 0.999. The blank control tube consisted of 2 ml of methanol. The quantity of flavonoids in the plant extract was determined in gram-equivalent quercetin (EQ) per 100 g dry matter (DM) (g EQ / 100 g DM)" [9].

2.4 Evaluation of Antioxidant Activities

2.4.1 FRAP test (ferric reducing antioxidant power)

The FRAP (Ferric Reducing Antioxidant Power) method is based on the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). This method evaluates the reducing power of the compounds. The reducing power of the samples was evaluated according to the spectrophometric method described by Apati et al., [10]. A volume of 1 ml of the extract was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide [$K_3Fe(CN)_6$] 1%. The mixture was incubated at 50 ° C for 20 min; 2.5 ml of 10% trichloroacetic acid (TCA) were then added before centrifuging the mixture at 3000 rpm for 10 min. A volume of 2.5 ml of the obtained supernatant was then taken and mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride ($FeCl_3$) 0,1%. In the white control tube, the extract was replaced with 1 ml of the extraction solvent (distilled water or ethanol). The absorbance was read at 700 nm using the spectrophotometer (Agilent 8453) against a standard curve of ascorbic acid (0-0.2 mg / ml). For each plant, the measurements were carried out in quadruple from two independent tests. An increase in absorbance reflects an increase in the reducing power of the sample. The results are expressed in ascorbic acid equivalents (Ascorbate Acid Equivalent = AAE) per milliliter. When the AAE value is equal to 1 AAE.ml-1, the reducing power of 1 ml of extract is equivalent to 1 µmol of ascorbic acid. The value of the AAE is inversely proportional to the reducing power.

2.4.2 DPPH test (2,2-diphényl-1-picrylhydrazyl)

"Free radical scavenging activity assessment (DPPH' assay): The antioxidant activity of the crude extracts and fractions was assessed by the mean of 2,2-dipheny1-1-picrylhydrazyl (DPPH') colorimetric method" as described by Velazquez et al., [11], slightly modified. This method depends on the reduction of purple DPPH to a yellow-colored diphenyl picrylhydrazine and the

remaining DPPH', which showed maximum absorption at 517 nm was measured (spectrophotometer Agilent 8453E). About 2 mL of a 20 mg mL -1 DPPH' solution were added to 1 mL of a methanolic solution of each extract (1-100 lig mL -1). A mixture of 2 mL of DPPH' and 1 mL of methanol served as control. The mixture was shaken vigorously then incubated for 15 min in darkness at room temperature. Absorbance was measured at 517 nm. Methanol was used as blank. Ascorbic acid solution was used as positive controls. Each experiment was performed in triplicate. The DPPH' radical scavenging activity was calculated. Decreasing of the DPPH solution absorption indicates an increase of DPPH radical scavenging activity. For each plant, the measurements are carried out in quadruple from two independent tests. The results are expressed in mg content of antioxidants ascorbic acid equivalent (AAE).

2.5 Statistical Analysis

All the assays were carried out in triplicate and each experiment was independently repeated at least three times, through which means and standard deviations (SD) were generated. The results were analyzed with GraphPad Prism version 6, using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test with $P = 0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

The residual moisture content of the different powders varied from 4,29±0,24 with *Tamarindus indica* to 7,43±0,18 for *Gymnosporia senegalensis* and was all less than 10%.

3.1.1 Phenolic compounds in plant extracts

The results of the total phenolic and flavonoid contents of the aqueous extracts of the trunk bark of the plants are shown in Figs. 1 and 2. The total phenolic contents of the different extracts varied from 1,30 ± 0,10 g ETA/100 g DM for extracts of *Balanites aegyptiaca* to 21.28 ± 0,18 g ETA/100 g DM for extracts of *Acacia nilotica.* There is no statistically significant difference ($p < 0.05$) between the total phenolic contents of extracts of *Khaya senegalensis* (14.91 ± 0.11 g ETA / 100 g DM) and *Piliostigma reticulatum* (15.06 ± 0.6 g ETA / 100 g DM) and between those of *Adansonia digitata* (1.64 ± 0.4 g ETA / 100 g DM) and *Balanites aegyptiaca* (1.30 ± 0.10 g ETA / 100 g DM). As regards the flavonoid contents of the extracts of the trunk bark, they varied from 0.018 ± 0.001 g EQ / 100 g DM for extracts of *Balanites aegyptiaca* to 0.209 ± 0.005 g EQ / 100 g DM for extracts of *Cassia sieberiana*. There is no statistically significant difference ($p < 0.05$) between the flavonoid content of extracts of *Acacia nilotica* (0.207 ± 0.003 g EQ / 100 g DM) and *Cassia sieberiana* (0.209 ± 0.005 g EQ / 100 g DM), between *Gymnosporia senegalensis* (0.069 ± 0.0009 g EQ / 100 g DM) and *Piliostigma reticulatum* (0.07 ± 0.007 g EQ / 100 g DM).

3.1.2 Antioxidant's activities in plant extracts

The antioxidant activity of the aqueous extracts of the bark of the trunks of different medicinal plants was evaluated using the DPPH free radical scavenging method and the method of the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). The results of the antioxidant activities are shown in Fig. 2. The anti-free radical activity by the DPPH method of the different plants ranged from 0.069 ± 0.006 AAE for *Balanites aegyptiaca* to 1.08 ± 0.03 AAE for *Acacia nilotica*. There is no statistically significant difference ($p < 0.05$) between the anti-free radical activities of extracts from the trunk bark of *Bombax costatum* (0.518 ± 0.001 AAE), *Cassia sieberiana* (0.544 ± 0.05 AAE), *Daniellia oliveri* (0.503 ± 0.03 AAE), *Khaya senegalensis* (0.5 ± 0.02 AAE), and *Piliostigma reticulatum* (0.534 ± 0.09 AAE) and between *Tamarindus indica* (0.263 ± 0.08 AAE) and *Adansonia digitata* (0.27 ± 0.002 AAE). The reducing capacity of Fe3 + in Fe2 + of the extracts varied from 0.0023 ± 0.0006 AAE for *Balanites aegyptiaca* to 0.107 ± 0.03 AAE for *Acacia nilotica*. There is a statistically significant difference ($p < 0.05$) between extracts of *Daniellia oliveri* (0.077 ± 0.003 AAE), *Khaya senegalensis* (0.063 ± 0.002 AAE), *Gymnosporia senegalensis* (0.0704 ± 0.0009 AAE) and *Piliostigma reticulatum* (0.059 ± 0.007 AAE), between extracts of *Bombax costatum* (0.040 ± 0.001 AAE) and *Cassia sieberiana* (0.032 ± 0.005 AAE) and finally between extracts of *Tamarindus indica* (0.016 ± 0.007 AAE), *Adansonia digitata* (0.0063 ± 0.0002 AAE) and *Balanites aegyptiaca* (0.0023 ± 0.0006 AAE).

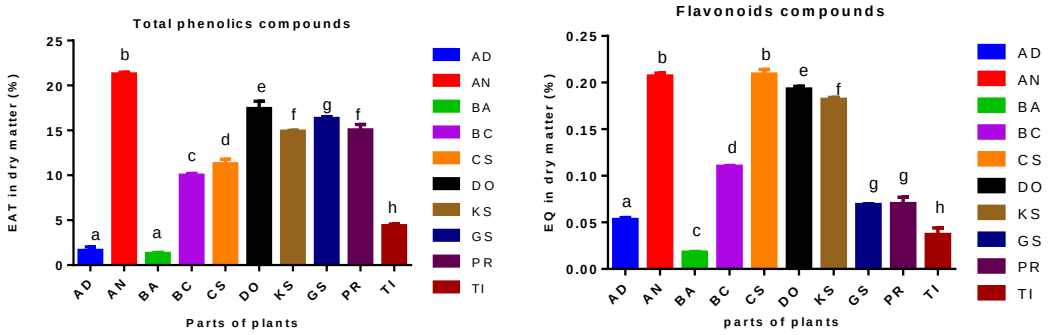


Fig. 1. Total phenolic and flavonoid content of extracts from different plants

**AD: Adansonia digitata, AN: Acacia nilotica, BA: Balanites aegyptiaca, BC: Bombax costatum, CS: Cassia sieberiana, DO: Daniellia oliveri, KS: Khaya senegalensis, GS: Gymnosporia senegalensis, PR: Piliostigma reticulatum, TI: Tamarindus indica; Test drugs: significant from each drug, * P < 0.05; Mean ± S.E.M = Mean values ± Standard error of means of 3 experiments*

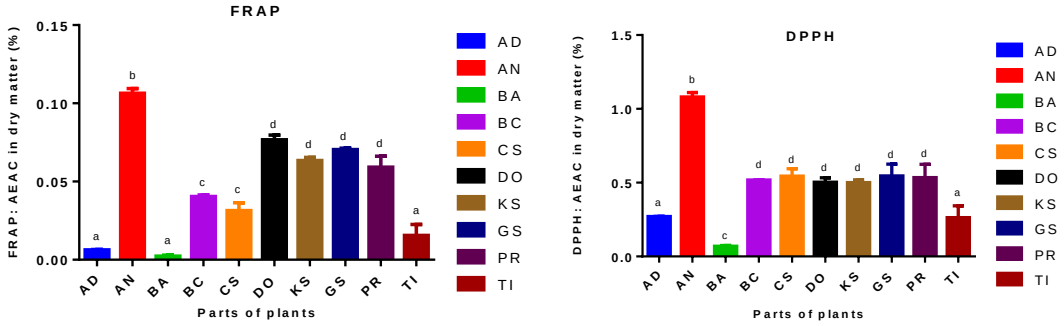
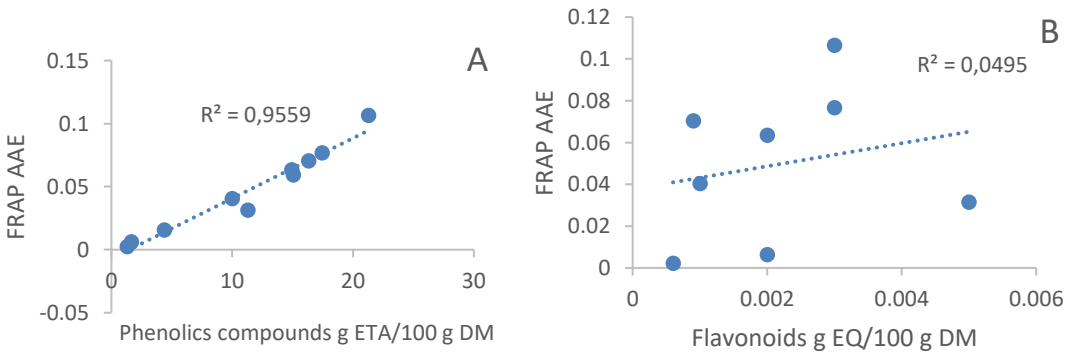


Fig. 2. Antioxidant activity by the FRAP and DPPH method of extracts from different plants

*AD: Adansonia digitata, AN: Acacia nilotica, BA: Balanites aegyptiaca, BC: Bombax costatum, CS: Cassia sieberiana, DO: Daniellia oliveri, KS: Khaya senegalensis, GS: Gymnosporia senegalensis, PR: Piliostigma reticulatum, TI: Tamarindus indica; Test drugs: significant from each drug, * P < 0.05; Mean ± S.E.M = Mean values ± Standard error of means of 3 experiments*

3.1.3 Relationship between compound content and antioxidant activity

The correlation between the contents of total phenolics, flavonoids and antioxidant activities by DPPH and FRAP is shown in Fig. 3. A weak correlation was found between DPPH and total phenolics (r^2 = 0.5054) and between DPPH and flavonoids (r^2 = 0.6245). A strong correlation was found between FRAP and total phenolics (r^2 = 0.9559) and an r^2 = 0.0495 observed between FRAP and flavonoids.



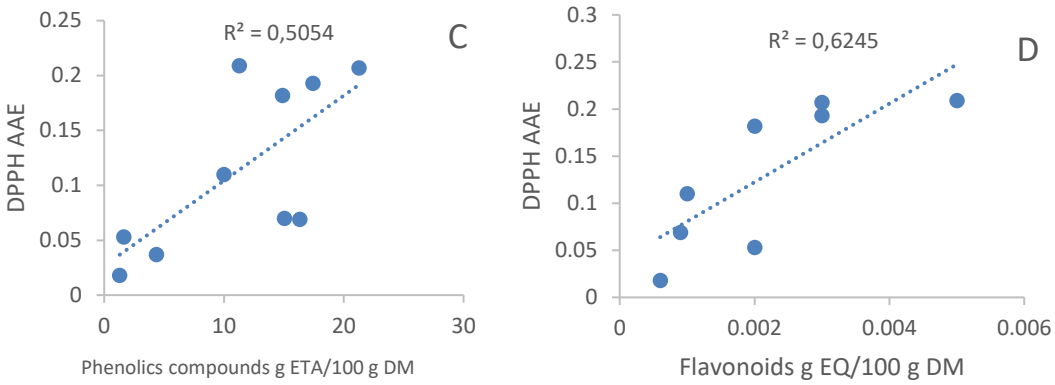


Fig. 3. Correlation between FRAP and the total phenolics content (A) and between FRAP and total flavonoid content (B); Correlation between DPPH and the total phenolics content (C) and between DPPH f and total flavonoid content (D)

3.2 Discussion

The RMC of the powders of the trunk bark of the different plants was less than 10 taking into account the storage conditions of the samples after drying and spraying. This low humidity would indicate better storage and it helps prevent possible enzymatic reactions as well as the development of microorganisms [12]. In fact, too high a water content could promote the deterioration of the microbiological quality, enzymatic reactions having consequences on the variation of secondary metabolites during the shelf life of the plant material powder. Thus, residual humidity would also promote the proliferation of microorganisms such as bacteria, yeasts and molds [13].

Phenolic compounds are biologically active molecules which are widely used in therapy as vasoconstrictors, anti-inflammatories, enzymatic inhibitors, antioxidants and anti-radicals, antimicrobials, etc. Found in all parts of plants (roots, stems, leaves, flowers, pollens, fruits, grains and wood), phenolic compounds form the most important group of phytochemicals in plants [14]. The levels of phenolic compounds vary from species to species, from part of the plant to another and depending on edaphic factors as well [15]. A comparison of the contents of phenolic compounds, in particular the total phenolics and the flavonoids of the aqueous extracts of the bark of the trunks of different plants from the same family or not, is very important in the choice of the plant for possible pharmacological activities. The determination of total phenolics showed that *Acacia nilotica* had the highest levels followed by *Daniellia oliveri* and that the lowest levels were with *Balanites aegyptiaca* and *Adansonia digitata*. These results corroborate those of Boly et al. [16] who had found higher levels of phenolic compounds with extracts of *Acacia nilotica* and those of Traoré et al. [17] who after a comparative study on 5 plants found that *Balanites aegyptiaca* had the lowest levels. By comparing the flavonoid contents of the 10 species, it turns out that it is *Acacia nilotica* and *Cassia sieberiana* which gave the best levels and the lowest level always with *Balanites aegptiaca*. These results always corroborate those of the two authors who in their respective studies had similar results. These phenolic compounds are the main compounds having their aromatic nucleus which allows them to stabilize and relocate unpaired electrons from their structures by giving up hydrogen atoms and electrons from their hydroxyl groups [15]. “They are known to act as antioxidants not only because of their ability to donate electrons, but also because of their stable radical intermediates, which can effectively prevent oxidation at the cellular and physiological level” [18].

Phenolic compounds, in particular phenols, can adjust the concentration of ROS, thus activating a network of biochemical events to increase tolerance, hence the importance of studying the antioxidant activity of plant species [15]. The two methods used make it possible to have a certain complementarity of the antioxidant activities of different plants. The results of the antioxidant activities by the two methods (DPPH and FRAP) showed that the aqueous extracts of *Acacia nilotica* had the best activities and the most activities were with the aqueous extracts of

Balanites aegyptiaca. Several authors have shown that Acacia nilotica extracts have good antioxidant activity [16, 19]. The work of Traoré et al. showed low antioxidant activity in aqueous extracts of *Balanites aegyptiaca* [17]. Six plants had the same statistically significant activities by the DPPH method and among the six there are four which have the same activity by the FRAP method. The difference between the antioxidant activities of the different ones is probably due to their composition and their content of phenolic compounds and this reduction of the DPPH radical and the ferric ions to ferrous ions is generally not due to the action of a single compound but to the interactions that may exist from one extract to another [20]. "*Acacia nilotica* which had the high contents of total phenolics and flavonoids, gave the best antioxidant activities and *Balanites aegyptiaca* which had the lowest contents, also gave the lowest antioxidant activities. The high antioxidant capacities of the extracts of these plants would explain their ability to trap ROS. Several herbal studies have found a correlation between total phenolics and antioxidant power" [21, 22]. "Phenolic acids, flavonoids and tannins are considered to be the main factors contributing to the antioxidant activity of medicinal plants. Indeed, the redox properties of these phenolic compounds would allow them to act as reducing agents, hydrogen donors, singlet oxygen deactivators and also metal chelators" [23]. "Indeed, a good linear correlation between total phenolics and antioxidant activity would show that most of the antioxidant activity is due in part to the presence of a large part of total phenolics" [8]. Phenolic compounds could probably act, in synergy or in isolation, through their antioxidant properties to protect hepatocytes against free radicals and reactive oxygen forms generated by attacks in the body.

4. CONCLUSION

This study highlighted the antioxidant capacity of the aqueous extract of the trunk bark of 10 plants used in traditional medicine for the treatment of liver pathologies. All plants had antioxidant activities that were proportional to the levels of phenolic compounds they contain and these varied from one plant to another. Seven (07) plants stood out due to their activity and among these, *Acacia nilotica* had the best antioxidant capacity and the highest phenolic compound content. These plants could be the subject of in-depth research to evaluate their anti-inflammatory or even hepatoprotective activity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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