Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2021; 13(4); 22-28
ISSN: 0975-4873

Research Article

Chemical and Anti-Free Radical Constituents of Three Organs of *Entada Africana*. Guill and Perr (Fabaceae), Used in the Traditional Treatment of Hepatic Disease

Doumbia S[1]*, Fofie NBY[2], Haidara M[1], Dembele DL[1], Denou A[1], Sanogo R[1,3]

[1]*Faculty of Pharmacy, University of Science, Technical and Technology of Bamako (USTTB), PO Box 1805 Mali*
[2]*Laboratory of Pharmacognosy UFR Pharmaceutical and Biological Sciences, University Félix Houphouët Boigny, BP V 34 Côte d'Ivoire*
[3]*Department of Traditional Medicine, Bamako, BP 1746 Mali*

Received: 20th Oct, 21; Revised 9th Nov, 21; Accepted: 15th Dec, 21; Available Online:25th Dec, 21

ABSTRACT
Entada africana is used in the management of jaundice and liver problems. Preclinical and clinical studies have confirmed the effectiveness of root extracts in relieving jaundice and hepatitis. The objective of the present study was to characterize the chemical and anti-free radical constituents of the leaves, trunk bark and roots of the plant. Color tube reactions and thin layer chromatography were used to characterize the chemical and anti-free radical constituents by reduction of the 1,1-diphenyl-2-picrylhydrazyl radical. The inhibitory concentration 50 (IC_{50}) of the anti-free radical constituents of the extracts was determined with a spectrophotometer. The major constituents found are saponosides, polyphenols, coumarins, sterols and triterpenes. The decoction of the trunk bark and the hydroethanolic extract of the leaves are the most active with IC_{50} of 2.535 and 2.588 µg / ml respectively. Extracts rich in anti-free radical constituents may contribute to the antioxidant, anti-inflammatory and hepatoprotective properties of *Entada africana* extracts in the management of liver diseases.

Keywords: Mali - *Entada africana* - saponosides - polyphenols - anti-free radical constituents' hepatic diseases.

INTRODUCTION

Entada africana is a very widespread plant in Africa[1]. It is used for the management of numerous pathologies including hepatitis[2]. The powdered roots are febrifuge, used against jaundice, snake bites, arthritis, malaria and anemia[3]. In Senegal, the trunk bark is used as a cough suppressant, for the treatment of bronchitis, antiseptic, healing wounds and injuries[4]. The leaves in direct application prevent suppuration of wounds and as an infusion to treat stomach aches[3].

In Mali, the Department of Traditional Medicine (DTM) has conducted preclinical and clinical studies to confirm the use of roots in the management of liver diseases[5-9]. The results have enabled the development of the Improved Traditional Medicinal Product (ITM) SAMNERE® based on the roots of the plant, used in the management of liver diseases. The roots of *Entada africana* have since been the subject of much study. Those concerning phytochemistry, carried out particularly on the roots' bark and to a lesser extent on the trunks' bark and the leaves of *Entada africana*, made it possible to characterize in their extracts polyphenols, flavonoids, tannins, saponosides, sterols and triterpenes, cardiotonic heterosides, alkaloids, coumarins, anthraquinones, leucoanthocyanins, carbohydrate[7,8,10-12]. Bioactive constituents have been isolated. They are polysaccharides[13], flavonoids (apigenin, robinetin, naringenin-7- O-glucoside, myricetins)[14,15], saponins[16], sterols (β-sitosterol, α-spinasterol)[17,18] and triterpenes (butelin)[19].

The extracts and some bioactive constituents have shown properties; hepatoprotective[6,20,21], complement fixing[13], antiproliferative[16,22], anti-free radical[23], antiulcer[24], hemolytic[25], antibacterial and antifungal[25-27], antiplasmodial[25,28], anti-inflammatory[29,30], antivirals[31], anti-angiogenic[15], induction of nuclear translocation[20,21], against endometriosis[32], and inhibition of cytochromes P450 2E1[21].

Faced with the significant uses of the roots of *Entada africana* to prepare SAMNERE®, which is in great demand for the treatment management of jaundice and hepatitis, it is important to analyze the other organs of the plant.

The aim of this study was to characterize the chemical and anti-free radical constituents of the leaves, trunk bark and roots of *Entada africana* from the Kati region.

MATERIAL AND METHODS

1. Material

1.1 Plant material

The leaf, trunk bark and root samples were collected in Kati (15 km from Bamako) in November 2019. They were then identified by the ethnobotany and raw materials department of DMT. Specimens of the drugs are available at the DMT herbarium under the numbers; Herbarium No. 2368 / DMT, Drugstore No. 0046 / DMT and

**Author for Correspondence:* s2doumbia@yahoo.fr

Drugstore No. 0047 / DMT; for leaves, trunk bark and roots respectively. The samples were allowed to dry in the shade for 4 weeks and then crushed with a Resch 143O upm crusher.

1.2 Technical material

They are composed of: SARTORIUS type analytical balance, oven set at 103° C plus or minus 2°C, electric oven set at 200° C, water bath Buchi 461, rotary evaporator Büchi R-200, freezer type Zanker, freeze dryer Heto Drywinner, visible UV spectrophotometer Zanway type, Aluminum plate with silica gel 60 F_{254} Merck as support, Glass chromatographic tank, dryer Solis type.

1.3 Reagents and solvents

The solvents and reagents are in particular: water, ethanol at 70 °, methanol, petroleum ether, dichloromethane, DPPH (2,2-diphenyl-1-picrylhydrazyl), butanol, acetic acid, methyl-ethyl-ketone, ethyl acetate, formic acid. All reagents were analytical grade.

2. Methods

2.1 Drug quality control

The water content was determined by drying in an oven at 103 ± 2 ° C for 24 hours. The total ash contents and ash insoluble in 10% hydrochloric acid were determined by calcination in an oven at 600 ° C for 6 hours[33].

2.2 Extraction

- **Aqueous extracts**

o **Decoction**

A volume of 500 ml water was added to 50 g of drug powder. The whole was brought to a boil for 15 minutes. The mixture was filtered through a compress after cooling. The filtrate obtained was concentrated using a rotary evaporator and then lyophilized. The yield was calculated.

o **Infusion**

This method used 500 ml of boiling water which was poured over 50 g of sample powder. The whole was left for 15 minutes. The filtrate was concentrated, lyophilized then the yield was determined.

o **Maceration**

This preparation put 50 g of drug powder in 500 ml of water. The whole was left to macerate by stirring for 24 hours at room temperature before being filtered, concentrated, lyophilized and after yield was calculated.

- **Hydro ethanolic extract**

This extract was obtained by macerating 50 g of drug powder with 500 ml of 70o ethanol. The resulting filtrate was subjected to concentration, lyophilization before calculating the yield.

- **Extracts of organic solvents with increasing polarity**

The solvents used were petroleum ether, methylene chlorhide (MC), MC / methanol (50/50), methanol and water.

An amount of 50 g of the sample was macerated in an Erlenmeyer flask with 50 ml of petroleum ether under magnetic stirring for one hour before being filtered to give the petroleum ether extract. The same operation was repeated on the marc successively with methylene chlorhide (MC), a methylene chlorhide-methanol mixture (50:50) then methanol to obtain the corresponding extracts.

With water, a digestion at 50 ° C was carried out successively on the marc obtained after exhaustion with organic solvents, then a decoction with 50 mL of water each time. Each operation lasted 15 minutes. The extracts as above were concentrated at 50° C, lyophilized and yields was calculated.

2.3 Characterization of chemical and anti-free radical constituents

- **Chemical constituents**

The chemical constituents were characterized by tube reactions and by thin layer chromatography.

o **Tube reactions**

Tube reactions were used to characterize the chemical constituents of samples according to methods reported in the African Pharmacopoeia[34].

The results were classified according to the intensity of the reaction: strongly positive (+ + + +), positive (+ ++), moderately positive (++), weakly positive (+) or negative (-).

o **Thin layer chromatography** (TLC)

With the exception of the infused ones, all extracts were identified on the TLC. The TLC plate consisted of 0.25 mm thick silica gel 60 F_{254} spread on aluminum foil.

The solutions to be analyzed were made up on one hand by the organic extracts dissolved in their corresponding solvents and on the other hand by the aqueous extracts dissolved in a water / methanol mixture (1/1). From each reconstituted solution, 10 µl were placed on the chromatography plates and then dried. These chromatography plates thus prepared were introduced into the migration tanks.

The migration was carried out in two systems. One was Petroleum Ether - Ethyl Acetate (1: 1) and the other, Ethyl Acetate - Methyl Ethyl Ketone - Formic Acid - Water (5: 3: 1: 1)

After migration, the plates were dried and revealed by Godin's reagent (polyvalent) or ferric chloride (specific for tannins and other polyphenolic compounds)[35].

- **Anti-free radical constituents**

The extracts used were the aqueous (infused, decoction, macerated) and hydroethanolic extracts.

o **Qualitative determination of the anti-free radical constituents**

Chromatograms were performed as usual. Only one migration solvent system was used, ethyl acetate, methyl ethyl ketone, formic acid, water (50: -30: 10: 10). The plates, after migration and drying revealed, with a methanolic solution of DPPH at 2 mg / ml, the anti-free radical constituents as yellow stains on a purple background[35,36].

o Quantitative determination of the anti-free radical constituents

It was carried out according to the method described by **Velázquez and al.**[37], slightly modified. Only the extracts rich in anti-free radical constituents were used.

A volume of 1400 microliters of the methanolic solution of DPPH at 25 mg / L was added to 700 microliters of extract at concentrations varying between 0.1 and 100 µg

/ mL. The prepared solutions were stirred and then incubated for 30 minutes in the dark.
The absorbance was measured at 517 nanometers. Methanol was used to set the spectrophotometer to zero. Gallic acid was used as a positive control. Three tests were carried out for each sample. The percentage inhibition of the DPPH radical was calculated according to the following equation:

$$\%\,\mathbf{Inhibition} = \frac{(\mathbf{A}-\mathbf{B}) \times \mathbf{100}}{\mathbf{A}}$$

With: A: Mean absorbance of the negative control,
B: average absorbance of the sample
The curves obtained from the percentages of inhibition made it possible to deduce the concentrations of extracts or gallic acid which resulted in the 50% reduction of the DPPH radical, denoted IC_{50}. Results were analyzed using Graph Pad Prism software.

RESULTS AND DISCUSSION

1. Extract

The extracts obtained after the extraction are the decocted, infused, aqueous macerated, the hydroethanolic macerates and the extracts of the solvents with increasing polarity (see **Table 1**).
The highest extraction yield was observed with leaf decoction (9.8%). For trunk bark and roots, the highest yields were obtained with hydroethanolic extract (9%) and infused (8.8%), respectively.
For the extraction with the solvents of increasing polarity, the yields range from 0.01% for the etheric extract of the trunk bark to 0.31% for the methanolic extract of the same organ. The yields of the aqueous extracts after exhaustion with organic solvents range from 0.013% for the root decoction to 1.08% for that of the leaves.
The root decoction showed a yield of 7.7%. This figure was lower than that obtained by Sanogo et al. (1998) who had a yield of 9.8% with the decoction of the same organ. The infused had the highest yield with root extracts at 8.8%. In 2016, Sogoba worked on 16 root samples and also had the best yields with the infused ones but most of their yields (37/46) were higher than ours.

Table 1: Yield (in percentage) of the extracts

Organs	Decocted	Infused	Aqueous macerat	EtOH 70% macerat	Solvents with increasing polarity					
					Ether	MC	MC/MeOH	MeOH	Digest	Decocted
Roots	7,7	8,8	7,6	7,2	0,02	0,02	0,03	0,016	0,30	0,013
Trunk bark	8	7,2	7,8	9	0,01	0,1	0,08	0,31	0,29	0,16
Leaves	9,8	8,4	8	8,6	0,11	0,09	0,11	0,21	0,46	1,08

EtOH : ethanol, MC : Methylene chlorhide, MeOH ; methanol

2. Physicochemical quality of drugs

The results of the water and ash contents are reported in Table II.
The water contents of the various samples were all less than 10%. The various drugs were therefore well dried.
The total ash content was highest in the leaves followed by trunk bark and roots (see **Table 2**). The ash insoluble in hydrochloric acid content was very low (<1%) in all three samples (see **Table 2**). This could be due to a low contamination of the plant material by siliceous elements (sand, dust).
Sangaré (2005)[8] had carried out studies on three root samples and also found water contents less than 10%, and total ash and hydrochloric ash contents of 3.9; 4; 7% and 0.4; 1.0 and 2.8 respectively. The results obtained with the roots are also similar to those of Sogoba (2016)[10] who worked on 16 samples of *E afriacana* roots and obtained total ash contents between 6.73 and 2.65 and hydrochloric ash contents less than 1%. On the other hand, a slight difference with the results of Sidibé (2020) concerning the hydrochloric ash contents is to be noted. Indeed, Sidibé's results showed that out of 8 samples analyzed, 3 had hydrochloric ash contents greater than 1%, ie 1.67; 1.33 and 2.17%. This could be due to the contamination of these samples by siliceous elements.

Table 2: The water and ash contents of the roots, trunk bark and leaves of *Entada africana*

Organs	Content (%)		
	Water	Total ash	HCL ash 10 %
Roots	5,40	3,52	0,26
Trunk bark	7,97	4,22	0,53
Leaves	6,60	4,83	0,40

HCl: hydrochloric ash

3. Chemical constituents

3.1 According to tube reactions

The chemical constituents characterized on the different samples are reported in **Table 3.**

Coumarins, saponosides, tannins, sterols and triterpenes are present in all samples analyzed. However, in our analysis, flavonoids are not found in the trunk bark. The same is true for the detection of anthracenosides and leucoanthocyanins in the leaves and the detection of alkaloids in all the samples (see **Table 3**).

The results obtained for the leaves are similar to those of Tibiri et al.[23] on the other hand, they are slightly different from those of Olanrewaju et al.[38] and Obidike and Emeje[24] who found the presence of alkaloids.

The results obtained for the trunk bark are slightly different from those of Njayou et al.[20] and Tibiri et al.[23] who found the presence of flavonoids. The presence of alkaloids and anthraquinones in trunk bark[39] is contradictory to our results.

The results obtained for the roots are similar to those of Sangaré[8] and Tibiri et al.[23] on the other hand, they are slightly different from those of Sogoba[10] who did not find the presence of flavonoids in the roots of *Entada africana* collected in sixteen localities in Mali, Ba[7] had obtained positive reactions with alkaloids while working on the roots of this plant.

Table 3: Chemical constituents characterized in leaves, trunk bark and roots

Chemical groups	Roots	Trunk bark	Leaves
Alkaloids	-	-	-
Free anthracenosides	+	-	-
Coumarins	+	+	+
Flavonoids	+++	-	++
Leucoanthocyans	++	+++	-
Saponosides (foam index)	+++	++	++++
Sterols and triterpenes	+++	++	++
Tanins	++	++	++++

++++: Strongly positive reaction +++: Positive reaction ++: Moderately positive reaction +: weakly positive -: Negative reaction

3.2 According to the TLC

TLC also showed the presence of chemical constituents in the samples.

The purple, green, red and yellow colorations observed on the chromatogram of the extracts revealed with Godin's reagent could be compounds with a triterpene or steroid genin, tannins and flavonoids, respectively (**Figure 1**). The blackish colorings observed on the chromatogram of the extracts revealed with $FeCl_3$ could be tannins and other polyphenols (**Figure 2**). Sangaré[8] had also found greenish colorations (compounds with steroid genin) on chromatograms of ethanolic extracts of roots revealed by Godin's reagent. These results also agree with those of Sogoba[10] who also found the presence of tannins and other polyphenolic compounds, saponosides and other compounds with a steroid or triterpene genin on chromatograms of aqueous and organic extracts of *E africana* roots revealed with the $FeCl_3$ and Godin's reagent.

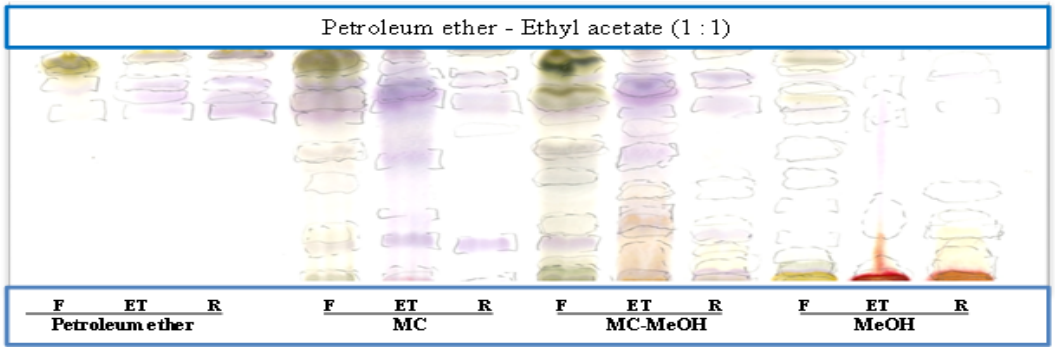

Figure 1: Chromatogram of extracts from the different organs of *E africana* revealed with Godin's reagent F: leaf, ET: trunk bark, R: root, MC: methylene chlorhide, MeOH: Methanol

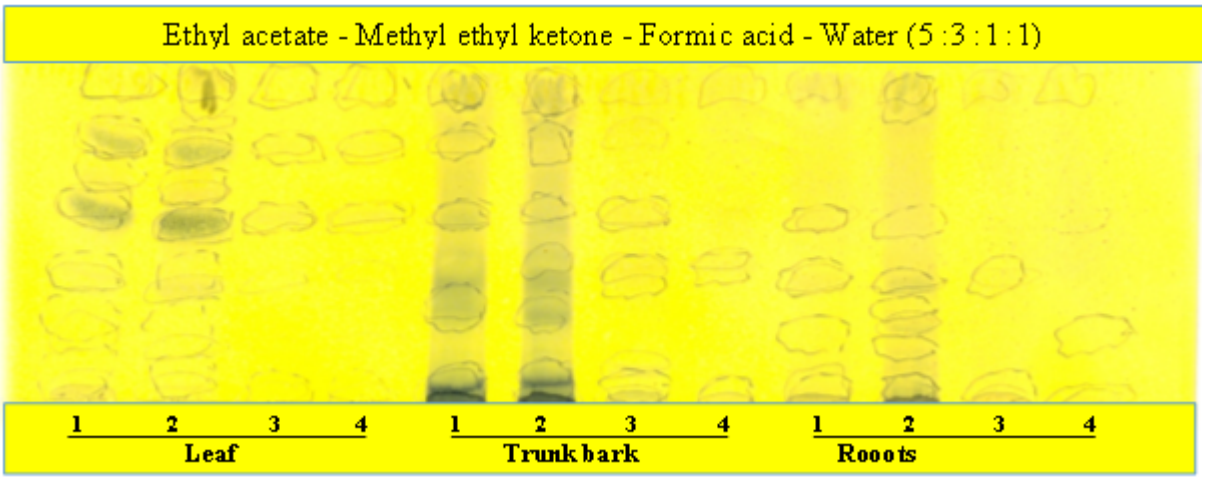

Figure 2: Chromatogram of aqueous and ethanolic extracts of leaves, trunk bark and roots of *E africana* revealed with $FeCl_3$

1: Decocted 2: Ethanolic extract 3: Digested 4: Decocted exhausted

4. Antioxidant constituents

The chromatograms revealed with DPPH showed several yellow spots on a purple background, which testifies to the richness of the extracts in anti-free radical constituents. All the extracts are rich in it as shown on the chromatogram (**Figures 3**); but the ethanolic leaf and root extracts, the decoction and the infused trunk bark showed the best anti-free radical activities.

With the quantitative determination of the anti-free radical constituents, the infused trunk bark and the ethanolic leaf extract are the most active with the respective IC_{50} of 2.535 ± 1.24 and 2.588 ± 0.51 µg / mL. The trunk bark decoction showed an IC_{50} of 4.332 ± 0.49 µg / ml while the ethanolic root extract was the least active at 31.29 ± 9.96 µg / mL. The reference IC_{50} for gallic acid was 1.435 ± 0.19 µg / mL (**Table IV**).

Many studies have also demonstrated the anti-free radical activity of leaves[23,25], roots[23] and trunk bark[20,23] of *Entada Africana.*

The richness of our extracts in polyphenolic substances and saponosides could contribute to this anti-free radical activity. The anti-free radical and antioxidant activity of these chemical groups has been demonstrated by authors including Quideau et al.[40] and Baguia-Broune et al.[41].

Studies have already demonstrated the role of anti-free radical in pathologies such as inflammatory diseases and liver diseases.[42,43] The antioxidant activity of the extracts could therefore be beneficial in the management of hepatic diseases.

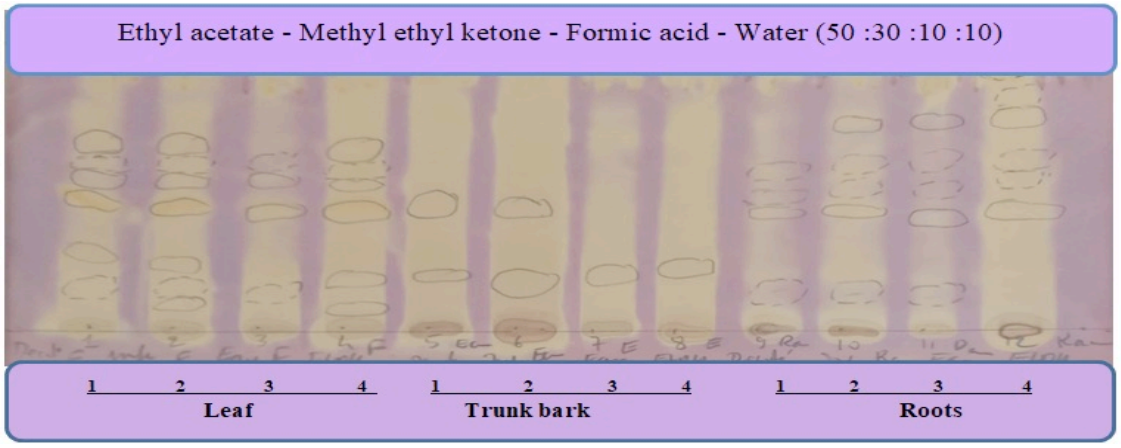

Figure 3: Chromatogram, of the different extracts of the three organs of *Entada africana*, migrated in the Acoet-MEC-

AF-Water solvent system (50: 30: 10: 10) and revealed with DPPH
1: decocted 2: infused 3: macerated water 4: macerated 70% ethanol

Table 4: Inhibitory concentration (IC_{50}) of the extracts rich in anti-free radical constituents

EXTRACT	Leaf EtOH	Trunk bark dec.	Trunk bark Inf.	Root EtOH	**Gallic acid**
IC_{50} (µg/mL)	**2,588 ± 0,51**	4,332 ± 0,49	**2,535 ± 1,24**	31,29 ± 9,96	**1,435 ± 0,19**

EtOH: ethanolic extract; Dec: decocted, Inf: infusion

CONCLUSION

Extracts from the leaves, trunk bark and roots of *Entada africana* are rich in anti-free radical constituents. Extracts rich in anti-free radical constituents may contribute to the antioxidant, anti-inflammatory and hepatoprotective properties of *Entada africana* extracts in the management of liver diseases.

ACKNOWLEDGMENT

The authors appreciate the technical assistance of the Traditional Medicine Department.

This work benefited from financial and material support from the Training of Trainers Program (TTP) which was obtained through the University of Sciences, Techniques and Technologies of Bamako (USTTB)

The spectrophotometer used is a donation to the Traditional Medicine Department within the framework of the IFS 3717-2 grant from Pr Rokia SANOGO, in 2007

REFERENCES

1. Maydell H von. Arbres et arbustes du Sahel: leurs caractéristiques et leurs utilisations. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ)/GmbH, Eschborn. 1983;
2. Adjanohoun, E., M.R.A. Ahyi, J.J. Floret , S. Guinko, M. Koumaré, A. M. R. Ahyi, J. Raynal,. Contribution aux études ethnobotaniques et floristiques au Mali. Paris, Agence de Coopération Culturelle et Technique. Paris; 1981. 291.
3. Malgras D. In: Arbres et arbustes guérisseurs des savanes maliennes. Paris: Edits. Karthala; 1992.
4. Kerharo J, Adams J. In: La Pharmacopée Senegalaise Traditionnelle Plantes médicales et Toxiques. Paris: Edition Vigot et Frères; 1974.
5. Douaré I. Contribution à l'étude clinique d'une préparation traditionnelle utilisant les racines de Entada africana (Guil et Perr). [Thèse de Médecine]. [Mali]: Bamako; 1991.
6. Sanogo R, Germanò MP, D'Angelo V, Guglielmo M, De Pasquale R. Antihepatotoxic properties of Entada africana (Mimosaceae). Phytotherapy Research. 1998;12(S1):S157‐9.
7. Ba A. Contribution à l'Étude de Quelques Plantes Molluscicides du Mali. These, Pharmacie, Bamako. 1998;90.
8. Sangaré O. Evaluation de Cochlospermum tinctorium, Entada africana et Combretum micranthum dans le traitement des hépatites à Bamako. Thèse de doctorat, Université de Bamako, Mali; 2005.
9. Doumbia M. Evaluation clinique de l'hépatite « B » sous traitement traditionnel [Doctorat d'Etat en Médecine]. [Mali]: Bamako; 2006.
10. Sogoba MN. Contrôle de qualité du MTA SAMANERE : racines de Entada africana (Guill. Et Perr), (Léguminosae) récoltées dans seize localités du Mali. [Thèse de Pharmacie]. [Mali]: Bamako; 2016.
11. Mbatchou VC, Ayebila AJ, Apea OB. Salmonella typhi. Journal of Animal & Plant Sciences. 2011;10(1):1248‐58.
12. Baidoo MF, Asante-Kwatia E, Mensah AY, Sam GH, Amponsah IK. Pharmacognostic characterization and development of standardization parameters for the quality control of Entada africana Guill. & Perr. Journal of Applied Research on Medicinal and Aromatic Plants. 2019;12:36‐42.
13. Diallo D, Paulsen BS, Liljebäck TH, Michaelsen TE. Polysaccharides from the roots of Entada africana Guill. et Perr., Mimosaceae, with complement fixing activity. Journal of Ethnopharmacology. 200; 74(2):159‐71.
14. Montoro P, Braca A, Pizza C, De Tommasi N. Structure–antioxidant activity relationships of flavonoids isolated from different plant species. Food chemistry. 2005;92(2):349‐55.
15. Germanò MP, Certo G, D'Angelo V, Sanogo R, Malafronte N, De Tommasi N, et al. Anti-angiogenic activity of Entada africana root. Natural product research. 2015;29(16):1551‐6.
16. Cioffi G, Dal Piaz F, De Caprariis P, Sanogo R, Marzocco S, Autore G, et al. Antiproliferative triterpene saponins from Entada africana. Journal of natural products. 2006;69(9):1323‐9.
17. Hassan LG, Mshelia HE, Umar KJ, Kangiwa SM, Ogbiko C, Yusuf AJ. Phytochemical Screening, Isolation and Characterization of Beta-Sitosterol from ethyl acetate Extract of Stem Bark of Entada africana (Fabaceae) Guill. et Perr. Journal of Chemical Society of Nigeria. 2018;43(3).
18. Kwaji A, Adamu H, Chindo I. Isolation and characterization of Stigmasta-7, 22-dien-3-ol (α-Spinasterol) from Entada africana stem bark crude extract. Journal of Applied and Advanced Research. 2019;4(3):85‐8.
19. Kwaji A, Adamu HM, Chindo IY, Atiko R. Isolation, characterization and biological properties of betulin from Entada africana Guill. and Perr.(Mimosaceae). Journal of Applied and Advanced Research. 2018;3(2):28‐31.
20. Njayou FN, Kouam AF, Simo BFN, Tchana AN, Moundipa PF. Active chemical fractions of stem bark extract of Khaya grandifoliola C. DC and Entada africana Guill. et Perr. synergistically protect primary rat hepatocytes against paracetamol-induced damage. BMC complementary and alternative medicine. 2016;16(1):1‐11.
21. Kouam AF, Owona BA, Fifen R, Njayou FN, Moundipa PF. Inhibition of CYP2E1 and activation of Nrf2 signaling pathways by a fraction from Entada africana alleviate carbon tetrachloride-induced hepatotoxicity. Heliyon. 2020;6(8):e04602.
22. Haïdara M. Caractérisation sur un modèle cellulaire d'hépatocarcinome humain de l'effet cytotoxique des extraits de Entada africana Guill et Perr., Erythrina senegalensis DC. et Securidaca longipedunculata

Fresen utilisés en médecine traditionnelle au Mali [Master 2 Recherche en Pharmacologie]. [Toulouse]: Université Paul Sabatier; 2013.
23. Tibiri A, Sawadogo RW, Ouedraogo N, Banzouzi JT, Guissou IP, Nacoulma GO. Evaluation of antioxidant activity, total phenolic and flavonoid contents of Entada africana Guill. et Perr.(Mimosaceae) organ extracts. Research Journal of Medical Sciences. 2010;4(2):81‑7.
24. Obidike IC, Emeje MO. Microencapsulation enhances the anti-ulcerogenic properties of Entada africana leaf extract. Journal of ethnopharmacology. 2011;137(1):553‑61.
25. Karou SD, Tchacondo T, Ouattara L, Anani K, Savadogo A, Agbonon A, et al. Antimicrobial, antiplasmodial, haemolytic and antioxidant activities of crude extracts from three selected Togolese medicinal plants. Asian Pacific Journal of Tropical Medicine. oct 2011;4(10):808‑13.
26. Kwaji A, Adamu HM, Chindo IY. Phytochemical analysis, Antibacterial and Antioxidant activities of Entada africana Guill. and Perrott Stem bark extracts. Research Journal of Chemical Sciences. ISSN. 2017;7(10):10‑5.
27. Tchana ME, Fankam AG, Mbaveng AT, Nkwengoua ET, Seukep JA, Tchouani FK, et al. Activities of selected medicinal plants against multi-drug resistant Gram-negative bacteria in Cameroon. African Health sciences. 2014;14(1):167.
28. Ezenyi IC, Ranarivelo L, Oluwakanyinsola SA, Emeje M. Analgesic, anti-inflammatory, and heme biomineralization inhibitory properties of Entada africana ethanol leaf extract with antiplasmodial activity against Plasmodium falciparum. Journal of basic and clinical physiology and pharmacology. 2013;25(2):217‑23.
29. Owona BA, Njayou NF, Laufer S, Moundipa PF, Schluesener HJ. A fraction of stem bark extract of Entada africana suppresses lipopolysaccharide-induced inflammation in RAW 264.7 cells. Journal of ethnopharmacology. 2013;149(1):162‑8.
30. Owona BA, Njayou NF, Laufer SA, Schluesener HJ, Moundipa PF. Entada africana fraction CH 2 Cl 2/MEOH 5% inhibits inducible nitric oxide synthase and pro-inflammatory cytokines gene expression induced by lipopolysaccharide in microglia. BMC complementary and alternative medicine. 2013;13(1):1‑7.
31. Galani Tietcheu BR, Sass G, Njayou NF, Mkounga P, Tiegs G, Moundipa PF. Anti-hepatitis C virus activity of crude extract and fractions of Entada africana in genotype 1b replicon systems. The American journal of Chinese medicine. 2014;42(04):853‑68.
32. Mvondo MA, Minko Essono S, Bomba Tatsinkou FD, Ateba SB, Njamen D. The root aqueous extract of Entada africana Guill. Et Perr.(Mimosaceae) inhibits implant growth, alleviates dysmenorrhea, and restores ovarian dynamic in a rat model of endometriosis. Evidence-Based Complementary and Alternative Medicine. 2017;2017.
33. Somboro AA, Patel K, Diallo D, Sidibe L, Chalchat JC, Figueredo G, et al. An ethnobotanical and phytochemical study of the African medicinal plant Guiera senegalensis JF Gmel. Journal of Medicinal Plants Research. 2011;5(9):1639‑51.
34. Pharmacopoeia A. General methods of analysis 1st edition. Organization of African Unity Scientific Technical and Research Commission (OAU/STRC) Lagos, Nigeria. 1986;2:142.
35. Marston A. Thin-layer chromatography with biological detection in phytochemistry. Journal of Chromatography A. 2011;1218(19):2676‑83.
36. Blois M. Antioxidant determinations by the use of stable free radicals. Nature. 1958;181(4617).
37. Velázquez E, Tournier HA, De Buschiazzo PM, Saavedra G, Schinella GR. Antioxidant activity of Paraguayan plant extracts. Fitoterapia. 2003;74(1‑2):91‑7.
38. Olanrewaju CA, Ahmed F. Proximate analysis and phytochemical screening of some medicinal plants commonly used by Guaris of Fct, Nigeria. International Journal of Current Research. 2014;6(6):6964‑7.
39. Ifemeje JC, Egbuna C, Udedi SC, Iheukwumere HI. Phytochemical and in vitro antibacterial evaluation of the ethanolic extract of the stem bark of Entada africana Guill. & Perr and Sarcocephalus latifolus. International Journal of Biochemistry Research & Review. 2014;4(6):584.
40. Quideau S, Deffieux D, Douat-Casassus C, Pouységu L. Plant polyphenols: chemical properties, biological activities, and synthesis. Angewandte Chemie International Edition. 2011;50(3):586‑621.
41. Baguia-Broune FDM, N'Gaman-Kouassi KCC, Mamyrbekova-Békro JA, Virieux D, Békro Y-A. Saponines des racines de Securidaca longipedunculata (Polygalaceae): quantification et évaluation antioxydante. Nature & Technology. 2018;(19):26‑31.
42. Libetta C, Sepe V, Esposito P, Galli F, Dal Canton A. Oxidative stress and inflammation: implications in uremia and hemodialysis. Clinical biochemistry. 2011;44(14‑15):1189‑98.
43. Onu A, Saidu Y, Ladan MJ, Bilbis LS, Aliero AA, Sahabi SM. Effect of aqueous stem bark extract of Khaya senegalensis on some biochemical, haematological, and histopathological parameters of rats. Journal of toxicology. 2013;2013.