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Phytochemical screening, phenolic content and antioxidant activity of *Entada africana* and *Sterculia stigera* two plants used in the treatment of cough

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ABSTRACT

Objective: This study aim's was to determine the phytochemical constituents, the total phenol content and to perform the antioxidant activity of *Entada africana* Guill. & Perr. (Samanèrè, dimijama Bambara, sinnégo; mooré) and Sterculia setigera Del. (karaya gum tree, Ponsimponrgo, mooré), two plants used in folk medicine in Burkina Faso to control various diseases.

Methodology and results: The extracts were obtained by maceration in ethanol and hydroethanol, and by decoction. Phytochemical screening was performed by tube test method and thin layer chromatography (TLC). The Folin Ciocalteu Reagent technique was used to evaluate the content of phenolic compounds. Antioxidant activity was determined by ABTS and DPPH methods. Phytochemical composition showed the presence of tannins, alkaloids and flavonoids in the extracts. *S. setigera* was the plant that contained the highest content of phenolic compounds with a variation of 34.53 ± 1.75 to 3506.70 µg GAE/g extract. It recorded also the best free radical scavenging activity towards DPPH- radical with an IC50 of 0.011±0.0002 followed by the ethanol extract of *E. africana* with an IC50 of 0.019±0.0002. The same ethanol extract of *S. setigera* shows a very high antioxidant activity with an Antioxidant Activity Index (AAI) of 3.63 followed by the ethanol extract of *E. africana* with an AAI of 2.10.

Conclusion and applications of findings: The results of this work suggest that these plants are natural sources of antioxidant compounds and justify their use in traditional medicine. This study provides information that can be used as a lead in the discovery and development of new, more affordable and safer phytomedicines in the treatment of free radical and cough.
Keywords: phenolic compounds; antioxidant activity; *Sterculia setigera; Entada africana;* cough

INTRODUCTION

The free radicals are chemical species highly reactive and potentially damaging. Indeed, they are at the origin of various chronic human diseases such as cardiovascular diseases, neurodegenerative diseases, cancer, diabetes, sclerosis, which are considered as public health problems (Pajares *et al.,* 2018). Free radicals are also generated in large quantities during respiratory tract infections such as cough (Forcados *et al.,* 2021). These free radicals generated by the human body are eliminated or trapped by molecules with antioxidant properties (Hermes-Lima, 2005). The use of synthetic antioxidant molecules in various fields, which was supposed to provide a solution to this situation, has been widely criticised in recent years. Indeed, because of the potential health risks and toxicity they are capable of causing, the synthetic antioxidant molecules available are currently being questioned (Kicel *et al.*, 2016; Liu and Yang, 2018). It is therefore necessary to look for an alternative to the use of these synthetic antioxidants. Moreover, medicinal plants contain chemical compounds with a wide range of physico-chemical properties, which present a wide range of biological activities such as antitumour, antiviral, antiparasitic, antibacterial, antifungal, antioxidant, insecticidal, anti-inflammatory. (Bruneton, 2006). To this end, the search for natural substances with antioxidant activity derived from plants is a major scientific challenge. Hence the interest of this study, which aims to evaluate the antioxidant properties of extracts from the trunks of *Entada africana* and *Sterculia setigera* used in traditional medicine to fight against several diseases such as cough. *S. setigera* contains a gum that swells easily in water. It is capable of absorbing up to two hundred and fifty times its weight in water, hence its use in the preparation of certain compound medicines. It is not surprising in these conditions, that one recognizes with its bark of the emollient and calming properties put at profit in the treatment of the cough, the feverish states, the pneumopathies, vomiting, the enteropathies, oligurias, dysurias, coryza, bronchitis, diarrhoea, whooping cough, rickets, syphilis, measles, dermatitises and asthma (Musa *et al.,* 2011). *Entada africana* is used to cure several diseases in traditional medicine. In Senegal, the bark of the trunk is used as a cough suppressant, for the treatment of bronchitis, as an antiseptic and as a wound healer. The barks are also used against bronchitis, coughs, gonorrhoea, nephritis, rheumatism, diarrhoea, rickets in children, metrorrhoea, haemorrhoids, angina, glossitis, dry colic, wounds, trachoma and are used in the preparation of remedies for the treatment of angina and other mouth and throat diseases. The roots are used to treat hysteria, nervousness, anaemia and are reputed to be diuretic and anti-syphilic (Ahua *et al.,* 2007; Inngjerdingen *et al.,* 2004).

MATERIALS AND METHODS

Plant material: The plant material consisted of *E. africana* and *S. setigera* trunk barks collected in Gonsé and Zorgho in December 2016. This plant material was identified and authenticated in comparison with specimens in the national herbarium by KOURA S. Paulin, botanist at the herbarium of "Centre National de la Recherche Scientifique et Technologique) (CNRST). Reference specimens have been deposited at the CNRST herbarium.

Preparation of extracts: The cleaned plant material was dried in the laboratory at room temperature, protected from the sun for a fortnight, then ground into powder, and stored in clean bags. Extraction was performed by maceration with ethanol and ethanol-water (1:1 v/v) and by decoction. Thus, 10 grams of powdered plant material were successively extracted with 100 ml of ethanol and water-ethanol respectively under stirring for 24 hours. After filtration on cotton, the extracts obtained were concentrated under reduced pressure using a rotary evaporator (rotavapor Büchi R-134) to obtain dry extracts. As for the decoction, 10 g of the plant material in 100 ml of distilled water were boiled (100°C) for 30 minutes. After filtration, the extracts obtained were freeze-dried using a freeze-dryer. The extracts were kept at 4°C in a refrigerator. The extraction yield was determined (Landoulsi, 2018).

Phytochemical screening: Phytochemical screening by solution reactions in test tubes and by Thin Layer Chromatography was performed according to the classical methods (Ciulei, 1982; Pachaly, 1997).

Identification by Thin Layer Chromatography (TLC)

Identification of tannins: The eluent used was ethyl acetate, formic acid and acetic acid and water in the proportions (100: 11: 11: 26). After deposition and elution, the plates were dried and then sprayed with a 2% methanolic FeCl3 solution. At the end of the operation, the appearance of a greenish-blue spot in the light indicates the presence of tannins.

Identification of flavonoids: The mobile phase used for the identification of flavonoids was a mixture of ethyl acetate, formic acid, acetic acid and water in the proportions (100:11:11:26). At the end of the operation, the blue, yellow or yellow-orange coloration of the spots under UV at 365 nm is synonymous with the presence of flavonoids.

Identification of alkaloids: For alkaloids, the eluent used was a mixture of toluene, ethyl acetate and diethylamine in the proportions (70:20:10). After elution, the plates were dried and then sprayed with Dragendorff's reagent. At the end of the operation, the appearance of orange or orange-brown stains indicates the presence of alkaloids in visible light.

Identification of terpenes and sterols: For the identification of terpenes and sterols, the eluent used was a mixture of hexane, ethyl acetate and methanol in the proportions (35: 10: 5). After elution, the plates were dried and then sprayed with Libermann-Buchard reagent. At the end of the operation, the appearance of blue, violet, red or red-violet spots is characteristic of terpenes and sterols under UV light at 365 nm.

Tube characterisation tests: It was performed in 20 ml test tubes. For each test, 2 test tubes were used. One test tube containing the extract to be analysed and 1 control test tube containing the standard.

Tannin characterisation test ($FeCl_3$ test): In 2 test tubes, containing 400 µl of Gallic acid and 400 µl of the extract to be analysed, a few drops of 2% FeCl3 were added. The presence of a blue-black or green-blackish colour suggests the availability of tannins.

Flavonoid characterisation test (Shibata test): In two test tubes, containing respectively 400 µl of quercetin and 400 µl of the extract to be analysed, were added a few drops of concentrated hydrochloric acid (HCl) and a few fragments of magnesium turnings. The occurrence of a red coloration indicates the content of flavonoids.

Alkaloid characterisation test: 20 ml of the extract was dry evaporated. The residue was taken up with 10 ml of 10% HCl to which a few drops of concentrated ammonia were added. The resulting solution was extracted with ether. About 10 ml of this solution was evaporated and the residue was dissolved in 20 ml of 10% HCl. This solution was partitioned into two sample tubes as described below. Tube 1 was taken as a control and in tube 2 a one or two droplets of Dragendorff's product were mixed in. The formation of a white-yellow precipitate in the second tube shows the existence of alkaloids.

Saponosides characterisation test: The extract was dissolved in 1 ml of distilled water. The resulting solution was stirred strongly for a few minutes. The apparition of a column of moss of at less than 1 cm in height and lasting at least 15 minutes is an indication of the existence of saponosides.

Determination of total phenolic compounds: Stock solutions were prepared at a concentration of 5 mg/ml in ethanol for ethanol extracts, in a water-ethanol mixture (1:1 v/v) for hydroethanol extracts and in water for aqueous extracts. The total phenol concentrations were determined by the Folin-Ciocalteu technique according to the method described by Turkmen *et al.* (*2007*). Gallic acid was utilized as a reference sample; a standardized plot was previously established by preparing a range of Gallic acid solutions with concentrations ranging from 0.01 to 1 mg/ml. Then, 60 µl of Folin-Ciocalteu reagent (FCR) was added to the Gallic acid liquid at different concentrations. The mix was held at ambient temperature during 8 min, and then 120 µl of 7.5% (w/v) Na2CO3 solution was added to neutralize the residual reagent. Absorbances were determined at 760 nm with a SAFAS MP96 spectrophotometer after incubation for 30 minutes at 37°C. For the extracts, the same procedure was used instead of Gallic acid. The results, determined from the equation of the calibration curve, were expressed as mg Gallic Acid Equivalents per gram of extract (mg GAE/g extract) as mean ± standard deviation.

***In vitro* antioxidant activity of extracts**

Antioxidant activity by the ABTS^{-+} method: A volume of 200 µL of ABTS reagent was added to 50 µl of extract at different concentrations ranging from 0.01 to 1 mg/ml (cascade dilution of the order of 1/2) and the absorbance of the intense blue-green coloration was read 10 minutes later at 734 nm against a blank using an MP96 SAFAS (Miller *et al.*, 1993). The antioxidant content of each extract was assessed by plotting the absorbance readings against the standard curve established with Trolox.

Antioxidant activity by the DPPH$^{\bullet}$ method: A volume of 200 µL of DPPH reagent was added to 50 µl of extract at different concentrations ranging from 0.01 to 1 mg/ml (cascade dilution of the order of 1/2) and the absorbance of the purple coloration was read 10 minutes later at 510 nm against a blank using an MP96 SAFAS (Dudonné *et al.*, 2009). The antioxidant content of each extract was assessed by plotting the absorbance readings against the standard curve established with Trolox.

Antiradical activity of extracts: The free radical scavenging activity of the extracts was determined by the 50% Inhibitory Concentration (IC50), which is calculated by plotting half the absorbance of the DPPH solution alone against the regression curve of the extracts and standards. It defines the

concentration of antioxidants needed to reduce the initial DPPH level down to 50%. Antioxidant activity was expressed as the Antioxidant Activity Index (AAI), calculated as follows:

$$\mathbf{IAA} = \frac{\text{final concentration of DPPH (µg/ml)}}{\text{IC50 (µg/ml)}}$$

Thus, the IAA made it possible to classify the antioxidant activity of the extracts.

- For an IAA < 0.5: the extract has a low antioxidant activity;
- For an IAA between 0.5 and 1.0: the extract has a moderate antioxidant activity;
- For an IAA between 1.0 and 2.0: the extract has a high antioxidant activity;
- For an IAA > 2.0: the extract has a very high antioxidant activity (Scherer and Godoy, 2009).

Statistical analysis of data: The data were entered into Microsoft Office Excel 2016 and the means and standard deviations were calculated. The results are reported as average ± standard error. Analyses of variance (ANOVA) were performed using SPSS version 20 software. Means were compared using Fisher's smallest significant difference (LSD) method at the 95% confidence level.

RESULTS AND DISCUSSION

Extraction yield: A total of six extracts were obtained by successive maceration extraction using ethanol and hydroethanol and decoction method. The extraction yield findings are reported in Table 1.

Table 1: Extraction yield of the plants

	Yield (%)		
	Ethanol	**water-ethanol**	**water**
S. setigera	4.7	2.7	1.4
E. africana	9.,3	8.8	1.7

The results show that extraction yields vary depending on the plant and the solvent used. Using a mass of 10 g of plant material, the extraction yields varied from 1.4 to 9.3%. Regardless of the plant, the highest yields were obtained with ethanol extracts. Furthermore, irrespective of the solvent, the highest yields were recorded by *E. africana*. Thus, the ethanol extract of *E. africana* recorded the highest yield with a value of 9.3%. The aqueous extract of *S. setigera* recorded the lowest yield with a value of 1.4 %. The results obtained were in agreement with the study of Diallo (2020) who obtained an extraction yield of *S. setigera* barks in the order of 4% for decoction and 11.4% for maceration with 70% ethanol. Diarra (2011) also observed a 10% yield for 70% ethanol maceration and a 9.86% yield for decoction. Ethanol is a solvent that is widely used for reasons of safety, toxicity, quality and abundance. In addition, ethanol is a bio-solvent that can be generated by the fermentation of several raw materials containing sugar or starch, with recycling possibilities. Thus, its use can be a fundamental element for the development of a sustainable process. Ethanol could be a good extraction solvent. The distribution of chemical compounds in plants and in the plant kingdom in general, as well as other factors such as particle size, extraction time, temperature, pH of the extraction medium can also influence extraction performance (Handa *et al.*, 2008).

Phytochemistry of extracts: Phytochemical screening of the extracts of the two plants studied revealed the presence of several

secondary metabolites, notably tannins, flavonoids and saponosides. With the exception of terpenes and sterols which were absent in the extracts, and saponosides which were absent in all ethanolic and hydroethanolic extracts, chemical compounds such as tannins, flavonoids, were present in all extracts. Tube reactions and observation of TLC plates after using Dragendorff's reagent revealed alkaloids in all the extracts studied. The results of these phytochemical tests carried out on the extracts are reported in Table 2.

Table 2: Phytochemical screening of plants extract.

Plant	Extraction Solvent	Chemical groups				
		Tannin	Flavonoid	Alkaloid	Terpenes and sterols	Saponin
E. africana	**EtOH**	+	+	+	-	-
	EtOH-water	+	+	+	-	-
	water	+	+	+	-	+
S. setigera	**EtOH**	+	+	+	-	-
	EtOH-water	+	+	+	-	-
	water	+	+	+	-	+

+: presence -: absence

Previous studies on aqueous extracts of *S. setigera* leaves have revealed the presence of alkaloids, catechic tannins, gall tannins, anthocyanosides, flavonoids, anthocyanins, leucoanthocyanins, quinone derivatives, mucilages and essential oils (Gbenou *et al.*, 2011). In the barks of the plant, chemical constituents such as coumarins in the ethereal extracts, flavonoids, saponosides, tannins, oses and holosides, mucilages in the aqueous extracts, free anthracenosides and O-heterosides have been identified (Diallo, 2020)**.** Phytochemical analysis of ethanolic, hydroethanolic and aqueous extracts of *Entada africana* trunk bark revealed some chemical groups such as catechic tannins, flavonoids, anthocyanins, leucoanthocyanins and saponosides. In both hydroethanol and aqueous extracts, the trace of alkaloids was found. In addition to these chemical groups, the phytochemical screening revealed terpenes and sterols in the ethanolic and hydroethanolic extracts, mucilages in the aqueous extracts, and coumarins in the aqueous and ethanolic extracts (Yovo *et al.*, 2020).

In the roots of *Entada africana*, the presence of flavonoids, anthracenosides, tannins, saponins, oses and holosides, mucilages, sterols and triterpenes, coumarins, leucoanthocyanins and heterosides in the aqueous extracts has been demonstrated (Diarra, 2011). The presence of these compounds in these extracts can be explained by the fact that during their growth, plants synthesise secondary metabolites that are involved in reproduction, growth and defence against various biotic and abiotic factors (Hartmann *et al.*, 2008). The observed differences in phytochemical composition with the reported results could be justified by the fact that the phytochemical composition

of the same plant collected from different parts is influenced by environmental factors (Borokini and Ayodele, 2012).

Total phenolic compound content of extracts: The values of total phenolic compounds (TPC) in the extracts are summarised in Table 3. The values in this table are given in µg Gallic acid equivalent per gram of dry matter (µg GAE/g extract) as mean ± standard deviation.

Table 3: Total phenolic compound content (TPC) of extracts

Plants	Solvents	TPC (µg GAE/g extract)
Sterculia setigera	Ethanol	3506.70 ± 9.06^{c}
	water	34.53 ± 1.75^{a}
	Ethanol-water	3083.54 ± 99.53^{d}
Entada africana	Ethanol	1278.57 ± 41.79^{b}
	water	30.93 ± 1.25^{a}
	Ethanol-water	1245.73 ± 35.32^{b}

Means with the same letter in the same column are not significantly different at the α=0.05 significance level.

Table 3 shows that the contents of total phenolic compounds varied from 30.93 to 3506.70 µg GAE/g extract. The best content was recorded by the ethanolic extract of *S. setigera* with a value of 3506.70 µg GAE/g extract. The lowest value was obtained with the aqueous extract of *E. africana* with 30.93 µg GAE/g extract. In general, depending on the solvent, ethanol has the highest content with an average value of 2392.635 µg GAE/g extract. The other solvents have relatively low values of around 2164.635 µg GAE/g extract and 32.73 µg GAE/g extract for hydroethanol and aqueous extracts respectively. Furthermore, depending on the plant, *S. setigera* has the best phenolic compound content with an average value of 2208.256 µg GAE/g extract, followed by *E. africana* with 851.743 µg GAE/g extract. All findings appear to be in agreement with the literature. Indeed, the study of Yovo *et al.*, (2020) on *E. africana* extracts showed that the ethanol extract of *E. africana* had the best phenolic compound content with a value of 7256.6 ± 24.67 µg GAE/g extract. However, our results remain lower than their values. This difference could be explained by the different climatic conditions or the genetics of the plants and the harvest period. Indeed, the phenolic level of a plant is influenced by a certain type of factors, both internal (genetic) and external (climate conditions, cultivation practices, harvest maturity and storing factors) (Falleh *et al.*, 2008).

Antioxidant content of extracts: The results, expressed as µg trolox equivalent per gram of extract (µg TE/g extract) are reported in Table 4 as mean ± standard deviation.

Table 4: Antioxidant content of extracts by the ABTS and DPPH method

Plants	**Solvent**	**AOC** (µg ET/g extract)	
		ABTS	**DPPH**
Sterculia setigera	Ethanol	365.26 ± 6.92^{e}	187.16 ± 0.65^{b}
	water	104.18 ± 12.74^{d}	55.98 ± 2.81^{a}
	Ethanol-water	272.62 ± 17.19^{b}	138.34 ± 6.83^{d}
Entada africana	Ethanol	369.58 ± 5.39^{c}	139.90 ± 3.75^{b}
	water	182.29 ± 5.27^{a}	64.14 ± 4.48^{a}
	Ethanol-Water	$301.61 \pm 0,25b^{b}$	111.12 ± 2.44^{c}

Means with the same letter in the same column are not significantly different at the significance level α=0.05

The results in the table 4 show that the antioxidant levels in the plants studied vary depending on the extraction solvent and the method of determination. According to the DPPH method, the table shows that antioxidant levels range from 55.98 ± 2.81 to 187.16 ± 0.65 µg TE/g extract. The ethanolic extracts recorded the highest contents, i.e. 187.16 ± 0.65 µg TE/g extract for *S. setigera* and 139.90 ± 3.75 µg TE/g extract for *E. africana*, followed by the hydroethanolic extracts with 138.34 ± 6.83 µg TE/g extract for *S. setigera* and 111.12 ± 2.44 µg TE/g extract for *E. africana*. The aqueous extracts recorded the lowest levels with 55.98 ± 2.81 µg TE/g extract for *S. setigera* and 64.14 ± 4.48 µg TE/g extract for *E. africana*. According to the ABTS method, the table 4 shows that the antioxidant contents vary from 104.18 ± 12.74 to 369.58 ± 5.39 µg TE/g extract. The ethanolic extracts recorded the highest contents, i.e. 365.26 ± 6.92 µg ET/g extract for *S. setigera* and 369.58 ± 5.39 µg ET/g extract for *E. africana*, followed by the hydroethanolic extracts with 272.62 ± 17.19 µg ET/g extract for *S. setigera* and 301.61 ± 0.25 µg ET/g extract for *E. africana*. The aqueous extracts recorded the lowest levels with 104.18 ± 12.74 µg TE/g extract for *S. setigera* and 182.29 ± 5.27 µg TE/g extract for *E. africana*. The analysis of the values in Table 4 shows that the antioxidant contents of *E. africana* and *S. setigera* obtained by the ABTS method reflect those obtained by the DPPH method. These findings appear to be in accord with data in the literature. Cai *et al.* (2004) showed a strong correlation between total polyphenol content (estimated by the Folin-Ciocalteu method) and antioxidant activity estimated by the ABTS. Based on Turkmen et al. (2007), polyphenols also seem to be good hydrogen donators for the DPPH radical, because of their perfect structural chemistry. This could explain why this study extracts with high contents of phenolic compounds contain high antioxidant activity. Furthermore, the phytochemical screening of the extracts revealed the presence of tannins and flavonoids, which are chemical compounds with antioxidant properties. The presence of these two chemical groups, which are usually soluble in polar solvents (Madjid *et al.*, 2015; Stanković, 2011) could justify this antioxidant property of these extracts. Furthermore, the proven activity of an extract may as well reflect a small amount of highly active constituents as a large amount of constituents with low activity. The activity of a crude extract depends on the inherent activity of the active compounds and their relative amounts in the extract (Dabire *et al.*, 2015). In addition, the results show that the extracts with the best scavenging capacity towards DPPH and ABTS free radicals also have a reducing capacity for Fe3+. This would indicate the presence in the extracts

analysed of antioxidant molecules that can intervene by two types of action mechanism.

Antiradical activity of extracts (IC_{50}): The results of the antiradical activity from the 50% Inhibitory Concentration (IC50) and Antioxidant Activity Index (AAI) of the plant extracts are mentioned in table 5.

Table 5: 50% Inhibitory Concentration (IC50) and Antioxidant Activity Index (AAI)

		Solvent	IC_{50}	AAI
Plant	*S. setigera*	water	0.054	0.74
		ethanol-water	0.017	2.35
		Ethanol	0.011	3.63
	E. africana	water	0.048	0.83
		ethanol-water	0.025	1.6
		Ethanol	0.019	2.10
Standards	Gallic acid	Methanol	0.001	40
	Trolox	Methanol	0.003	13.33

By comparing the IC50 values of the extracts with those of the standards used (Trolox, Gallic acid), it emerges from the analysis of Table 5 that the antiradical activity of the plant extracts studied is lower than the DPPH- radical scavenging capacity of the standards. A low IC50 value of an extract indicates its high antiradical activity (Sahgal *et al.*, 2009). Thus, among the plant extracts studied, the ethanol extract of *S. setigera* recorded the best antiradical activity towards the DPPH- radical with an IC50 of 0.011±0.0002 followed by the ethanol extract of *E. africana* with an IC50 of 0.019±0.0002. The ethanol extract of *S. setigera* then shows a very high antioxidant activity with an AAI of 3.63 followed by the ethanol extract of *E. africana* with an AAI of 2.10.

CONCLUSION AND APPLICATION OF RESULTS

The purpose of our research was to determine the phytochemical composition and to evaluate the antioxidant effect of two plants traditionally used in medicine. At the end of our study, it was found that both plants contained secondary metabolites such as tannins, alkaloids, flavonoids, saponosides, terpenes and sterols that have pharmacological properties. For the determination of phenolic compounds, ethanol extracts revealed the greatest amount of phenolic constituents relative to hydroethanol and aqueous extracts. As for the evaluation of antioxidant content, ethanol extracts also showed a better scavenging power towards ABTS and DPPH free radicals and thus exhibit high antioxidant activities. The results of this work suggest that these plants are natural sources of antioxidant compounds and justify their use in traditional medicine against respiratory diseases in general and cough in a specific way. Indeed, these plants could be recommended for the treatment of cough and as good sources of natural antioxidants. This research provides also data that can be used as a lead for the discovery and development of new, more affordable and safe phtytomedicines for the treatment of free radicals and cough.

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