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Research Article

Phytochemistry and Biological Activities of Extracts from Two Combretaceae Found in Burkina Faso: *Anogeissus leiocarpus* (DC) Guill. and Perr. And *Combretum glutinosum* Perr. Ex DC

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Abstract:

Many studies indicate that Combretaceae species are commonly used in Africa to treat various diseases. Nonetheless, no phytochemical and/or biological activity data are available from species found in Burkina Faso to secure their ethnomedicinal uses. In addition, drugs derived from these species, especially those of the genus *Combretum*, are sometimes the objects of adulteration by herbalists dealing in large cities of the country. This paper reports the pharmacognostic, phytochemical and biological activity of extracts from two of the most commonly used Combretaceae species found in Burkina Faso: *Anogeissus leiocarpus* (DC) Guill. and Perr. and *Combretum glutinosum* Perr. ex DC. Through the method of Ciulei, the characterization of the two drugs was made possible, and it was found that the phenolic compounds and flavonoids are the main active ingredients in the methanol extracts. The levels of total phenolic extracts ranged from 49.63 ± 0.21 to 68.27 ± 0.90 EAG/100 mg for *Anogeissus leiocarpus* and *Combretum glutinosum*, respectively. Evaluation of the antibacterial activity showed a Minimum Inhibitory concentration (MIC) value of 0.86 mg/ml for *Anogeissus leiocarpus* (against *Staphylococcus aureus* clinical isolate) and 1.41 mg/ml for *Combretum glutinosum* (against *Staphylococcus aureus* ATCC 6538). TLC analysis provided evidence of the presence of two flavonols, quercitrin and rutin, and gallic acid, all of which are recognized as bioactive compounds. These data were utilized to justify the use of the two species in traditional medicine.

Keywords: Antibacterial activity, Phytochemistry, *Anogeissus leiocarpus*, *Combretum glutinosum*

1.0 Introduction:

An ethnobotanical survey in the Mouhoun region of Burkina Faso showed that many dye plants are commonly used in traditional medicine (SORE, 2010). Among these plants, two belonging to the family Combretaceae family appear to be the most frequently used: *Anogeissus leiocarpus* (DC) Guill. and Perr. and *Combretum glutinosum* Perr. ex DC. *Anogeissus leiocarpus* is a tree that is considered to be sacred, hence the common name Siiga (soul) in Moore, the main language of Burkina Faso. It is widely used to dye textiles and leather as well as in the traditional treatment of many diseases. The decoction and maceration of the stem bark are used against anorexia, constipation, malaria, jaundice, itching, wounds, eczema, psoriasis, carbuncles, boils and various forms of ulcers (Kerharo et al., 1974, Nacoulma, 1996). The decoction of the leaves is used in the treatment of

jaundice, various forms of hepatitis and amoebic dysentery.

The leaves of the *Combretum glutinosum* plant are used to treat malaria, anemia, anorexia, cough, bronchitis, fever, liver disease, diarrhea, and liver failure and to combat microbial agents (Kerharo et al. 1974; Nacoulma, 1996). The leaves or stem bark are crushed and used for dressing wounds (Bukill, 2000; PROTA 3, 2005). These two species are present in most African savannah ecosystems. In Burkina Faso, however, these species have not been studied for their pharmacognostic characterization or complete biological activity. Indeed, there are several genera in the Combretaceae family (e.g., *Combretum*, *Anogeissus*, *Terminalia*) that may be occasionally confused with some frequently utilized species including many *Combretum* species, for which high market demands exist in the larger cities. The

pharmacognostic characterization of these two species should help fight against the adulteration of these drugs. In addition, the evaluation of the two plants' antibacterial and anti-radical activities is expected to justify their traditional uses in the treatment of infectious diseases and/or against oxidative stress.

2.0 Material and Methods:

2.1. Plant Material Collection and Authentication

Anogeissus leiocarpus and *Combretum glutinosum* leafy twigs were collected in September 2010 in Kamboinsin (10 km from Ouagadougou), and voucher specimens, with respective identification codes SH 01 and SH 02, were deposited in the herbarium of "Université de Ouagadougou".

2.3. Drying And Pulverization

The leafy twigs were dried in the laboratory and were then pulverized using a mortar in October-November 2010. The resulting powders were packaged in plastic and stored away from light. Pharmacognostical and phytochemical investigations proceeded between March 2011 and May 2012 in University of Ouagadougou (Burkina Faso).

2.4. Pharmacognostical Investigations

2.4.1. Microscopic Study

Microscopic studies were performed by preparing thin cross sections from the roots, stems and leaves of each species. Every section was cleared with chloral hydrate solution, stained with phloroglucinol and hydrochloric acid, and mounted with glycerin. Separate sections were then prepared and stained with specific reagents to identify some phytochemicals: NaOH was used to locate the flavonoids, Lugol reagent for alkaloids and FeCl₃ for tannins (Ciulei, 1982).

2.4.2. Physiochemical Investigation

The moisture content, total ash, water-soluble ash, acid-insoluble ash, and the alcohol- and water-soluble extractive values were determined to be part of the physiochemical parameters.

2.4.2.1. Water-Soluble Extractive Value

Twenty-five grams of plant powder was extracted by boiling in 250 ml of distilled water for 45 min. The decoction was then filtered, and the filtrate was used for the various antibacterial tests.

2.4.2.2. Alcohol Extractive Value

The methanol extraction was performed using a Soxhlet system with 25 g of plant material and 250 ml of methanol for 10 hours. The extracts were then evaporated until dry.

The methanol extract was used for the phytochemical screening tests and the assays of biological activity.

2.4.2.3. Moisture Content

This analysis was used to determine the loss of mass of a known amount of powder by drying in an oven at a temperature of 100 °C for 24 hours.

2.4.2.4. Total Ash

The total ash analysis involved the determination of residual, non-volatile substances contained in a drug after it was burned in a furnace at 600 °C for 6 hours. Five replicates were performed in the same way to determine the mean value.

2.4.2.5. Acid-Insoluble Ash

The acid-insoluble part of the ash consists of silica, sand and dust that could contaminate the drug. The analysis consists of boiling a mixture of total ash in HCl, 10% (v/v), for 15 min and then filtering the solution using filter paper previously dried until its weight became constant. The content of acid-insoluble ash is calculated by comparing the weights of the filter paper after filtration (and complete desiccation) with its initial mass prior to filtration. The obtained value is compared to the total mass of ash.

2.5. Phytochemical Investigations

2.5.1. Phytochemical Screening Of The Extracts

Tannins, flavonoids, alkaloids, coumarins, saponins, sterols and triterpenes were screened according to the methods of Ciulei (1982).

2.5.2. Dosage of Phenolic Compounds

Different types of phenolic compounds were measured in extracts using the following methods: the method of Singleton et al. (1999) for total phenolics, the method of Arvouet et al. (1994) for total flavonoids, the method of Abarca et al. (2007) for total flavonols, and the method of the European Commission (2000) for total tannins.

2.5.3. Chromatographic Analysis of Extracts

To search for particular phenolic compounds in which biological activities are well established, analysis by thin layer chromatography (TLC) was performed following the method of Wagner and Bladt (1996). Two types of effluent systems were used: the first system contained hexane, ethyl acetate and acetic acid in proportions of 6.2/1.8/1, respectively, and the second system consisted of hexane, ethyl acetate, acetic acid and water in the respective proportions of 2/10/2/0.4.

2.5.4. Analysis of the Antiradical Scavenging Capacity of Extracts

Evaluation of the antiradical activity (ARA) was accomplished using the DPPH method described by Velazquez et al. (2003). After a series of dilutions, 0.5 ml of methanol extract was mixed with 1 ml of methanol solution containing DPPH (20 mg/l). After a 15 min incubation in the dark, the absorbance was read at 517 nm against a blank containing methanol instead of the extract. The percent inhibition is determined by the following relationship:

$$\% = (1 - A/A_0) \times 100.$$

% = percentage inhibition, A_0 = absorbance of blank, and A = sample absorbance.

The IC_{50} is the concentration of plant extract that reduces the DPPH radicals to 50% of the initial amount contained in the test solution. The lower the IC_{50} value, the greater the radical-scavenging power of the extract.

Three trials were conducted to determine an average value of IC_{50} in each case.

2.5.5. Analysis of the Antimicrobial Activity Of Extracts

2.5.5.1. Strains of Bacteria

The following bacteria strains were used for the tests: *Staphylococcus aureus* (clinical isolate), *Staphylococcus aureus* ATCC No. 6538, *E. coli* (clinical isolate), *E. coli* ATCC No. 25922, and *Salmonella typhi* (clinical isolate).

2.5.5.2. Antibacterial Tests

Antibacterial activity was tested using traditional extracts (aqueous extracts) from the two plants following the agar diffusion method of Perez et al. (1990) as described by Poppola et al. (2007). The antibiotics ampicillin and gentamicin were used as references and the inhibition zone diameters were measured (in mm) with a ruler. The reading of the inhibition zone diameters was made after 24 h and

confirmed after 48 h. For each concentration and bacteria strain, the measurements of the inhibition zone diameters were performed in triplicate. The minimum inhibitory concentrations (MIC) were also determined by the method of Perez et al. (1990). The MIC is defined as the minimum concentration of extract in which, by eye-sight alone, prevention of bacterial growth is observed. The concentration of extract causing an inhibition zone diameter of 8 mm is considered as the MIC of the extract (Perez et al., 1990). Lower MIC scores define higher antibacterial activity in the extracts.

2.6. Statistical Analysis of Data

The following software was used: MS Excel, to calculate the equivalents of gallic acid, tannins and quercetin, to determine the percentage of DPPH free radical inhibition and to determine the linear regression equation. One-way ANOVA (Tukey's test) was used to determine the level of statistical significance of differences between the means (at $p < 0.05$) using the software SigmaStat 2.0 (Jandel Scientific Software).

3.0 Results and Discussion :

3.1. Pharmacognostical Results

3.1.1 Physicochemical Characteristics of the Drug

Table 1: Physicochemical characterization

Physicochemical parameter	<i>Anogeissus leiocarpus</i>	<i>Combretum glutinosum</i>
Water-soluble extract	44,12%	31,62%
Alcohol-soluble extract	32,08%	26,71%
Moisture	6,80%	6,00%
Total ash	5,89%	5,24%
Acid-insoluble ash	3,00%	4,25%

3.1.2. Histochemical Characteristics

The flavonoids' location on the sectioned tissue is marked by the presence of yellow precipitates after treatment with NaOH (2%). This coloration is observed in the cortical parenchyma and in the sclerenchyma of *Anogeissus leiocarpus* (Fig. 1D) and in the medullary parenchyma of *Combretum glutinosum* (photo 2H). The tannins and polyphenols were located in the medullary parenchyma of both *Anogeissus leiocarpus* (photo 1B) and *Combretum glutinosum* (Fig. 2 F). The alkaloids were localized in the collenchyma, cortical parenchyma and the medullary parenchyma of *Anogeissus leiocarpus* stem

(picture 1C) and in the same root tissues of *Combretum glutinosum* (photo 2G).

3.2. Phytochemical Screening and Dosage

Table 2 (below) contains the results of the phytochemical screening. These results largely confirm those of the previous histochemical analysis. Thus, the presence of gallic and ellagic tannins, coumarins and saponins was observed in the methanol extracts from both plants and the presence of flavonoids, aglycones, sterols and triterpenes in *Combretum glutinosum*. The tests were, however, negative for alkaloids possibly due to the methods of extraction. Alkaloid extraction usually needs an alkaline or acidic solvent. Table 3 summarizes the results of total phenolics, total flavonoids, flavonols and total tannin dosages.

3.3. TLC Analysis

Analysis of the chromatograms shows that the methanol extracts of both species, especially those from *Combretum glutinosum*, are very rich in flavonoid compounds. Comparisons of the R_f (frontal reference) values of the standards with those of separated substances from the extracts allow the identification of some of the extract components: *Anogeissus leiocarpus* extract contains gallic acid, and *Combretum glutinosum* extract contains genistein, rutin and quercetrin (photo 3).

3.4. Radical-Scavenging Activity of the Extracts

Figure 1 (below) summarizes the obtained results, which show that the methanol extract of

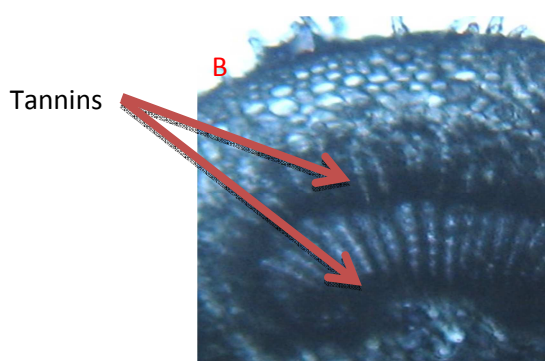
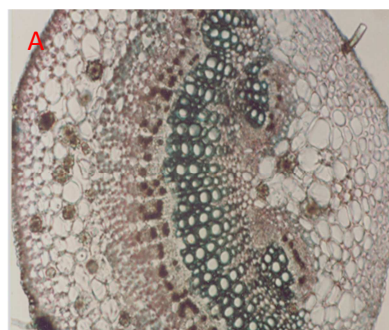
Anogeissus leiocarpus (IC₅₀=0.9 ± 0.12 g/ml) is more active than that of *Combretum glutinosum* (with 2.58 ± 0.31 g/ml as the mean value of IC₅₀). The radical-scavenging activities of these two plants are quite interesting compared to those of the reference compound, quercetin, used in this test (with 0.88 ± 0.11 g/ml as the mean value of IC₅₀). The reference compound is a pure substance, while the plant extracts consist of mixtures of substances with impurities.

3.5. Antibacterial Activity

Table 4 summarizes the test results of antibacterial activity. The diameters of inhibition zones caused by the aqueous extract of *Anogeissus leiocarpus* ranged from 7.5 mm to 16 mm according to the tested bacteria strains, while the inhibition zones caused by the aqueous extract of *Combretum glutinosum* ranged from 08 ± 00 mm to 15 ± 00 mm depending on the concentration of extracts.

3.6. MIC Determination of the Plant Extracts

Table 5 shows the MIC values of the different plant extracts for each type of bacteria strain. MICs of aqueous extracts from the two plants ranged from 0.86 mg/ml to 5 mg/ml. The low value of MIC of *Anogeissus leiocarpus* extract (0.86 mg/ml) on *Staphylococcus aureus* indicates a fairly good antibacterial activity. Another interesting MIC value was also obtained on *Staphylococcus aureus* ATCC 6538 (1.41 ± 00 mg/ml) and *Salmonella typhi* (1.41 ± 00 mg/ml) with the aqueous extract of *Combretum glutinosum*.



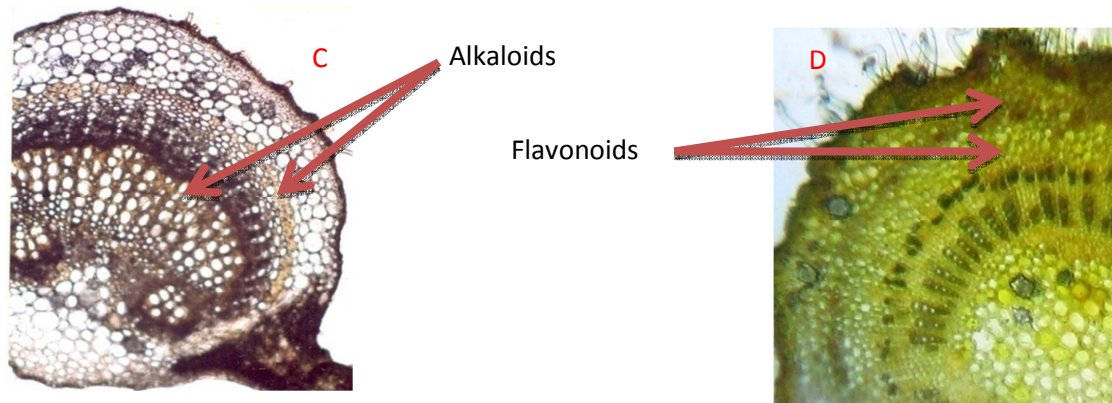


Photo 1: Histochemical analysis of plant organ sections of *A. leiocarpus*. The location of the following phenolic compounds is shown (A, leaf sections treated with Carmino-green; B, leaf section treated with FeCl_3 ; C, stem section treated with Lugol; and D, stem section treated with NaOH).

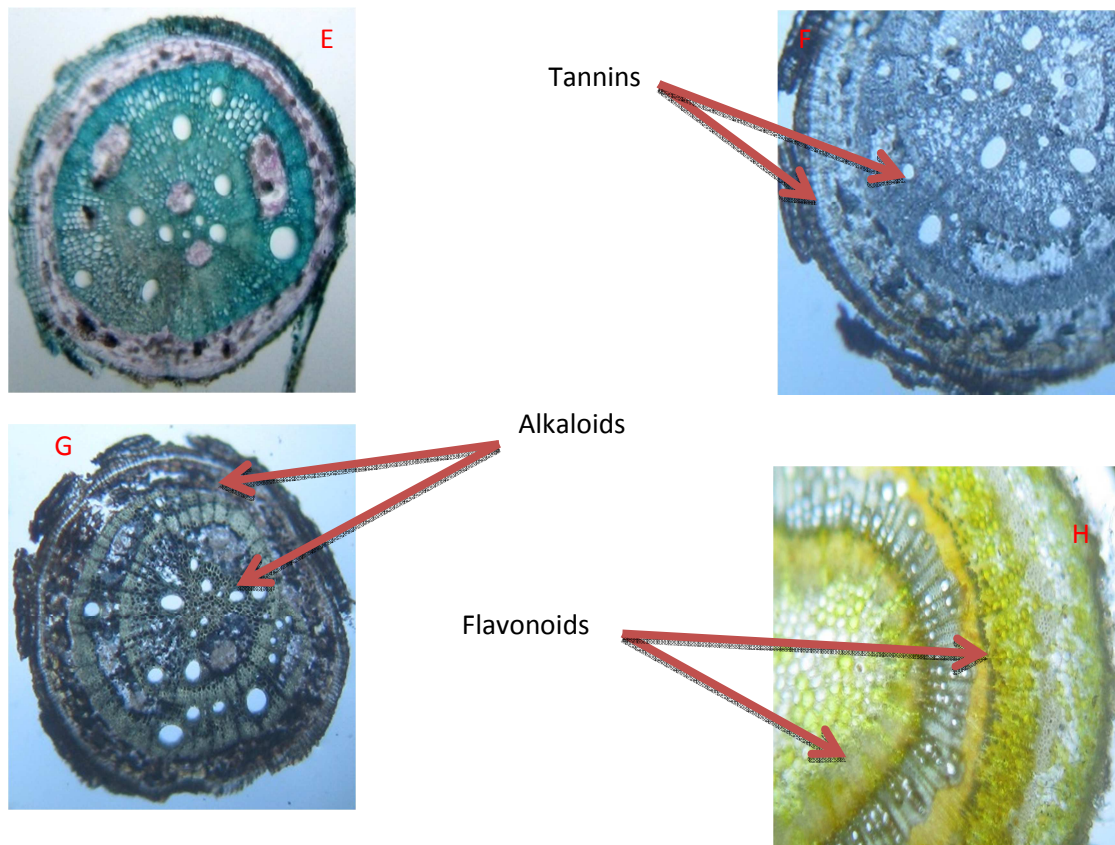


Photo 2: Histochemical analysis of plant organ sections of *C. glutinosum*. The location of the following phenolic compounds is shown (E, root section treated with Carmino-green; F, root section treated with FeCl_3 ; G, stem section treated with Lugol; and H, root section treated with NaOH).

Table 2: Screening results from methanol extracts

compounds	Plants	
	<i>Anogeissus leiocarpus</i>	<i>Combretum glutinosum</i>
Sterols and triperthenes	–	+
flavonoid aglycons	–	+
Taninns	+	+
Saponines	+	+
Anthracenosides	–	–
Alcaloids	–	–
Coumarines	+	+

Table 3: Dosage results (in mg/100 g of extract)

	Total phenolics	Total flavonoids	Total flavonols	Total tannins
<i>Anogeissus leiocarpus</i>	68.27 ± 0.90 ^a	12.92 ± 0.07 ^a	2.18 ± 0.14 ^a	29.37 ± 0.35 ^a
<i>Combretum glutinosum</i>	49.63 ± 0.21 ^b	6.24 ± 0.2 ^b	1.22 ± 0.006 ^b	11.12 ± 0.51 ^b

Every value is the mean of three measures (n = 3).
The superscript letters indicate a significant value (p<0.05)

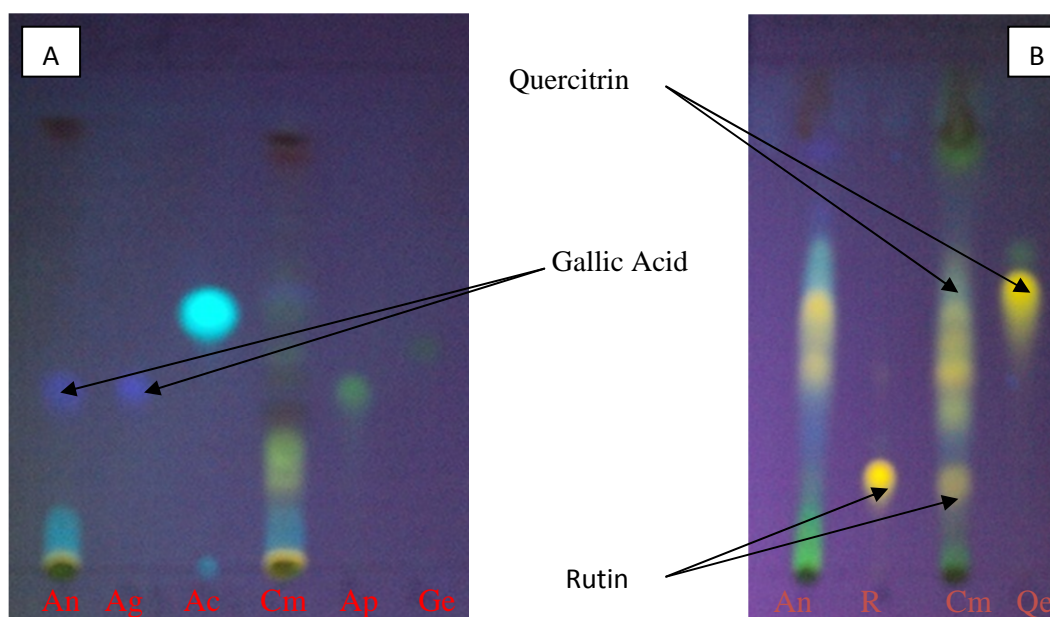


Photo 3: TLC chromatograms of methanol extract (After spraying with NEU reagent).

A= apolar system: hexane/ethyl acetate/acetic acid (62/18/10);

B= polar system: hexane/ethyl acetate/acetic acid/water (2/10/2/0.4)

An: *Anogeissus leiocarpus*, Ag: gallic acid, Ac: caffeic acid, Cm: *Combretum glutinosum*, Ap: Apigenin, Ge: genistein, R: Rutin, Qe: Quercitrin

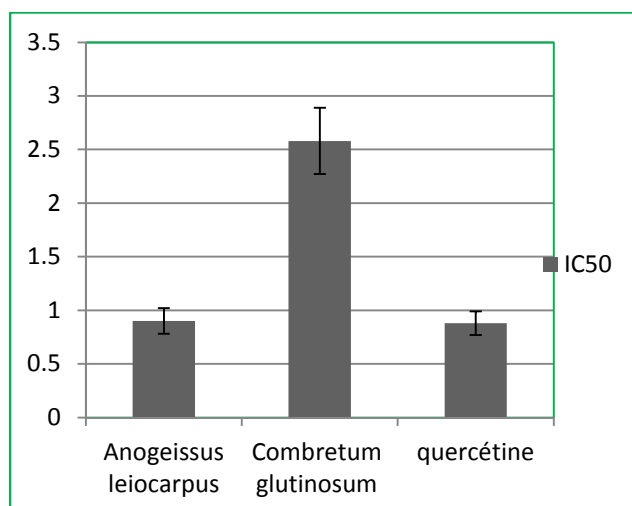


Figure 1: Antiradical activity of methanol extracts from *Anogeissus leiocarpus* and *Combretum glutinosum* (as compared to quercetin).

The water-soluble extracts in *Anogeissus leiocarpus* and *Combretum glutinosum* are, respectively, 44% and 31.64%. These results show a wealth of water-soluble substances from both species, which usually consist of flavonoids, tannins and coumarins. This result could justify why the traditional mode of preparation of these drugs for the treatment of many diseases is decoction (Kerharo et al., 1974).

With moisture content below 10%, drugs of both species are well suited to conservation without any risk of alteration of the active ingredients. The high proportions of total ash indicate a high mineral content of the plant materials. The high content of hydrochloric ash may correspond to the contamination of the drug by silica (Paris et al., 1965). The results of the tube tests confirm the presence of phenolic compounds in the extracts that were previously localized using histochemical cross sections.

Tannins have antiseptic healing (Kerharo et al., 1974; Bruneton 1993), and anti-inflammatory properties (Araùja et al., 2008). Their presence in *Anogeissus leiocarpus* and *Combretum glutinosum* could explain the traditional use of these plants in the treatment of boils, abscesses, and carbuncles. The protein-precipitating properties of tannins enhance the conservation of leather, thus justifying the use of these plant extracts in traditional leathertanning. Flavonoids and coumarins are well known for their antioxidant,

vasodilatory, anti-inflammatory, antibacterial and immune-stimulating activities (Bruneton, 1993). They can also act as chelators of toxic ions.

The interesting antiradical activity of extracts from the two species is related to their high content of total phenolics and flavonoids, which are known for their good antiradical activity (Hukkanen et al., 2006, Schwarz et al., 2009). Thus, rutin, as evidenced by the TLC analysis of *Combretum glutinosum* extract, is a powerful antioxidant used in Chinese medicine to treat high blood pressure and inhibit the damage induced by the oxidative effects of UV radiation (Bors et al., 1990). This compound is usually in the form of a glucoside flavonoid but is rapidly hydrolyzed by intestinal microflora (Manach et al., 1997) to an aglycone, which is the principle antioxidant. It acts by increasing the activity of catalase and decreasing the oxidative effect of metal ions in the liver (Zhonghong, 2002; Gao, 1999). Gallic acid, also identified by the TLC analysis, is known for its anticarcinogenic properties (Salucci et al., 2002). More recent studies have shown that genistein (here identified in the extracts by TLC) improves the activity of antioxidant enzymes such as catalases and glutathione superoxidase. The presence of such bioactive flavonoids and the strong scavenging activity measured in this study may explain the frequent use of these two species in traditional medicine to treat diseases of oxidative stress.

The antibacterial activity of extracts from the two species could be explained by the strong presence of phenolic compounds, especially tannins, in these extracts. Genistein, an isoflavone found in the methanol extract of *Combretum glutinosum*, is known to have inhibitory activity on the growth of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella enterica* (Katarzyna et al., 2007). Similarly, studies have shown an antibacterial property of gallic acid on *Staphylococcus aureus* and *E. coli* (Rodriguez et al., 2009). Rutin is known for its inhibitory activity on bacterial topoisomerase IV (Bernard et al., 1997). Interesting antibacterial activities of the constituents of these two species could justify their traditional use in the treatment of many infectious diseases such as intestinal disorders and wound and skin care.

Table 4: Inhibition zone diameters produced by the extracts on the tested bacteria strains

Extracts	Concentration (mg/ml)	<i>Staphylococcus aureus</i> clinical isolate	<i>Staphylococcus aureus</i> ATCC 6538	<i>Escherichia coli</i> clinical isolate	<i>Escherichia coli</i> ATCC 25922	<i>Salmonella typhi</i>
<i>Anogeissus leiocarpus</i>	55	16 ± 00 ^a	10.5 ± 0.71 ^d	14.5 ± 0.71 ^{ab}	11.5 ± 0.71 ^{cd}	13 ± 00 ^{bc}
	27,50	14.5 ± 0,71 ^a	8.5 ± 0,71 ^b	13 ± 00 ^a	9.5 ± 0.71 ^b	11 ± 00 ^b
	13,75	13.5 ± 0.71 ^a	7.5 ± 0.71 ^c	11 ± 00 ^b	9 ± 00 ^c	9 ± 00 ^c
<i>Combretum glutinosum</i>	30	11.5 ± 0.71 ^b	15 ± 00 ^a	10 ± 00 ^b	10.5 ± 0.71 ^b	10 ± 00 ^b
	15	10.5 ± 0.71 ^b	13.5 ± 0.71 ^a	9.25 ± 0.35 ^b	8.75 ± 0.35 ^b	10 ± 00 ^b
	7.50	10 ± 00 ^b	11 ± 00 ^a	9 ± 00 ^c	8.25 ± 0.35 ^c	9 ± 00 ^c
Ampicillin		–	16.5 ± 2.12 ^a	11 ± 1.41 ^b	–	–
Gentamicin		20 ± 00 ^b	–	19.5 ± 0.71 ^b	7 ± 00 ^c	26 ± 1.41 ^a
Antibiotics	Concentration	inhibition diameter (in mm)				

- = No action detected

Each result consists of the mean of three measures (n = 3).
The superscript letters indicate a significant value (p<0.05)

Table 5: MIC value of the extracts

Bacteriums Plants	<i>Staphylococcus aureus</i> (clinical-isolate)	<i>Staphylococcus aureus</i> ATCC 6538	<i>E.coli</i> (clinical isolate)	<i>E.coli</i> ATCC 25922	<i>Salmonella typhi</i> (clinical isolate)
<i>Anogeissus leiocarpus</i>	0.86 ± 00 ^c	2.63 ± 00 ^b	2.58 ± 00 ^b	3.87 ± 1.82 ^a	4.01 ± 00 ^a
<i>Combretum glutinosum</i>	2.35 ± 0.66 ^b	1.41 ± 00 ^c	2.82 ± 00 ^b	5.00 ± 0.88 ^a	1.41 ± 00 ^c
MIC value (in mg/ml)					

Each result consists of the mean of three measures (n = 3).

The superscript letters indicate a significant value (p<0.05)

4.0 Conclusion:

The results of this study allowed us to determine the pharmacognostical characteristics of two Burkina Faso medicinal species. These results should help in the fight against adulteration (which is becoming more common) of the drugs sold by herbalists in the big cities of the country. Similarly, phytochemical screening and biological activity tests have revealed well-known pharmacologically active compounds that could justify the traditional uses of these two Combretaceae to treat infectious diseases and/or oxidative stress. TLC chromatograms of the methanol extracts, especially from *Combretum glutinosum*, showed a wide variety of flavonoid compounds, which are different from the tested reference compounds. Ultimately, it could be interesting to continue fractioning the extracts to isolate the most active substances for radical-scavenging and/or antibacterial activities.

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