



Antioxidative and antibacterial activities of phenolic compounds from *Ficus sur* Forssk. and *Ficus sycomorus* L. (Moraceae) : potential for sickle cell disease treatment in Burkina Faso

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ABSTRACT

Ficus sur and *Ficus sycomorus*, two medicinal species used in Burkinabe traditional medicine for the treatment of sickle cell disease were investigated. The determination of total phenolic and tannins contents, using the method of Folin-Ciocalteu (FCR), indicate values significantly ($p < 0.05$) higher in extracts of *Ficus sycomorus* compared to those of *Ficus sur*, with respective values of 336.80 ± 0.80 mg TAE/g of extract and 203.74 ± 0.9 mg TAE/g of extract against 247.00 ± 0.52 mg TAE/g of extract and 120.8 ± 0.83 mg TAE/g of extract. Similarly, using the method of 1,1-diphenyl-2-picrylhydrazyl (DPPH), the results of the analysis indicate that extracts of *Ficus sycomorus* produce the highest antiradical activity with IC_{50} value of 9.60 ± 0.02 μ g/mL against 31.83 ± 0.55 μ g/mL for *Ficus sur*. The IC_{50} value of quercetin, used as reference antioxidant, was of 4.6 ± 0.08 μ g/mL. The latex of *Ficus sycomorus* presented the lowest minimum inhibitory concentrations (MIC) against *Staphylococcus aureus* and *Escherichia coli*, with respective values of 0.13 mg/mL and 0.25 mg/mL. The difference in phenolic content could explain the difference in biological activity between the two *Ficus* species.

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INTRODUCTION

Sickle cell disease is an inherited disorder linked to mutations in the hemoglobin gene. Several complications, including chronic haemolysis, anemia and severe acute vaso-occlusive injuries were

found as due to this disease (Giro et al., 2003). One of the main characteristics of this pathology is producing a huge amount of free radicals, leading to a severe oxidative stress (Sess et al., 1998). Usually, many infections (e.g. due to *Staphylococcus aureus*,

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Salmonella sp. and *Escherichia coli*) are also associated with this disease, as consequences of the complications listed above (Schmugge et al., 2008). Sickle cell disease is the most common genetic hemoglobin disorders encountered in Africa. In Burkina Faso, more than 25% of the population has a mutation in hemoglobin and about 3% of children have major sickle cell syndrome (MSS) of homozygous (SS, S indicates a mutation of valine) or composite heterozygous (SC, C indicates a mutation of lysine) (CID, 2010).

Being an endemic disease, and specifically present in tropical countries, sickle cell disease is not taken into account in research programs of global pharmaceutical companies, due to the fact that related trading profits seem weak. This is a “neglected” disease, in terms of scientific research. The few modern drugs available are very expensive for African patients. Being faced for centuries to this disease, African traditional medicine has had to deal with thanks to medicinal plants. Indeed, medicinal recipes are often used in Africa for the management of the disease, to mitigate, for example, recurrent inflammation (pain, fever, and edema), anemia and frequent infections. Until today, however, only few scientific data on these medicinal plants are available. Then there is an urgent need to investigate, in this sense, the rich African flora.

An ethnobotanical survey, conducted in the region of the central plateau of Burkina Faso showed that the two species, *Ficus sur* Forssk. and *Ficus sycomorus* L., are used in folk medicine for the treatment of sickle cell disease. *Ficus sur* (called womsèèga in Moore language) is a tree of 5 to 8 m high, while *Ficus sycomorus* (kankanga in Moore language) is generally 10 to 30 m high and about 3 m in diameter. Their bark is smooth or rough; gray, pink or red. Their latex is white. Both species belong to the genus *Ficus* (constituting the largest genus in the botanical family Moraceae, with over 1500 species). They are distributed in tropical and subtropical regions of the world and their

morphological characteristics vary considerably (Arbonnier, 2000). Previous work relating to *Ficus sur* and *Ficus sycomorus* showed antispasmodic and anti-plasmodial activities from aqueous extracts of bark and leaves (Sanon et al., 2003; Ayinde et al., 2009). Alkaloids were considered responsible for the antimalarial activity of *Ficus sycomorus* (Sandabe et al., 2006). However, neither of these species was further studied for its potential in the treatment of associated disorders of sickle cell disease. We then undertook this study with the aim to investigate the antioxidant and antibacterial activities of aqueous extracts of leaves of both *Ficus* species.

MATERIALS AND METHODS

Materials

Culture media and microorganisms

Broth and Mueller Hinton agar were used for antibacterial tests. Strains of *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* were obtained from Children's Hospital and the National Public Health laboratory at Ouagadougou. These wild strains were isolated from urine, feces and blood. In addition, the following American Type Culture Collection (ATCC) reference strains were provided by the same laboratories: *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25932 and *Salmonella typhimurium* ATCC 13311.

Plant material

The leafy stems of *Ficus sycomorus* were harvested in Tanghin situated on the outskirts of Ouagadougou, in July 2009. Those of the *Ficus sur* were harvested in August 2009 at Pabré, located at 25 km from Ouagadougou. After identification by Professor Millogo J., botanist, specimens have been deposited in the herbarium of the University of Ouagadougou, under registration number 01/2009 and 02/2009, respectively for *Ficus sycomorus* and *Ficus sur*.

Extraction and phytochemical screening of extracts

The leaves of both species were washed, dried away from sunlight for two weeks and then finely ground. 25 grams of powdered leaves of each species were used to perform successive extractions in a Soxhlet system with solvents of increasing polarity (chloroform, ethanol 90%, distilled water). Phytochemical screening was performed by following the method described by Ciulei (1982) for tube tests, and according to the method described par Wagner et al. (1996) for thin layer chromatography (TLC) on silica gel plates 60 F₂₅₄ (Merck). Eluent systems (n-BuOHsec:MeOH:AcOOH:H₂O, 50:6.25:6.25:17.5) and (n-hexane: toluene: ethyl acetate, 6:2:2) were used for the migration of flavonoids, phenolic acids, sterols and triterpens. Phenolic acids and flavonoids were revealed on TLC plates with Neu's reagent in the presence of UV light (365 nm). Sterols and triterpenes were revealed by 3% H₂SO₄ in EtOH (96%).

Estimation of total amount of phenolic compounds, flavonoids, flavonols and tannins

25g of the drug powder were introduced into a 500 mL-flask. 250 mL of distilled water was added and the mixture was heated to reflux for 30 min. The solution was left to cool at room temperature, then filtered and centrifuged (Centriguge OSI, Italy) for 10 min at 2000 g. The extract obtained was frozen, and then lyophilized for phenolic compound contents determination and biological activities assessment. The latex of *Ficus sycomorus* was collected from unripe fruits.

Determination of total phenolic content

Total phenolic content was determined by the method of Singleton et al. (1999). The reaction mixture consists of 1 mL of extract, 1 mL of FCR (Folin Ciocalteu Reagent) 2N and 3 mL of a 20% sodium carbonate solution. This mixture is left to stand at room temperature for 40 min and then the

absorbance was measured (spectrophotometer Agilent 8453) at 760 nm. In the control tube, the extract volume was replaced by distilled water. A standard curve was plotted using tannic acid (1-5 µg/mL). Tests were performed in triplicate. Total phenolic content of the extract was calculated using the formula:

$$T_{PT} = \frac{C_{Tube}}{C_i} \times D$$

where T_{PT} is the total phenolic content of the extract expressed as tannic acid equivalent (mg TAE)/g, C_{Tube} is the concentration (mg/mL) in the test tube, D the dilution factor and C_i the concentration in mg/mL in the stock solution.

Determination of tannin content

Tannin content was assessed as described by Tibiri et al. (2007): Polyvinyl pyrrolidone (PVPP) can precipitate tannins by formation of a complex; 100 mg of PVPP can complex 2 mg of total phenolics. To 1 mL of extract (0.50 mg/mL), is added the quantity of PVPP to complex the total phenolics present in the extract (and determined as described above). The mixture was vortexed, kept at 4 ° C for 15 min and then centrifuged at 3000 g for 10 min. The supernatant contains phenolic compounds other than tannins (which have been precipitated by PVPP). A test tube was performed like for total phenolics. After 40 minutes, it was centrifuged and the absorbance of the supernatant was measured at 760 nm. Total phenolic content of the supernatant is given by the above formula. Tannin content was determined as the difference between the first value of total phenolics (containing tannins) and the second value of total phenolics (in the absence of tannins)

Determination of flavonoid content

Flavonoid content was determined as described by Abdel-Hameed (2009). 100 µL of extract (10 mg/mL) in methanol were mixed with 100 µL of 20% aluminum trichloride in methanol and a drop of acetic

acid, and then the volume was adjusted to 5 mL with methanol. After 40 min, the absorbance was recorded at 415 nm (with a spectrophotometer Agilent 8453). Blank consists of 100 μ L of extract, a drop of acetic acid and adjusted to 5 mL with methanol. The absorbance of quercetin (0.10 mg/mL), used as reference compound, is measured under the same conditions. The tests were performed in triplicate. The flavonoids content expressed as quercetin equivalent (QE) is calculated using the following formula:

$$T_{Flav} = \frac{A \cdot m_0}{A_0 \cdot m}$$

T_{Flav} is the flavonoid content of extract in mg QE/mg; A , the absorbance of the extract; A_0 the absorbance of quercetin; m the mass in mg of the extract and m_0 the mass of quercetin in mg.

Determination of flavonol content

The flavonol content was determined as described by Abdel-Hameed (2009) using quercetin as reference compound. The dosage is based on the formation of a complex with maximum absorption at 440 nm. 1 mL of methanol extract (10 mg/mL) was mixed with 1 mL of aluminum trichloride (20 mg/mL) and 3 mL of sodium acetate (50 mg/mL). The absorption was measured after 2 h 30 min. The absorbance of quercetin (0.025 mg/mL in methanol) was measured under the same conditions. The tests were performed in triplicate. The content of flavonols in the extract (expressed as quercetin equivalent, QE) is calculated using the same formula as for the flavonoids determination (above).

Antiradical and antibacterial activities

Antiradical activity by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) test

The antiradical activity by DPPH test was evaluated using the method described by Kim et al. (2003). The freeze-dried leaves extract was prepared with an initial concentration of 21 mg/mL in dimethyl sulfoxide (DMSO). Concentrations range of extracts or standard (quercetin) was prepared

by successive dilution using microplate technique. The absorbance of residual DPPH in each well was measured at 490 nm (with a spectrophotometer BIO-RAD Model 680) after incubation at 37°C for 30 min. The antiradical activity of a sample (calculated by the following formula) is given as percentage of reduced DPPH:

$$I\% = \frac{A_0 - A_s}{A_0} \times 100; \text{ where } I \text{ is the}$$

percentage of inhibition, A_0 absorbance of control, A_s the absorbance of the sample. For each sample, the concentration (in μ g/mL) required to reduce by 50% the activity of DPPH (IC_{50}) was determined.

Antibacterial activity

The antibacterial activity was evaluated using the method of diffusion through agar as described by Perez et al. (1990). It consists of determining the Minimum Inhibitory Concentration (MIC) which is the lowest concentration of an extract or an antibiotic which does not allow a visible growth (when looking the media with naked eye) (Karaman et al., 2003). The larger the zone of inhibition, the greater the extract is active. The lyophilized decoction of leaves was prepared at a concentration of 10 mg/mL from which different twofold dilutions were prepared. 1 mL of latex was introduced into a sterile tube (whose weight was initially determined) and evaporated to dryness. 10 mL of distilled water were added to the tube and vortexed to dissolve the latex. From this extract (16 mg/mL), twofold dilutions were prepared. The reference controls, gentamicin (10 μ g/disc) and ampicillin (10 μ g/disc) were tested in parallel.

Analysis and expression of results

The results are expressed as mean \pm SEM ($n = 3$). The Data were analyzed using ANOVA followed by Dunnett's posttest for multiple comparisons using Graph Pad Prism version 5.0 for Windows, Graph Pad Software, San Diego California United States. Differences were considered statistically

significant for a p -value less than or equal to 0.05.

RESULTS

Phytochemical profile of extracts

In the present investigation, preliminary phytochemical screening, using tube tests and TLC was performed on leaf extracts of both species. This analysis showed the presence of phytochemical constituents including alkaloids, carbohydrates, flavonoids, saponins, steroids, tannins, phenols, triterpenoids, anthracenosides, anthocyanins, and coumarins (Table 1). Similar results were obtained with extracts of bark of *Ficus racemosa* (Poongothai et al., 2011). These results show a wealth of both species extracts in key phytochemicals.

Antioxidant activity and phenolic content

DPPH scavenging activities of all the extracts were concentration dependent (Table 2). However, the highest DPPH scavenging activity was shown by the extract from *Ficus sycamoros* ($9.60 \pm 0.02 \mu\text{g/mL}$, IC_{50}). Both

extracts showed a weak activity as compared to those of Quercetin ($4.60 \pm 0.08 \mu\text{g/mL}$, IC_{50}) which is a reference antioxidant.

Phenolic compounds are very important plant constituents because they exhibit antioxidant activity by inactivating free radicals or prevent decomposition of hydroperoxides into free radicals (Pokorny et al., 2001). The total phenolic, tannin, flavonoid, and flavonol content of the two extracts were determined in this study (Table 2). The concentration of phenolic compounds, tannins, and flavonoids were higher in the extract from *Ficus sycamoros* when compared with the same extract from *Ficus sur*. For flavonol content, *Ficus sur* ($0.27 \pm 0.01 \text{ mg QE/g}$) is richer than *Ficus sycamoros* ($0.23 \pm 0.00 \text{ mg QE/g}$).

Antibacterial activity

The results of the antibacterial assay of the leaves extracts of both *Ficus* species and the latex of *Ficus sycamoros* are presented in Table 3. Of the three extracts tested, the latex

Table 1: Chemical composition of leaf extracts of *Ficus sur* and *Ficus sycamoros*.

Chemical groups	Plants	
	<i>Ficus sur</i> Forsk.	<i>Ficus sycamoros</i> L.
Sterols and triterpenes	+	+
Carotenoids	+	+
Alkaloid bases	-	-
Flavones aglycones	-	-
Anthracenosids aglycones	-	-
Coumarins	-	-
Tannins	+	+
Alkaloid salts	+	+
Reducing compounds	+	+
Flavonosides	+	+
Anthracenosides	+	+
Coumarin derivatives	+	+
Cardenolides	+	+
Anthocyanins	+	+
Saponins	+	+

+ present, - absent

Table 2: Phenolic contents of leaf aqueous extracts and scavenging activity by the DPPH test.

	Total phenolic mg TAE/g	Tannins mg TAE/g	Flavonoids mg QE/g of	Flavonols mg QE/g	Antiradical activity against DPPH IC₅₀ (µg/ml)
<i>Ficus sur</i>	247,00 ±0,52 ^a	120,80 ±0,83 ^a	19,78 ±0,16 ^a	0,27 ±0,01 ^a	31,83±0,55 ^{a,b}
<i>Ficus sycomorus</i>	336,80 ±0,80	203, 74 ±0,90	22,37 ±0,10	0,23 ±0,00	9,60±0,02 ^b
Quercetin					4,60±0,08

TAE, Tannic Acid Equivalent; QE, Quercetin Equivalent; values are mean ±SEM (n = 3); a, p<0, 05 against *F. sycomorus*; b, p<0, 05 against quercetin

Table 3. MIC values (mg/mL) of leaf aqueous extracts and latex of *Ficus sycomorus* against various bacterial strains.

Plant	Bacteria					
	<i>S. aureus</i>	<i>S. aureus</i> ATCC	<i>E. coli</i>	<i>E.coli</i> ATCC	<i>Sa. typhi</i>	<i>Sa.typhi</i> ATCC
<i>Ficus sur</i>	2.50	1.25	2.50	2.50	-	-
<i>Ficus sycomorus</i>	0.31	0.31	0.63	0.63	-	-
<i>Latex (Ficus sycomorus)</i>	0.13	0.13	0.25	0.25	-	-

Staphylococcus aureus (*S. aureus*), *Escherichia coli* (*E. coli*), *Salmonella typhimurium* (*Sa. typhi*); - no activity; Controls: Ampicillin (10µg/disk) and Gentamicin (10 µg//disk)

of *Ficus sycomorus* was the most potent because it has the best minimum inhibitory concentration (MIC) on *Staphylococcus aureus* (MIC of 0.13 mg/mL) and *Escherichia coli* (MIC of 0.25 mg/mL). The best antibacterial activity of the three extracts was against *Staphylococcus aureus* ATCC. Furthermore, none extract had any inhibition activity against *Salmonella typhimurium*.

DISCUSSION

Herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Much of the therapeutic effects of medicinal plants species have been attributed to phytochemicals, which are the non-nutrient plant compounds. The major phytochemicals revealed by the screening are known to possess a wide range of activities, which may

help in protection against chronic diseases. For example saponins, flavonoids, tannins and alkaloids have hypoglycemic and anti-inflammatory activities (Augusti et al., 2008). Reports show that saponins possess hypocholesterolemic and antidiabetic properties (Rupasinghe et al., 2003). The terpenoids have also been shown to decrease blood sugar level in animal studies. Steroids, triterpenoids and saponins showed analgesic properties and central nervous system activities (Argal et al., 2006). Moreover, the results of this phytochemical screening could support the use of the two species in traditional medicine for the treatment of sickle cell disease. Indeed, many works report the effectiveness of plant secondary metabolites in the treatment of this pathology. Several research results indicate that phenolic compounds and anthocyanins play a role in

the anti-sickle cell activities of some medicinal plants (Mpiana et al., 2008; Ouattara et al., 2009). The detection of phenolics and anthocyanins in extracts of both species indicates a potential of interesting phytochemicals recognized as active against sickle cell disease.

The results also showed that the antiradical scavenging activity is related to the amount of the phenolic content. Then, *Ficus sycomorus* which had the highest total phenolic content with 336.80 ± 0.80 mg TAE/g had also the best antiradical activity with an IC_{50} of 9.6 ± 0.02 mg/mL. Compared to the antiradical activity of the reference control, quercetin ($IC_{50} = 4.6 \pm 0.08$ g/ml), this result, obtained from a raw extract, shows a significant scavenging activity of the active chemicals in leave extract of *Ficus sycomorus*. Previous studies reported the antioxidant activity of some species of *Ficus* and also showed that this antioxidant activity is related to the phenolic content (Al-Fatimi et al., 2007; Abdel-Hameed, 2009). The antiradical activity of *Ficus sur* and *Ficus sycomorus* could be due to the high content of tannins and flavonoids in the leaves of both species. Flavonoids have important antioxidant and antiradical activities. Their protective effects in biological systems are linked to their ability to transfer electrons to free radicals, chelate metals, activate antioxidant enzymes, reducing radicals of alpha-tocopherol or to inhibit oxidases (Bruneton, 2009). Also, the effects of hypoxia are partially mitigated in response to treatment with antioxidants (Carriere et al., 2004). Bouchet et al. (1998) showed that hydrolysable or condensed tannins have antiradical and antioxidant properties expressed by their inhibiting effect on lipid peroxidation (induced by Fe^{2+}) and radical-scavenging ability on DPPH radical. In addition to the molecular abnormality that governs the synthesis of abnormal S hemoglobin, sickle cell anemia is one of the conditions in which the production of free radicals results in a state of oxidative stress. It was also showed that the excessive production

of superoxide anions and hydroxyl radicals in erythrocytes, resulting from the instability of S hemoglobin, can be the initiating factor of hemolysis, which releases in the plasma ionized iron and heme molecules generating in turn, free radicals Deby et al. (1986). Our results show that *Ficus sycomorus* and *Ficus sur* could be of interest in finding news molecules with antioxidant activity because of their high content in phenolics compounds.

About antibacterial properties, the interesting activity of latex is corroborated by other reports showing that the latex of *Ficus carica* L. is more active than leaf extracts on yeast and human pathogenic bacteria (Aref et al., 2010). Evaluation of the antibacterial activity of the two species is important because in addition to the painful crises and anemia, infections are recurrent pathologies of the sickle cell disease that slow down blood circulation. Infectious complications remain the leading cause of death among sickle cell children patients. Indeed meningitis, septicemia and osteomyelitis are common infections in these patients. *Salmonella typhi* and *Staphylococcus aureus* are the bacteria most implicated in septicemia and osteomyelitis (Diagne et al., 2003), while some serotypes of *Escherichia coli* (K1 in particular) are able of causing very serious neonatal infections that are potentially complicated by meningitis septicemia (Sansonetti, 1985). Several works reported antibacterial activities of phenolic compounds such as tannins (Kolodziej et al., 1999). The occurrence of phenolic compounds in both species of *Ficus* could support the observed antibacterial activity.

Conclusion

This study showed that the antiradical and antibacterial activity of *Ficus sur* and *Ficus sycomorus* extract can provide scientific support of their traditional use in the treatment of sickle cell disease. The highest content in total phenolics and tannins and the best antiradical activity were obtained with *Ficus sycomorus*. In addition, the latex of this plant

showed an antibacterial activity on some very important pathogenic germs related to sickle cell disease. These preliminary results call for further investigations of this plant, using biological models of sickle cell disease.

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