

Ficus Sycomorus L. Extracts: Phytochemical Screening, Total Polyphenols and Flavonoids Contents, Antioxidant and Antibacterial Activity

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Abstract: *Ficus Sycomorus L.* is a plant widely used in Senegal by traditional healers for the management of certain pathologies such as stomach aches or skin rashes. The objective of our work is to determine the contents of total polyphenols and flavonoids of the methanolic extracts of the different organs of *Ficus Sycomorus L.* Subsequently, the antioxidant and antimicrobial activities of these extracts were studied. Polyphenols, flavonoids, sterols and polyterpenes are present in all plant organs. The antioxidant capacities expressed as IC₅₀ of the methanolic extracts of the different organs of *Ficus Sycomorus L.* reveal that the stem bark extract has the best activity with an IC₅₀ of 0.237 ± 0.016 mg/mL. This good activity is in agreement with the high levels of total polyphenols in stem bark (193.4257 ± 0.6971 mg/g of gallic acid equivalent). The steam bark extract give the best results for the antimicrobial tests with a MIC value of 1.875 mg/mL on *Staphylococcus Aureus* ATCC 29212. The good scavenging activity of the *Ficus Sycomorus L.* extracts on free radical and the good biological activity justify the use of this plant in traditional medicine. The best antibacterial activities of stem bark of *Ficus Sycomorus L.* on the different bacterial strains can be explained by the higher polyphenol content of stem bark compared to leaves and fruits of *Ficus Sycomorus L.*

Keywords: *Ficus Sycomorus L.*, Polyphenols, Flavonoids, Antioxidant Activity, Antimicrobial Activity

1. Introduction

Traditional medicine occupies an important place in the Senegalese health system. Indeed, the high cost of drugs, the absence of health structures in rural zones and the poverty of the populations constitute a serious problem for access to quality health care. Consequently, Senegalese rural populations as well as urban populations are increasingly resorting to the use of plants which therapeutic effects are attributed. According to some authors, 80% of the world's developing countries uses traditional medicinal plants for health purposes and treatment diseases [1, 2].

The World Health Organization (WHO) considers that in many developing countries, traditional medicine is the primary sector of health referral [3]. In developing countries, knowledge and practices concerning the uses of plants in

traditional medicine are transmitted from generation to generation, often in the same family, in the form of oral tradition. This knowledge are not transmitted in an academic way, and some tend to disappear with the arrival of modern medicine. Plants are a source of medicine because they possess a multitude of bioactive molecules, most of which act as chemical defenses against predation and infection [4, 5]. These molecules are generally endowed with important pharmaceutical properties [6, 7]. For example, alkaloids have analgesic properties [6], tannins are healing [8], antibacterial [9] and antiseptic [10]. Flavonoids have anti-inflammatory and antibacterial properties [11, 12].

Terpenes and steroids are analgesic, anti-inflammatory [13, 14] and quinone derivatives are antibacterial [15].

Under physiological conditions, the body produces free radicals which are useful in certain metabolisms but also in

defense mechanisms against infectious agents. Moreover, in the event of oxidative stress, these free radicals become veritable poisons by attacking normal cellular components such as proteins, lipids, and DNA. It has been shown that oxidative stress is involved in the pathogenesis of several age-related diseases such as cancer or neurodegenerative diseases [16, 17]. For several decades, the resistance of bacteria to antibiotics has become a serious public health problem. Thus, it becomes necessary and urgent to find new antimicrobial molecules.

Various organs of *Ficus Sycomorus L.* are used in herbal medicine. Fruits, roots, stem barks and leaves have been used by different ethnic communities around the world for treatment of different diseases such as gastrointestinal, respiratory, or cardiovascular disorders [18]. *Ficus Sycomorus L.* is also known for its antimicrobial role in the treatment of fungal infections [19]. In view of the beneficial effects attributed to polyphenols, flavonoids, alkaloids, and tannins, and taking into account the intensive use of plants in Senegal for therapeutic purposes, it becomes necessary to make a detailed study of these plants. It is in this perspective that we have started a study on medicinal plants [20]. Herein, we report the results of the qualitative and quantitative study of the extracts of *Ficus Sycomorus L.* After the determination of total polyphenols and total flavonoids contents, in the methanolic extracts of leaves, fruits and stem barks of *Ficus Sycomorus L.*, we studied the antioxidant activities and antimicrobial properties of those extracts.

2. Material and Methods

2.1. Plant Material

The choice of *Ficus Sycomorus L.* is justified by an ethnobotanical survey coupled with a bibliographic study of the plants listed in certain traditional healers. The material used consists of powder from the *Ficus Sycomorus L.* plant (leaves, stem barks and fruit). The harvest of the different organs of this plant was carried out in November 2020 in a field located in the commune of Taïba Ndiaye, located in the region of Thiès, in the West of Senegal with geographical coordinates 15°3'0" North and 16°52'60" West. The three organs of the plant were dried away from direct sunlight and at room temperature. The dried drugs were pulverized using an electric grinder RM 100 Retsch®. The fine powder thus obtained after spraying is used as raw material for the rest of the work.

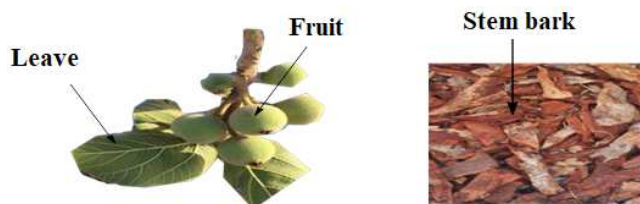


Figure 1. Photograph of the different organs (Leaves, fruits, and stem barks) of *Ficus Sycomorus L.*

2.2. Extraction Procedures

The extraction of the secondary metabolites was done by maceration in methanol to avoid a possible degradation of the thermolabile molecules present in the plant. 200g of plant material were macerated in 500 mL of methanol for 5 days at room temperature. After filtration, the methanol is removed using a rotary evaporator at a temperature of 40°C.

2.3. Phytochemical Studies

Phytochemical screening is a qualitative analysis based on precipitation or coloring reactions which confirm the presence or the absence of secondary metabolites. In this work, the screening concerns the search for alkaloids, polyphenols, tannins, flavonoids, sterols, polyterpenes, coumarins, leucoanthocyanins, catechols and mucilages. We have characterized these different chemical groups by referring to the techniques described by Ronchetti *et al.* [21]. Polyphenols and tannins were identified by the FeCl_3 test and Stiasny's reagent; flavonoids, leucoanthocyanins and catechols by reaction with cyanidin; sterols and polyterpenes by the Liebermann-Burchard test; coumarins by the test with ammonium hydroxide and alkaloids by the Mayer test [22].

2.4. Determination of the Content of Total Phenolic Compounds

The total phenolic content was determined with the Folin-Ciocalteu reagent [23]. Indeed, 40 μL of each extract are taken and supplemented to 200 μL with distilled water. A volume of 150 μL of Folin-Ciocalteu reagent, 600 μL of a 20% Na_2CO_3 solution and 2.32 mL of distilled water are added. After 30 minutes of incubation in the dark, the absorbance was read at 760 nm from a Perkin-Elmer Lambda 365 UV/Visible spectrometer. Gallic acid was used as a standard. The result was expressed as mg GAE/g \pm Standard deviations.

2.5. Determination of Flavonoid Content

The total flavonoid content was determined by the method described by Dirar *et al.* [24]. This method consists of adding 2.5 mL of a 2% ethanolic AlCl_3 solution to 500 μL of each extract. The mixtures are incubated for 1 hour at room temperature and the absorbance was read at 425 nm from a Perkin-Elmer Lambda 365 UV/Visible spectrometer. Quercetin (Q) was used as standard. The result was expressed as mg QE/g \pm Standard deviations.

2.6. Antioxidant Activity

The antioxidant test was performed using the DPPH \cdot method [25]. The DPPH \cdot solution was obtained by dissolving 10 mg of DPPH \cdot in 250 mL of methanol in the dark. For the different extracts, 60 mg of crude extract were dissolved in 3 mL of methanol yielding a solution of 20 mg/mL concentration. This mixture is vortexed. 1 mL of the above solution was mixed with 1 mL of methanol to obtain a solution S1 of 10 mg/mL concentration. Thus, cascade

dilution was carried out to obtain the following eight different solutions of concentrations in the range [5—0.0390625 mg/mL].

For each solution, 0.1 mL was introduced in test tube and mixed with 3.9 mL of the purple methanolic solution of DPPH·, previously prepared. The tubes were stored in dark for 30 min. The absorbance was read at 517 nm from a Perkin-Elmer Lambda 365 UV/Visible spectrometer using methanol as blank. Ascorbic acid is used as reference. DPPH scavenging activity was determined by calculating the percentage of inhibition:

$$\text{Scavenging activity} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}}$$

2.7. Antibacterial Activity

2.7.1. Susceptibility Test: Agar Diffusion Method (Antibiogram Test)

The test consists of depositing discs of defined quantities of antibiotic or extract on the surface of an agar medium previously inoculated with a suspension of bacteria or fungi to be studied. After a stay of 24 hours in the oven at 37°C, the activity of the fractions is judged by the diameter of the zone of inhibition of the culture around each disc. The diameter of the zone of inhibition varies according to the degree of sensitivity of the bacterium or fungus to the antibiotic.

The tests were carried out in a solid medium with Müller Hinton for bacteria and Sabouraud for fungi according to the method of Seck *et al.* with slight modifications [26]. The inoculum was prepared by dilution from an 18 to 24 hours young culture of the microorganism sample. A suspension in physiological water was made, then homogenized by vortexing and sterilized until a turbidity equal to 0.5 Mac Farland was reached. The suspension of germs corresponding to 0.5 Mac Farland was uniformly spread on the surface of the agar then left to incubate for 15 minutes at 37°C to allow the impregnation of the bacteria or fungus on the surface of the agar. The fraction to be tested (25 µL), is deposited on a disc of blotting paper. The discs are placed using sterile forceps, on the surface of the Mueller-Hinton agar previously inoculated, respecting the distance: 30 mm between two discs and 15 mm between the edge of the box and the disc. The dish is incubated at 37°C for 24 hours in an oven.

A stock sample solution of 30 mg/mL was prepared in dimethyl sulfoxide (DMSO) then a successive dilution to half of this solution is carried out twice in order to obtain mass concentrations of 15 mg/mL and 7.5 mg/mL. The controls used are povidone iodine 10, Vancomycin, Clindamycin, Levofloxacin, Amikacin.

2.7.2. Determination of the Minimum Inhibitory Concentration (MIC)

The MIC corresponds to the lowest concentration of the fraction where no turbidity was observed.

The MIC were sought at the level of the fractions which exhibit antimicrobial activities on certain strains. The method used is that described by Cordovana *et al.* [27]. To perform this part, 96-well plates were used. To determine the MIC, we proceeded as follows:

- 1) 24-hours cultures of susceptible germs are diluted in Mueller Hinton (MH) broth;
- 2) the inoculum was standardized to 0.5 at Mac Farland;
- 3) 100 µL of each fraction tested were diluted with 100 µL of MH broth then distributed horizontally in the 96-well microplates. These dilutions were carried out in order to have a value of the concentration divided by two compared to the concentration of the preceding well (30 mg/mL; 15 mg/mL; 7.5 mg/mL; 3.75 mg/mL...);
- 4) 20 µL of bacterial culture suspension is added to each well;
- 5) the plate is then incubated at 37°C for 24 hours in an oven;
- 6) finally, the reading is done.

3. Results and Discussion

3.1. Phytochemical Screening

The results of the phytochemical screening obtained from specific reactions are presented in Table 1. Polyphenols, flavonoids, sterols and polyterpenes are present in all studied parts of the plant. However, alkaloids and catechols are not present in the methanolic extracts of the barks. Catechols, catechic tannins and gallic tannins are absent from the methanolic fruit extract while Leucoanthocyanin and coumarin are absent from the leaves extract.

Table 1. Results of the phytochemical screening of the methanolic extract of the different organs of *Ficus Sycomorus L.*

Organs	Polyphenol	Flavonoid	Alkaloid	Sterol & polyterpene	Leucoanthocyanin	Catechol	Coumarin	Catechic Tannin	Gallic Tannin
Leaves	+	+	+	+	-	+	-	+	+
Stem barks	+	+	-	+	+	-	+	+	+
Fruits	+	+	+	+	+	-	+	-	-

+ Presence; - Absence.

3.2. Total Polyphenol Content, Total Flavonoid Content, and Antioxidant Activity

Several bioactive elements are found in the leaves, the fruits, and the stem barks of *Ficus Sycomorus L.* These

chemical molecules, in particular phenolic compounds often endowed with antioxidant power, play essential roles in physiological processes. Moreover, a deficit of antioxidants, which neutralize reactive oxygen species facilitates the development of cardiovascular diseases, cancer, inflammatory diseases among others [28].

The total polyphenol content (TPC) was expressed in mg GAE/g, while the total flavonoid content (TFC) was determined in mg QE/g equivalent.

The TPC and TFC results and the antioxidant capacities

expressed in IC50 of the methanolic extracts the leaves, the fruits, and the stem barks of *Ficus Sycomorus L.* are collated in Table 2 below.

Table 2. Summary table of the results of the dosage and the antioxidant activity of the different methanolic extracts of *Ficus Sycomorus L.*

Methanolic extract	Extraction yield (%)	Total polyphenol content (mg GAE/g)	Total Flavonoid content (mg QE/g)	CI50 (mg.mL ⁻¹)
Leaves	5.859	8.7931 ± 0.2874	6.0399 ± 0.0574	0.694 ± 0.033
Stem barks	16.76	193.4257 ± 0.6971	5.5232 ± 0.0574	0.237 ± 0.016
Fruits	1.35	11.9885 ± 0.1724	1.5180 ± 0.0459	0.377 ± 0.005

The results of the quantitative determination of TPC of the different studied parts of *Ficus Sycomorus L.* are given in Table 2. From these results, it appears that the stem barks extract shows a fairly high content of polyphenols with 193.4257 ± 0.6971 mg GAE/g. Furthermore, the extracts of the leaves and the fruits of *Ficus Sycomorus L.* were found to be poor in polyphenol compounds with TPC content values of 8.7931 ± 0.2874 mg GAE/g and 11.9885 ± 0.1724 mg GAE/g, respectively. These results show clearly that most of the polyphenolic compounds of *Ficus Sycomorus L.* were produced in the stem barks.

Since flavonoids are considered to be the most widely distributed group of phenolic compounds [29], we evaluated the flavonoid content of the leaves, the fruits, and the stem barks of *Ficus Sycomorus L.* As shown in Table 2, we note that the methanolic extracts reveal low flavonoid contents with a maximum value of 6.0399 ± 0.0574 mg QE/g for leaves methanolic extract. The TFC value of stem barks extract (5.5232 ± 0.0574 mg QE/g) is comparable to the value found for leaves extract. The fruits extract appears very poor in flavonoid with TFC value of 1.5180 ± 0.0459 mg QE/g. Thus, we can postulate that flavonoids are not the main phenolic compounds of *Ficus Sycomorus L.*

The antioxidant capacity was evaluated by the colorimetric technique using the radical DPPH[•] (2,2-diphenyl-1-picrylhydrazyl). The DPPH[•] radical is one of the most commonly used substrates for the rapid and direct assessment of

antioxidant activity due to its stability and simplicity of analysis. The violet-colored DPPH[•] radical is reduced to a yellow-colored compound in the presence of antiradical compounds. With regard to Table 2, we note that the extracts the leaves, the fruits, and the stem barks of *Ficus Sycomorus L.* present interesting antioxidant activities but remain below ascorbic acid (0.143 mg/mL). The highest antioxidant activity which correspond to the lowest IC50, was noted in the stem barks extract with a value of 0.237 ± 0.016 mg/mL. The result is in agreement with the highest TFC value of the stem barks extract. The lowest TFC value found for leaves extract is correlated with the highest IC50 value of 0.694 ± 0.033 mg/mL for this extract. The fruit extract which exhibits the intermediate TFC value shows the median IC50 value of 0.377 ± 0.005 mg/mL. An overview of the literature relates that phenolic compounds, because of their hydroxyl groups, express their antioxidant activity by trapping free radicals and by chelating certain ions. [30, 31]

3.3. Antimicrobial Activity

3.3.1. Sensitivity Test of the Different Strains on the Extract of *Ficus Sycomorus L.*

The sensitivity test was carried out on the methanolic extracts of *Ficus Sycomorus L.* by measuring the diameter of inhibition for the different strains. The results, based on the presence or absence of an inhibition zone around the discs, are shown in Table 3.

Table 3. Antibacterial sensitivity of the strain methanolic extracts of leaves, stem barks and fruits of *Ficus Sycomorus L.* at 30 mg/mL.

Bacteria	Inhibition zone diameter (mm)		
	Leaves	Fruits	Steam barks
<i>Escherichia coli</i> ATCC 25922	-	-	-
<i>Escherichia coli</i> ATCC 35218	9	8	11
<i>Staphylococcus aureus</i> ATCC 29212	8	-	11
<i>Enterococcus faecalis</i> ATCC 29213	8	-	11
<i>Pseudomonas aeruginosa</i> ATCC 27853	-	-	-
<i>Candida albicans</i> ATCC 24433	-	-	-
<i>Candida krusei</i> ATCC 6858	-	-	-

- absence of inhibition zone.

Bacterial resistance against frequently used antibiotics is a major problem and prompts the exploration of new antibacterial molecules. Sensitivity tests of methanolic extracts of leaves, stem barks and fruits were carried out on different bacterial and fungal strains (*Escherichia Coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomas aeruginosa*, *Candida krusei* and *Candida albicans*). Table 3

shows that *Escherichia coli* ATCC 35218 is the only strain sensitive to the extracts of the of leaves, stem barks and fruits of *Ficus Sycomorus L.* at the same time for a concentration of 30 mg/mL. However, strains such as *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 24433, *Candida krusei* ATCC 6858 are not sensitive to methanolic extracts solutions of 30 mg/L

concentration. *Staphylococcus aureus* ATCC 29212 and *Enterococcus faecalis* ATCC 29213 are sensitive to the methanolic extracts solution of leaves and stem barks with 30 mg/L concentration, while they are resistant to the methanolic extracts solution of fruits with the same

concentration.

3.3.2. Minimum Inhibitory Concentration (MIC)

A range of concentrations was prepared to determine the MICs and the results are shown in Tables 4-7.

Table 4. Application of the methanolic extracts of leaves, stem barks and fruits of *Ficus Sycomorus L.* on *Staphylococcus aureus* ATCC 29212 with different concentrations.

Organs	Concentration (mg/mL)											
	30	15	7.5	3.75	1.875	0.937	0.468	0.234	0.117	0.058	0.029	0.0146
Leaves	-	-	-	+	+	+	+	+	+	+	+	+
Fruits	+	+	+	+	+	+	+	+	+	+	+	+
Stem barks	-	-	-	-	-	+	+	+	+	+	+	+

- bacterial inhibition; + bacterial growth.

Table 5. Application of the methanolic extracts of leaves, stem barks and fruits of *Ficus Sycomorus L.* on *Escherichia coli* ATCC 35218 with different concentrations.

Organs	Concentration (mg/mL)											
	30	15	7.5	3.75	1.875	0.937	0.468	0.234	0.117	0.058	0.029	0.0146
Leaves	-	-	-	-	+	+	+	+	+	+	+	+
Fruits	-	+	+	+	+	+	+	+	+	+	+	+
Stem barks	-	-	-	-	+	+	+	+	+	+	+	+

- bacterial inhibition; + bacterial growth.

Table 6. Application of the methanolic extracts of leaves, stem barks and fruits of *Ficus Sycomorus L.* on *Enterococcus faecalis* ATCC 29213 with different concentrations.

Organs	Concentration (mg/mL)											
	30	15	7.5	3.75	1.875	0.937	0.468	0.234	0.117	0.058	0.029	0.0146
Leaves	-	-	-	+	+	+	+	+	+	+	+	+
Fruits	+	+	+	+	+	+	+	+	+	+	+	+
Stem barks	-	-	-	-	+	+	+	+	+	+	+	+

- bacterial inhibition; + bacterial growth.

Table 7. Minimum inhibitory concentration of the methanolic extracts of leaves, stem barks and fruits of *Ficus Sycomorus L.* against the different strains.

Bacteria	MIC (mg/mL)		
	Leaves	Fruits	Stem barks
<i>Escherichia Coli</i> ATCC 35218	3.75	30	3.75
<i>Staphylococcus Aureus</i> ATCC 29212	7.5	-	1.875
<i>Enterococcus Faecalis</i> ATCC 29213	7.5	-	3.75

The results obtained on the strains, with an inhibition diameter of 11 mm for *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC 29212 and *Enterococcus faecalis* ATCC 29213, show that the methanolic extract of the stem barks demonstrates the best antibacterial sensitivity. Indeed, the best MIC which is equal to 1.875 mg/mL, detected for the methanolic stem barks extract was observed on *Staphylococcus Aureus* ATCC 29212. For *Enterococcus faecalis* ATCC 29213, the MIC methanolic stem barks extract was evaluated at 3.75 mg/ mL. Furthermore, the methanolic fruits extract is almost ineffective against the strains tested, with an MIC of 30 mg/mL for *Escherichia Coli* ATCC 35218 and high bacterial resistance for the *Staphylococcus Aureus* ATCC 29212 and *Enterococcus Faecalis* ATCC 29213 strains. The methanolic leaves extract give a MIC values of 3.75 mg/mL for *Escherichia coli* ATCC 35218 and 7.5 mg/mL for *Staphylococcus aureus* ATCC 29212 and *Enterococcus faecalis* ATCC 29213. These results can be correlated with the TPC, as well as with the IC₅₀ obtained

from the methanolic extracts of stem barks, leaves, and fruits. The stem barks show an interesting potential for inhibition of certain microorganisms. However, the methanolic extracts of stem barks, leaves and fruits show lower activities than those of the antibiotics used as reference.

4. Conclusion

In this work, we tried to establish a correlation between the use in traditional medicine of *Ficus Sycomorus L.* and the biological activities of the methanolic extracts of the leaves, stem barks and fruits of *Ficus Sycomorus L.* Phytochemical screening showed the richness in secondary metabolites of the different studied parts of this plant. The total polyphenol content was correlated with the antioxidant capacity determined with DPPH method. The stem barks extract was the richest in polyphenols with a TPC of 193.4257 ± 0.6971 mg GAE/g and it give the lowest IC₅₀ value of 0.237 ± 0.016 mg/mL. The stem barks extract demonstrates the better

antimicrobial sensitivity, with an inhibition diameter of 11 mm for *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC 29212, and *Enterococcus faecalis* ATCC 29213. It shows the best MIC value of 1.875 mg/mL against *Staphylococcus Aureus* ATCC 29212. These various results obtained for the stem barks of *Ficus Sycomorus L.* justify the use of this plant in the management of certain pathologies in traditional medicine.

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