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Chemical composition and antioxidant activities of different parts of *Ficus sur*

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ABSTRACT

Introduction: *Ficus sur* is a plant widely used in traditional pharmacopoeia in Togo. So, this study aimed the assessment of antioxidant properties and identification of some compounds from the ethanolic extracts of different parts of the plant (leaves, fruits, roots, and barks).
Methods: The phenolic and flavonoid contents of the ethanolic extracts of different organs of *Ficus sur* were assessed using conventional known methods. The DPPH radical scavenging and the ferric-reducing antioxidant power (FRAP) assays were used to highlight the antioxidant activities. The different extract samples were also analysed by liquid chromatography coupled to a quadrupole-time of flight mass detector (ESI-QTOF).
Results: Total phenolic contents (TPCs) for 1 mg of dry extract ranged from 489.40 ± 7.48 µg GAE (gallic acid equivalents) for the bark to 62.34 ± 2.66 µg GAE for unripe fruits. The bark exhibited the highest flavonoid content, which was closed to 90.20 ± 3.72 µg QE (quercetin equivalents)/mg of dry extract. The radical scavenging activities of the bark and unripe fruits were 56.50 ± 0.29 and 7.3 ± 0.30 µg QE/mg of dry extract, respectively. In the same order, the FRAP values of the two organs were 104.57 ± 4.75 and 19.61 ± 0.22 µmol $FeSO_4$ Eq/mg of dry extract. Many compounds including notoginsenoside R10; 4',5,7-trihydroxyflavan-3-ol; catechin; and boviquinone 4 were identified.
Conclusion: The various organs of *Ficus sur* are a source of bioactive compounds especially phenolic compounds and flavonoids with antioxidant activity.

Implication for health policy/practice/research/medical education:
Well established as source of bioactive compounds, medicinal plants have long been used in treatment of human ailments. The validation of traditional use of medicinal plants can be achieved by screening a collection of extracts for bioactivity using *in vitro, in cellulo* and even *in vivo* assays. The results of this work are consistent with the traditional use of the various parts of *Ficus sur* to cure hepatic pain, cardiovascular and neurodegenerative diseases, cancer, and diabetes. Although the bark extract is more active than the extracts of the other organs, it would be better to use the leaves to preserve the plant, as these contain all the metabolites present in the bark and much more.
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Introduction

In recent years, there has been growing interest in alternative therapies by the use of natural products. So, during the last decade, focus has been made on the investigation of phytochemistry of plants for pharmaceutical and nutritional purposes. In Togo, plants are widely used in traditional medicine, especially in the rural areas. Among the plants used in Togolese pharmacopoeia, there is *Ficus sur* (Moraceae) commonly called "Petit sycamore" in French and "Wild fig" in English. This plant is usually found in tropical and subtropical areas along the rivers. The plant can grow up to 30-35 m of height while trunk diameter can reach 150 cm (1). The fruits and leaves of *Ficus sur* are used as food in the northern Togo. In addition, several medicinal properties have been attributed to various parts of the plant since

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its roots, barks, leaves, and fruits are used to treat, relieve and heal several pathologies. Indeed, the roots are braised and crushed with grilled corn cobs, then the sieved are used against female infertility. The crushed and macerated fruits in water are used for the care of women during childbirth and after delivery (promotion of lactation) (1). The beverage obtained by decoction after mixing roots and leafy twigs is used to treat eczema (1). Decocted trunk bark is used against amenorrhea, dysentery, hepatic, and cardiovascular pain (2-4).

It is known that oxidative stress is involved in many inflammatory processes related to chronic diseases such as cardiac dysfunction, neurodegenerative diseases or diabetes (5). Indeed, a large number of phenolic compounds such as flavonoids, isoflavones, and phenolic acids have shown antioxidant activity (6-9). Moreover, the benefits of phenolic antioxidant compounds from plants in prevention of chronic diseases have been reported (10,11).

In our preliminary work on this plant, two bioactive phytosterols were identified in the cyclohexanic extract of the leaves (12). The aim of the current work was to assess the antioxidant properties, the total phenolic and flavonoid contents, and then identify some more compounds from various organs of this medicinal plant.

Materials and Methods

Plant material

The leaves, roots, bark, ripe and unripe fruits of *Ficus sur* were harvested at north latitude 11°05'31" and east longitude 00°19'38" in the northern region of Togo (Dapaong). Samples were dried at room temperature inside the laboratory and then reduced to a fine powder using a mill (Thomas Scientific Laboratory Mill Model 4, USA) with a sieve of 1 mm porosity. The extracts were obtained by macerating in ethanol (95°) for 72 hours. After filtration, the solvent was evaporated under reduced pressure at 30°C.

Antioxidant activity

The antioxidant activities of the extracts were evaluated by the DPPH radical-scavenging activity and the ferric-reducing antioxidant power (FRAP).

Determination of DPPH radical-scavenging activity

The method, inspired by the works of Molyneux (13) and Constantin et al (14), is based on the reduction of the violet DPPH• radical by an H atom donor (AH) leading to the formation of the colorless 2,2-diphenyl-1-picrylhydrazine (DPPH-H) and the radical (A•) according to the following equation: DPPH• + AH ⟶ DPPH-H + A•

One hundred microliters (100 µL) of each extract or standard methanolic solutions was mixed and vortexed with a 2 mL methanolic solution of DPPH (10^{-4} M). Only methanol was used as extract solution in the control sample. The fading of the purple free radical DPPH solution by effect of the extracts was measured at 517 nm using a Genesys 10S UV-Vis spectrophotometer (USA), after incubating samples at room temperature in the dark for 30 minutes. A quercetin calibration curve was established for concentrations ranging from 0 to 30 µg/mL. Results were expressed in mg of quercetin equivalents/g of dry extract (mg QE/g).

Determination of ferric-reducing antioxidant power

The FRAP assay consists in reducing the intense blue tripyridyltriazine-ferric complex (Fe^{3+}-TPTZ). At pH 3.5, the Fe^{3+}-TPTZ complex is added to the sample whose antioxidant compounds have effect to reduce this complex into its reduced form Fe^{2+}-TPTZ. The test was carried out according to the method described by Nair et al (15). In tubes containing 300 µL of freshly prepared FRAP solution (25 mL of acetate buffer, 2.5 mL of 10 mM Fe^{3+}-TPTZ in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3$,$6H_2O$), 30 µL of distilled water and 10 µL of sample were added. The change in absorbance from the red to blue was followed at 593 nm after a process of 10 minutes of incubation. A calibration curve was established from a concentration range of 0 to 2000 µM of iron sulphate ($FeSO_4$,$7H_2O$) solution dissolved in methanol (blank). The obtained results were expressed in µmol of $FeSO_4$ equivalents/mg of dry extract.

Phytochemical profiles of the different organs of *Ficus sur*

An initial rapid screening of molecular families present in the different organ extracts was done using specific reagents and procedures described in the literature (16-18).

Determination of total phenolic content

The colorimetric method developed by Singleton et al (19) and slightly modified by Al-Farsi et al (20) was used. An aliquot of 100 µL of each extract at 1 mg/mL was mixed with 750 µL of Folin-Ciocalteu reagent diluted up to 1/10. After 5 minutes of incubation at 25°C, 750 µL of Na_2CO_3 aqueous solution (20%) was added. The obtained mixture was incubated for 90 minutes in the dark and the change in absorbance was followed at $\lambda = 765$ nm. The extract was substituted by distilled water for blank. Gallic acid at different concentrations (0-500 µg/mL) was used as standard and the results were expressed in µg of gallic acid equivalents per milligram of dry extract (µg of GAE/mg of dry extract) by extrapolating the calibration line ($y = 0.005018x + 0.055190$, $R^2 = 0.9925$) obtained from the different concentrations of gallic acid. The Genesys 10S UV-Vis Spectrophotometer, USA was used.

Determination of flavonoid content

The total flavonoid contents (TFCs) of the extracts were estimated via the colorimetric aluminum trichloride

($AlCl_3$) method described by Kim et al (21). One hundred microliters (100 µL) of ethanolic extracts solution was added to 400 µL of distilled water. Thirty microliters (30 µL) of a 5% sodium nitrite ($NaNO_2$) solution was added to the former mixture. After 5 minutes of incubation at room temperature, 20 µL of a 10% aqueous solution of $AlCl_3$ was added. Finally, 200 µL of a Na_2CO_3 aqueous solution (1M) and 5 minutes later, 250 µL of distilled water (blank) were added, successively. The whole mixture was vortexed and the absorbance was measured directly with UV-Vis spectrophotometer (Genesys 10S UV-Vis Spectrophotometer, USA) at λ = 510 nm. The concentration of total flavonoids was deduced from the calibration curve established with quercetin (0-500 µg/mL) and the results were expressed in µg quercetin equivalents/mg of dry extract (µg EQ/mg of extract).

Statistical analysis

Results were analyzed statistically using the OriginPro 9.0 software. Data were expressed as means ± standard deviation (SD) of experiments performed in triplicate. One-way analysis of variance (ANOVA), principal component analysis (PCA) and Pearson correlation coefficient (ρ) were used to evaluate and correlate the results with each other. Differences with P values less than 0.05 were considered significant.

HPLC-ESI$^+$-QTOF-HRMS analysis

A methanolic solution (1000 µg/mL) was prepared from the ethanolic extracts of different organs. The chromatographic analysis for the separation of the compounds was carried out in reverse phase on an Agilent series 1200 HPLC system equipped with a C18 column from Microchip Technology (Agilent, Zorbax 300 SB-C18, 5 µm, 43 mm, 75 µm). An Agilent 6530 quadrupole - time of flight (QTOF) mass detector (Agilent Technologies, Santa Clara, Calif., USA) coupled to an electrospray ionizer (ESI) was used. Chromatographic conditions were as follows: flow rate: 0.4 µL.min^{-1}; solvent A: 0.1% formic acid in water; solvent B: 0.1% formic acid in methanol. The solvent gradient (v/v) was generated starting from 20% B and reaching 100% B in 10 minutes and maintaining at 100% B for 10 minutes for a total run of 20 min. Two microliters of the samples were analyzed by ESI in positive mode. Mass spectral data were acquired in the range m/z 100–1500, with an acquisition rate of 1.35 spectra.s^{-1}, averaging 10 000 transients. The source parameters were adjusted as follows: drying gas temperature 250°C, drying gas flow rate 5 L.min^{-1}, nebuliser pressure 45 psi and fragmentor voltage 150 V. Data acquisition and processing were done by Agilent MassHunter Workstation Acquisition software v. B.02.00. The METLIN database (https://metlin.scripps.edu/index.php) was used to predict plausible structures of detected metabolites.

Results

Evaluation of antioxidant activity

To investigate the antioxidant activity of the various organs of *Ficus sur*, two *in vitro* assays were used, the DPPH radical scavenging and the FRAP assays. Results are resumed in Figure 1. The bark extract had the best DPPH radical scavenging activity closed to 56.50 ± 0.29 µg QE/mg of dry extract; the ripe fruit extract being the least active with an activity of 7.3 ± 0.30 µg QE/mg of dry extract. The evaluation of the ferric ion reduction power yielded FRAP values ranging from 104.57 ± 4.75 µmol $FeSO_4$ Eq/mg dry extract for the bark to 19.61 ± 0.22 µmol $FeSO_4$ Eq/mg of dry extract for ripe fruits.

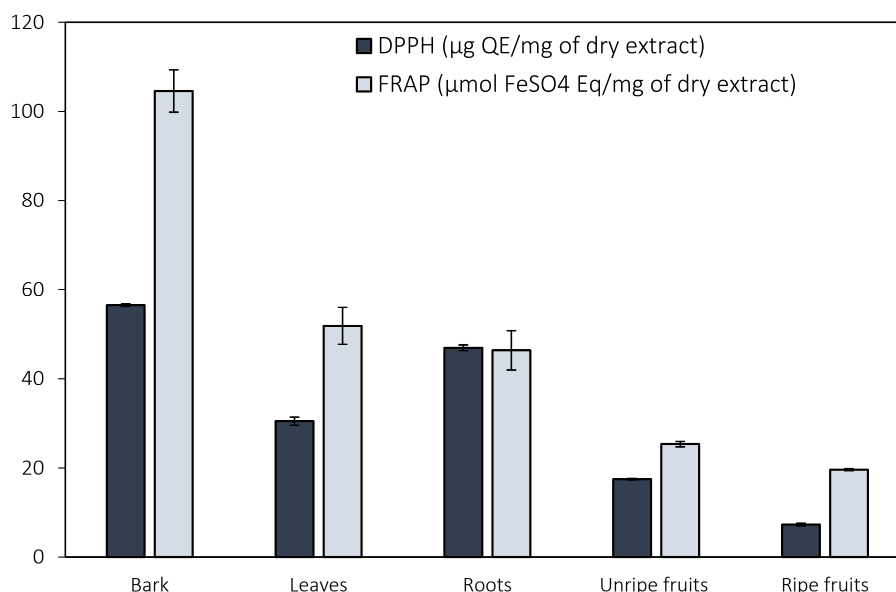

Figure 1. Antioxidant abilities in µg QE (quercetin equivalents)/mg of dry extract (for the DPPH radical scavenging assay) and in µmol $FeSO_4$ Eq/mg of dry extract (for the FRAP assay). Values are expressed as mean ± SEM; n = 3.

Table 1. Phytochemical analysis of the various organs of *Ficus sur*

Phyto-chemicals	Ripe fruits	Unripe fruits	Leaves	Bark	Root
Alkaloids	+	+	+	+	+
Flavonoids	+	+	+	+	+
Saponosides	+	+	+	+	+
Quinones	-	-	-	-	-
Glycosides	+	+	+	+	+
Tannins	+	+	+	+	+
Sterols and terpenoid	+	+	+	+	+
Anthocyanins	+	+	+	+	+

(+) = present ; (-) = absent.

Phytochemical screening

The phytochemical screening revealed the presence of alkaloids, flavonoids, saponosides, glycosides, anthocyanins, tannins, sterols, and terpenoid in the various organs of *Ficus sur* (Table 1).

Total phenolic content

Figure 2 reports the different values of total phenolic contents (TPCs) of the various organs of *Ficus sur*. For 1 mg of the dry extracts, the TPC ranged from 489.40 ± 7.48 µg GAE for the bark to 62.34 ± 2, 66 µg GAE for unripe fruits. For the other organs, the values are as follows: 177.79 ± 3.29 µg GAE for the roots, 84.86 ± 15.06 µg GAE for the ripe fruits and 66.06 ± 7.44 µg GAE for leaves.

Total flavonoid content

The TFCs of the various organs of *Ficus sur* are reported in Figure 2. The bark had the highest flavonoids content, 90.20 ± 3.72 µg QE/mg of dry extract. This was followed by ripe fruit (78.07 ± 10.23 µg QE/mg of dry extract), roots (48.55 ± 3.19 µg QE/mg of dry extract), unripe fruits (31.57 ± 5.72 µg QE/mg dry extract) and leaves (27.06 ± 5.19 µg QE/mg of dry extract).

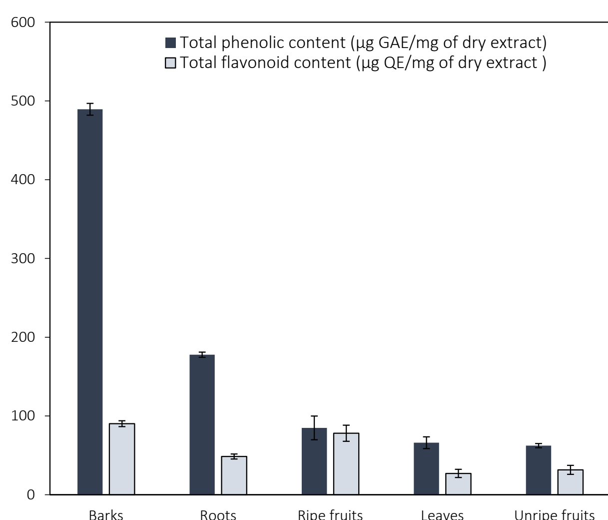

Figure 2. Total phenolic and flavonoid content of extracts from different organs of *Ficus sur*. Values are expressed as mean ± SEM; n = 3.

Results of the descriptive statistical analyses

Figure 3 shows the principal component analysis (PCA) carried out taking into account the different parameters (TFC, TPC, FRAP and DPPH). The Pearson correlation coefficients (ρ) determined in order to quantify correlation between those parameters are resumed in Table 2.

Identification of the main compounds of the various extracts

The HPLC analysis of each tested extract yielded the chromatograms which are shown in Figure 4. The comparative chromatographic profile of roots, barks, leaves, and ripe fruit shows (Figure 4), on the one hand, common peaks to all the four extracts (peaks 1-23) and on the other hands, some peaks that discriminate different organs (peaks a-q). This comparison shows that the root extract has the simplest chromatographic profile, followed by barks, leaves, and finally by fruits which exhibit complex profile.

The ESI$^+$-QTOF-HRMS analysis offered the possibility to determine the exact masses of the different compounds detected. Many characteristics of the different compounds identified are summarized in Tables 3 and 4.

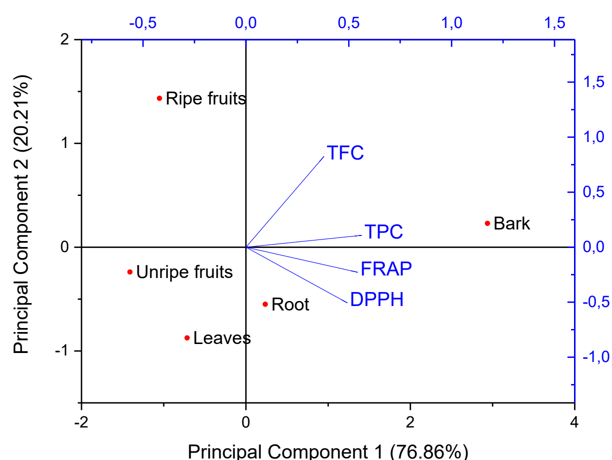

Figure 3. Comparison of the different organs by principal component analysis.

Table 2. Correlation matrix between DPPH, FRAP, TFC and TPC

	DPPH	TPC	TFC	FRAP
DPPH	1	0.79994	0.25466	0.8747
TPC	0.79994	1	0.72076	0.92075
TFC	0.25466	0.72076	1	0.46917
FRAP	0.8747	0.92075	0.46917	1

Discussion

All extracts from the various organs of *Ficus sur* showed antioxidant activities considering the two assays: DPPH and FRAP. Indeed, the different extracts exhibited positive effect to the total phenolics and flavonoids contents. The PCA allowed a better understanding of the relative behavior of the different parameters studied. In fact, the determination of the Pearson correlation coefficient between FRAP and DPPH ($\rho = 0.8747$) indicated that the two methods of assessing the antioxidant activity progress together and could be complementary. Moreover, the TPC is strongly correlated with the FRAP values ($\rho = 0.92075$) and DPPH values ($\rho = 0.79994$). The similar observations were made by Michel et al (22). Flavonoids are a subclass of phenolic compounds recognized as an important source of antioxidants (23). In this work, although the TFCs are well correlated with those of the total phenolic compounds ($\rho = 0.72076$), the antioxidant activity is very weakly related to the TFCs; the coefficients being 0.25466 between the TFC and the DPPH values and 0.46917 with those of the FRAP. This weak correlation could be explained by the non-specificity of the flavonoid assay method. Indeed, as illustrated by Cornard and Merlin (24), quercetin is able to form stable compounds in the presence of aluminum chloride since three different complexation sites (3-hydroxy-4-keto, 5-hydroxy-4-keto, 3′,4′-o-diphenolic) exist in its structure. The extracts showing positive responses to this assay might contain derivatives of quercetin and more generally flavonoids. However, it should be noted that some terpene compounds with adjacent hydroxyl, acidic or coumaryl groups that favour the complexation of aluminum may also respond

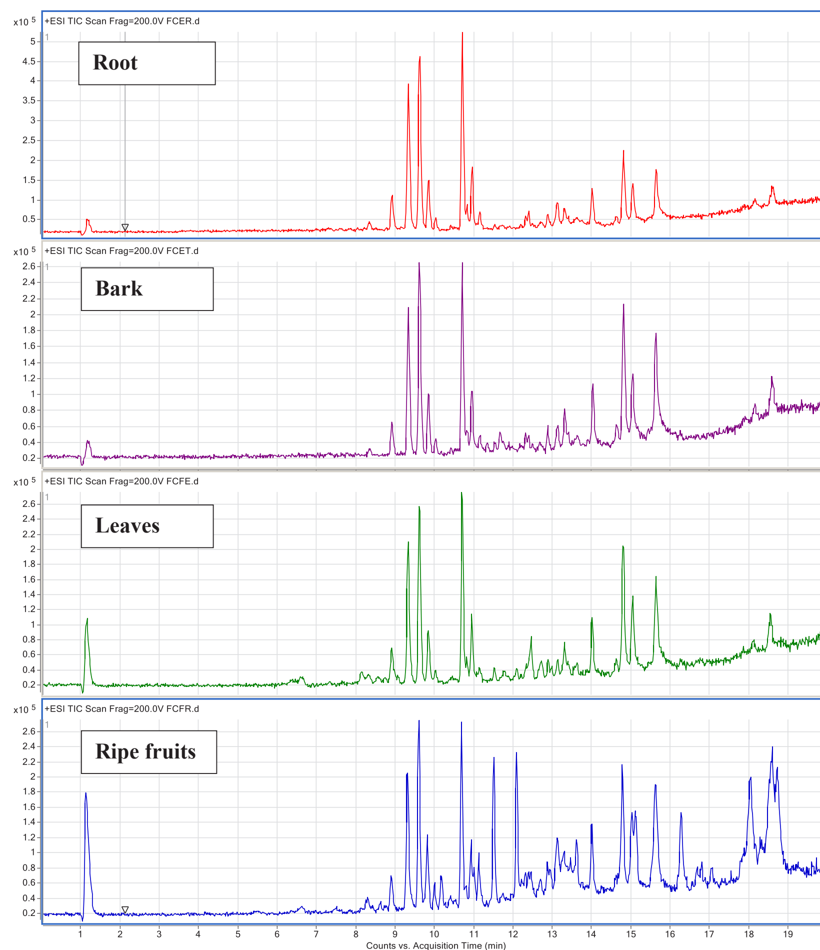

Figure 4. Chromatographic profiles of the extracts from various organs.

Table 3. Characteristics of the common compounds of the extracts of the various organs of *Ficus sur*

Peak	RT (min)	$[M + H]^+$ m/z	Proposed molecular formula	Proposed compound identification	Class
1	8.31	453.3470	$C_{20}H_{36}O_{11}$	2-Cyclohexyl-1-ethyl-β-D-maltoside	Glycoside
2	8.89	567.4323	N.I.	-	-
3	9.28	555.3575	$C_{30}H_{50}O_{9}$	Notoginsenoside R10	Glycoside
4	9.60	396.8035	N.I.	-	-
5	9.81	453.8481	N.I.	-	-
6	9.99	509.8878	N.I.	-	-
7	10.69	275.2768	$C_{15}H_{14}O_{5}$	4’,5,7-trihydroxyflavan-3-ol	Flavonoid
8	10.93	291.2711	$C_{15}H_{14}O_{6}$	Catechin or isomer	Flavonoid
9	11.13	304.3010	$C_{21}H_{37}N$	3,5-dipentyl-2-hexyl-pyridine	Alkaloid
10	12.35	579.2985	N.I.	-	-
11	12.69	277.2176	$C_{18}H_{28}O_{2}$	α-Parinaric acid	Fatty acid
12	12.86	425.2159	$C_{22}H_{32}O_{8}$	Dihydrovaltrate	Fatty acid
13	13.10	502.3760	N.I.	-	-
14	13.28	470.2553	N.I.	-	-
	13.29	491.1869	N.I.	-	-
15	13.60	637.3068	N.I.	-	-
16	13.98	257.2486	$C_{16}H_{32}O_{2}$	Palmitic acid	Fatty acid
	13.99	353.2673	$C_{21}H_{36}O_{4}$	Ceriporic acid C	Fatty acid
17	14.66	522.5984	N.I.	-	-
18	14.78	285.2799	$C_{18}H_{36}O_{2}$	Stearic acid or isomer	Fatty acid
	14.78	381.2989	$C_{23}H_{40}O_{4}$	Persine or isomer	Fatty acid
19	15.02	413.2679	$C_{26}H_{36}O_{4}$	Boviquinone 4	terpenoid
20	15.42	437.2887	$C_{25}H_{40}O_{6}$	Salvisyriacolide	terpenoid
21	18.16	520.4382	N.I.	-	-
22	18.59	685.4381	N.I.	-	-
23	19.9	569.4346	$C_{40}H_{56}O_{2}$	Lutein	Carotenoid

N.I. = Not Identified.

Table 4. Characteristics of compounds discriminating extracts of the various organs of *Ficus sur*

Peak	RT (min)	$[M + H]^+$ m /z	Proposed molecular formula	Proposed compound identification	Class	Organ
a	6.35	377.0863	$C_{18}H_{16}O_{9}$	5,7,3',6'-Tetrahydroxy-6,8,2'-trimethoxyflavone	Flavonoid	Le
b	6.63	235.0612	$C_{12}H_{10}O_{5}$	Armillarisin A or isomer	Coumarin	Le, Fr
c	8.18	608.3866	N.I.	-	-	Le
d	8.66	393.1897	N.I.	-	-	Le, Fr
e	8.76	481.1690	N.I.	-	-	Le
f	10.16	293.0432	N.I.	-	-	Fr
g	11.25	479.1722	N.I.	-	-	Ba
h	11.49	415.2132	$C_{24}H_{30}O_{6}$	Armillarine	Sesquiterpene aryl ester	Ba, Le, Fr
i	11.76	288.2570	N.I.	-	-	Ba, Le, Fr
j	12.07	452.3259	$C_{28}H_{41}N_{3}O_{2}$	Teleocidin B1 or isomer	Alkaloid	Le, Fr
k	13.37	468.3925	$C_{30}H_{49}N_{3}O$	Lucidine B	Alkaloid	Fr
ℓ	16.25	484.3889	N.I.	-	-	Fr
m	16.47	469.3321	$C_{30}H_{44}O_{4}$	Uralenolide	Terpenoid	Fr
n	16.77	429.3746	$C_{29}H_{48}O_{2}$	Saringosterol	Sterol	Fr
o	17.11	427.3940	$C_{30}H_{50}O$	alpha-amyrin or isomer	terpenoid	Fr
p	18.16	520.4382	N.I.	-	-	Fr
q	18.62	703.4176	N.I.	-	-	Fr

positively to this assay (25).

Figure 3 provides an excellent comparison of the various organs studied. In that figure one may notice the eccentricity of two organs: on the one hand, the bark which seems more antioxidant and richer in phenolic compounds and on the other hand the ripe fruits with less antioxidant activity and less rich in phenolic compounds. Unripe fruits, leaves and roots have more or less similar behaviour in relation to the parameters studied. This trend is confirmed by the one-way ANOVA. In fact, with the DPPH radical scavenging assay, the bark extract is significantly more antiradical ($P < 0.0001$) than the extracts of the other organs. All antiradical activities are significantly different from each other and decrease in the following order: bark, roots, leaves, unripe fruits, ripe fruits. This order is almost confirmed by the FRAP test, except that the leaves are significantly more active than the root. Similarly, for phenolic compound contents, the bark is significantly richer in those metabolites ($P < 0.0001$). The HPLC-ESI$^+$-QTOF-HRMS analysis provided important information on the nature of the metabolites contained in the various extracts. Positive ionization yielded proton molecular ions $[M + H]^+$ and the approximate value of the mass of each $[M + H]^+$ ion was given with the corresponding molecular structure in the METLIN database. Mass analysis showed more than one compound for some peaks (peaks 14 and 18), demonstrating that the problem of coelution of some compounds in HPLC can be solved by mass spectrometry. Based on the $[M + H]^+$ quasi-molecular ions provided by the high-resolution mass spectra, some common compounds from the various extracts might be notoginsenoside R10 (peak 3) and 4',5,7-trihydroxyflavan-3-ol (peak 7). According to previous studies, the latter compound (a flavonoid), has been identified in *Ficus sur* (26). In the present work, other major compounds such as catechin, palmitic acid, saringosterol and α-amyrin have been identified. The presence of some of these compounds in *Ficus sur* extracts has been previously reported (27,28).

Conclusion

This study shows that the different organs of *Ficus sur* constitute an important source of phenolic compounds and flavonoids. The immature fruit extract which is poor in phenolic compounds contains 62.34 ± 2.66 µg GAE for 1 mg of dry extract while the extract of the bark (the most active) contains 489.40 ± 7.48 µg GAE/mg dry extract. The correlation assessment shows that about 72% of the phenolic compounds found in the *Ficus sur* would be flavonoids. Moreover, the FRAP technique and the inhibition of the DPPH radical confirmed that the various organs of *Ficus sur* contain metabolites with potential antioxidant activity. Above all, statistical analyses such as PCA and ANOVA showed that the bark is the organ that contains both the most important phenolic content and presents the best antioxidant activity. The bark of *Ficus sur* would therefore be a natural source for the search for active ingredients against cardiovascular diseases, neurodegenerative diseases, cancer, and diabetes.

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Authors' contributions

KIS, PB, OS and MM conceived and designed the experiments; KIS, KE, KI, performed the experiments; KIS, OS, KE, KI and OT analysed the data; PB, OS, KK and AA supervised the experiments; all authors contributed to the writing of the manuscript and confirmed publication of the final version.

Conflict of interests

The authors have no conflicts of interest.

Ethical considerations

Ethical consideration has been completely observed by the authors.

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