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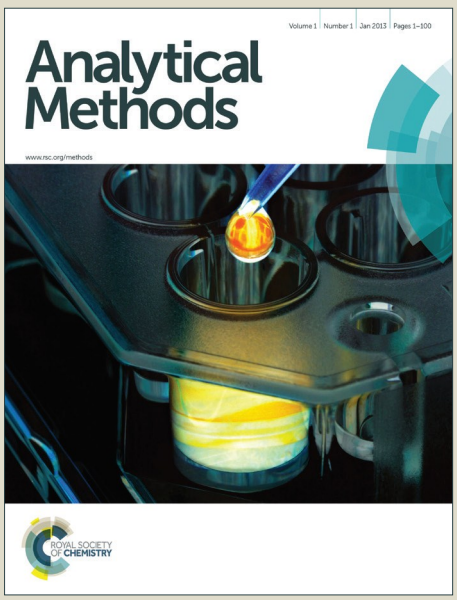

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Profiles of phenolic compounds by FT-ICR MS and Antioxidative and Antiproliferative Activities of *Stryphnodendron obovatum* Benth leave extracts

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Abstract

Stryphnodendron obovatum Benth is a Brazilian Cerrado tree, which is rich in catechin derivatives, being therefore widely used in folk medicine. Its seeds, beans and barks have been used for various medicinal purposes, but few reports have tried to correlate their biological activity with chemical composition. In this study we have profiled the main phenolic constituents of *S. obovatum* leaves extracts by ultrahigh resolution mass spectrometry (FT-ICR MS), and tried to establish a correlation of metabolites and antioxidant and *in vitro* antiproliferative activities. In the methanolic extract were detected flavonoid derivatives and they presented the most promissing scavenging activities for methanolic extracts (EC_{50} 0.86±0.01μg mL^{-1}), being slightly more active than Trolox (EC_{50} 1.13±0.09μg mL^{-1}). In the hidroetanolic extracts, catechin derivatives as well as protocatechuic acid-glucoside were detected and found to be a little less active than the control (EC_{50} 1.39±0.01μg mL^{-1}). The best performance in terms of antiproliferative activity was found for the methanolic extract for renal cell lines (TGI 40.6 g mL^{-1}).

Keywords: *Stryphnodendron obovatum* Benth, Antioxidative, Antiproliferative, FT-ICR MS.

Introduction

Stryphnodendron spp are Brazilian Cerrado plants traditionally used in folk medicine to treat several pathologies such as gonorrhea, hernia, diarrhea, bleeding wounds, and gastric ulcers.[1] Several biological properties such as antimicrobial, anti-ulcer, hypotensive and antioxidant activities,[2,3] have also been described for the extracts of species of *Stryphnodendron* barks. Seeds of *Stryphnodendron* spp have also been reported to display antifungal and antibacterial activities,[5] whereas it beans are known to be very toxic to animals.[6] Most phytochemical studies have been focused on the *S. obovatum* barks and their polyphenols. Some of them have been described, in such studies, to be responsible for the activities of its extracts. Among such phenols, the most comoon have been gallic acid, p-hydroxybenzoic acid, catechin derivatives and tannins isolated from acetone:water (7:3 v/v) and ethyl acetate extracts.[2,7,8] Numerous experimental and epidemiological studies have also shown that phenolics, flavonoids and catechin derivatives are commonly found in *Stryphnodendron* spp, and these constituents are normally described as antioxidants.[9,10]

Polyphenols and derivatives in plant extracts have been determined mainly via chromatographic protocols, but more recently direct infusion electrospray ionization mass spectrometry (ESI-MS) has been increasingly used to obtain profiles of phenolic compounds from plant extracts, particularly when elemental composition can be inferred from the MS measurements.[11] Direct infusion ESI-MS most particularly when ultrahigh resolution and high accuracy Fourier transform MS (FT-MS) analysis is employed has offered therefore a chromatography-free protocol for fast, versatile, sensitive, low-consuming solvent protocol for chemical screening of plant extracts.[13,14] The direct analysis also eliminates more elaborated sample preparation protocols required for chromatographic separations.[11] We have, therefore, extensively and successfully applied direct ESI-FT-MS fingerprinting in our laboratory for a variety of complex mixtures such as plant extracts,[12] herba matte[13], propolis,[15] wine,[16] sediments[17] and crude oils.[18]

Plant extracts, which are rich in phenolic compounds such as those from *S. obovatum* are also usually analyzed in terms of total phenolic contents and antioxidant assays. The level of total phenols is normally used to estimate the relative concentrations of flavonoids, phenolic acids and tanins due to their importance as scavengeers of free radicals and their action as antioxidants. The importance of the search for natural antioxidants is highlighted by their action in disposing, scavenging and suppressing the formation of ROS (reactive oxygen species) or in opposing their actions. Plant extracts are being, therefore, increasingly used in fighting against various diseases including cancer and their clinical manifestations.[19]

The search of phytochemicals has been raised because their potential use in the therapy as antioxidants or anticancer drugs. Experimental studies have also associated oxidative cellular damage arising from an imbalance between free radical generating and scavenging systems, as the primary cause of cardiovascular disease, cancer and aging.[20] Various plant secondary metabolites have also been reported to act as antioxidants and, amongst them, phenolic compounds form a major group. They exert different properties such as reducing agents, hydrogen donors, free radical scavengers, singlet oxygen quenchers and metal chelators.[21,22] The scavenging of DPPH radical is widely used for rapid evaluation of antioxidant activity of different compounds, and the inhibitory effect of tannins and flavonoids against DPPH radical is well established.[22,23]

In this context, the aim of this study was to evaluate the main phenolic compounds present in different *S. obovatum* Benth leaves extracts using direct infusion ESI-FT-ICR MS, and to establish a correlation among such compounds with the antioxidant and *in vitro* antiproliferative activities of such extracts.

Experimental

Plant Material and Extractions

The leaves of *S. obovatum* (Fabaceae) were collected at Faculty of Sciences and Letters UNESP (Assis, Brasil) in July 2006. A voucher was deposited at Dom Bento Pickel Herbarium – São Paulo

Forestry Institute on number 40892. Dried and crushed leaves (60g) were extracted by dynamic maceration with ethanol:water 70:30 v/v (1:10 plant/solvent ratio, 3 x 2h), at room temperature. After filtration, the extract was concentrated under reduced pressure until complete organic solvent elimination, providing the hydroethanolic extract (SOHE). For organic extracts, 100g of leaves were successively extracted by dynamic maceration with hexane, ethyl acetate and methanol (1:10 plant/solvent ratio, 3 x 2h), at room temperature. After filtration, combined extracts were concentrated under reduced pressure until complete organic solvent elimination, providing the hexane (SOH), ethyl acetate (SOA) and methanol (SOM) extracts.

ESI(-) FT- MS analysis

For this exploratory study, 2 µL of leaves extracts were dissolved in a MeOH/H_2O solution (1:1 v/v) containing NH_4OH 0.1 % in order to facilitate deprotonation of the more acidic compounds to yield $[M - H]^-$ anions. The FT-MS analysis were performed using a 7.2T LTQ FT Ultra ion cyclotron resonance (ICR) mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with a direct infusion electrospray ionization source (ESI) operating in the negative ion mode at the follow conditions: Spray Voltage, -3.6 kV; Capillary Potential, -40V; Tube lens potential, -100V; and Capillary Temperature, 280°C. The width isolation and fragmentation amplitude for the MS/MS experiments were adjusted to obtain the best condition for each experiment. The average resolving power (Rp) was 200.000 at *m/z* 400, where Rp was calculated as $m/\Delta m_{50\%}$, that is, the *m/z* value divided by the peak width at 50% peak height, and the acquisition in the ICR cell was of the 10 transients for each run. The instrument was carefully tuned to provide adequate ion peaks in the mass range of interest and to minimize possible fragmentations. Data acquisition were performed along the mass range of *m/z* 100-1000 and processed *via* the Xcalibur 2.0 software.

Phenolic content

It was performed as described by Prior et al.[24], with small modifications in order to allow the use of a microplate reader. Briefly, an aliquot (10µL) of the sample (1mg mL^{-1}) was diluted in distilled water (600µL). Then, this solution was placed in a 96-well plate (150µL $well^{-1}$), in triplicate, followed by Folin-Ciocalteau solution (12.5µL $well^{-1}$), sodium carbonate (37.5µL well, 1M) and water (50µL $well^{-1}$). After 2h of incubation at 37°C, absorbance was measured at 725nm with a microplate reader (VERSA Max, Molecular Devices). A calibrated gallic acid standard curve was made and results were expressed as mg equivalents of gallic acid per gram of sample.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Microplate DPPH assay was performed as described by Brand-Williams et al.[25] modified by Brem *et al*.[26] Briefly, in a 96-well plate, successively sample dilutions (100µL $well^{-1}$, 0.25, 2.5, 25 and 250µg mL^{-1}), tested in triplicate, received DPPH solution (40µM in methanol, 100µL $well^{-1}$) and absorbance was measured at 550nm with a microplate reader (VERSA Max, Molecular Devices). Results were determined each 5min for 150min in order to evaluate kinetic behavior of the reaction. The percentage of remaining DPPH was calculated as follows: %DPPHrem=100×([DPPH]sample/[DPPH]blank). A calibrated Trolox standard curve was also made. The sample concentration necessary to decrease the initial DPPH concentration by 50% (EC50) was calculated by exponential regression, using software Excel. The time needed to reach the steady state of EC_{50} (TEC_{50}) was verified experimentally and, in order to correlate TEC50 and EC50, the antiradical efficiency (AE) was calculated as follows: $AE = 1/(EC_{50} \times TEC_{50})$, as described by Brem.[26]

***In vitro* antiproliferative activity assay**

Human tumor cell lines U251 (glioma), UACC-62 (melanoma), MCF-7 (breast), NCI-ADR/RES (ovarian expressing phenotype with multiple drugs resistance), 786-0 (renal), NCI-H460 (lung, non-

small cells), PC-3 (prostate), OVCAR-03 (ovarian), K562 (leukemia) and a non-tumoral cell line VERO (epithelial cells of monkey kidney) were obtained from National Cancer Institute at Frederick, MA, USA. Stock cultures were grown in medium containing 5 mL of RPMI 1640 (GIBCO BRL) supplemented with 5% fetal bovine serum (FBS, GIBCO) at 37°C with 5% CO_2. Penicillin:streptomycin (1000 μg L^{-1}: 1000 U L^{-1}, 1 mL L^{-1}) was added to the experimental cultures. Cells in 96-well plates (100 μL cells $well^{-1}$) were exposed to the extracts in DMSO (Merck)/RPMI (0.25, 2.5, 25, and 250 μg mL^{-1}) at 37 ^{0}C, 5% of CO_2 in air for 48 h. Final DMSO concentration (0.2% in higher concentration) did not affect cell viability. Cells were then fixed with 50% trichloroacetic acid (Merck) and cell proliferation was determined by spectrophotometric quantification (540nm) of cellular protein content using sulforhodamine B assay. Using the concentration–response curve for each cell line, the values for the concentrations that produces total growth inhibition or cytostatic effect (TGI) were determined through non-linear regression analysis using software ORIGIN 8.5® (OriginLab Corporation).[27]

Results and Discussion

The greatest extraction yield was achieved by hydroethanolic extraction (27.8%) followed by extraction with the organic solvents, that is: methanol (23.2%), ethyl acetate (11.6%) and hexane (4.3 %).

Table 1 as well as Fig 1 to 3 show the main phenolic compounds identified via their molecular formula and dissociation patterns from all these extracts by ESI(-)-FT-MS/MS. Note that several of the flavonoids and catechin derivatives have already been described in previous studies using *S. obovatum* stem bark extracts.[28]

Table 1 – Phenolic compounds identified from *Stryphnodendron obovatum* leaves extracts.

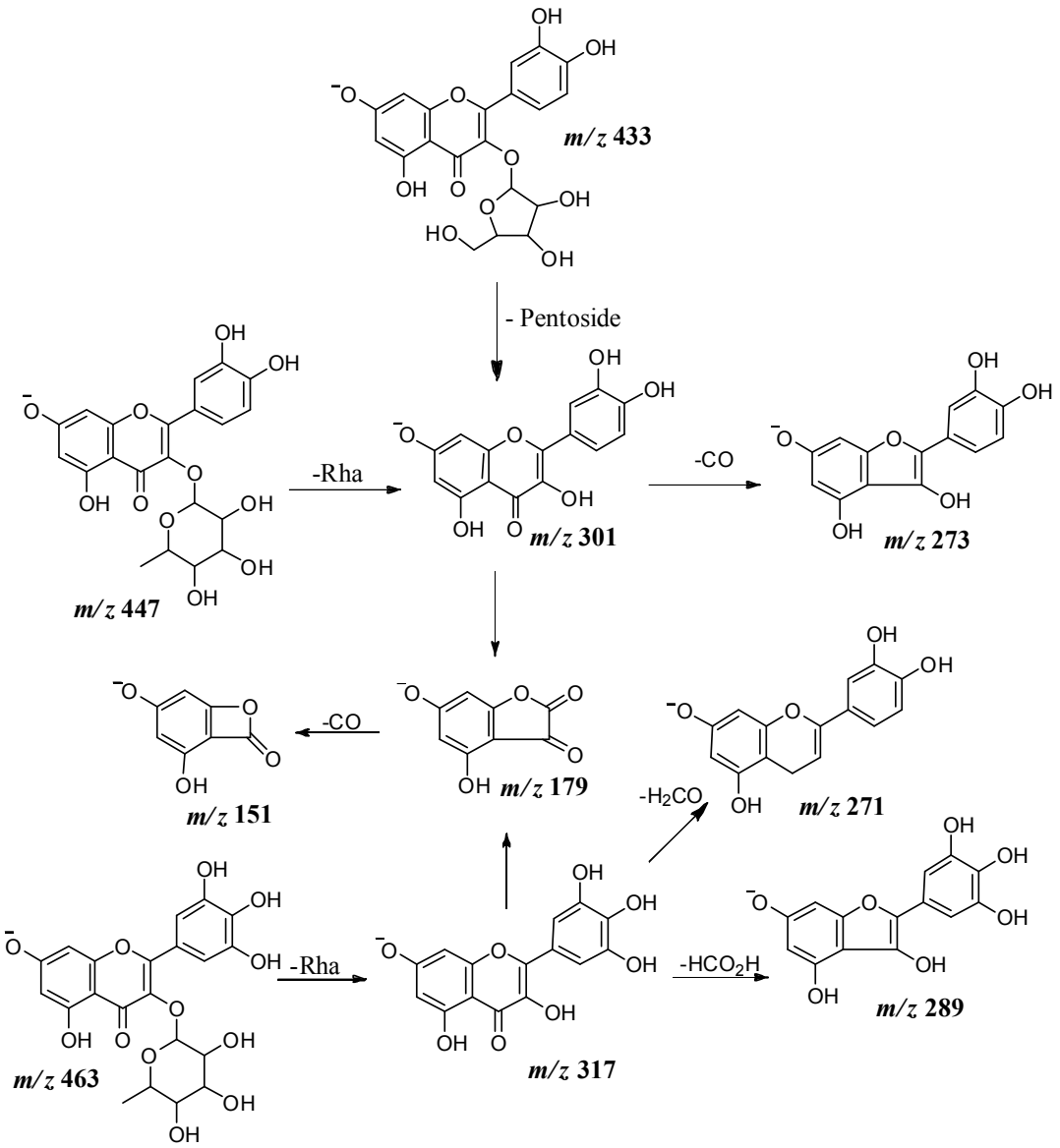
Formula $[M - H]^-$	Theoretical *m/z*	Experimental *m/z* $[M - H]^-$	Δm (ppm)	MS/MS fragments *m/z*	Compound identification	Extracts
$C_7H_5O_5$	169.0131	169.0143	0.02	151,125	gallic acid	SOHE, SOM
$C_7H_{11}O_6$	191.0561	191.0560	0.57	173,111	quinic acid	SOM
$C_9H_9O_5$	197.0444	197.0456	-0.08	169,153	syringic acid	SOHE
$C_{15}H_{13}O_6$	289.0718	289.0712	0.17	245, 186,125	catechin	SOHE
$C_{15}H_9O_7$	301.0353	301.0353	-0.02	179, 151, 125	quercetin	SOHE, SOM, SOA
$C_{15}H_{13}O_7$	305.0661	305.0667	0.14	261,179,125	epigallocatechin	SOHE
$C_{13}H_{15}O_9$	315.0716	315.0721	-0.27	300, 153, 109	protocatechuic acid-glucoside	SOHE, SOM
$C_{15}H_9O_8$	317.0292	317.0303	0.03	289, 179, 151, 137, 109	myricetin	SOHE, SOM
$C_{13}H_{15}O_{10}$	331.0665	331.0670	-0.24	169, 163	monogalloyl-glucose	SOHE, SOM
$C_{12}H_{22}O_{11}.Cl^-$	377.0856	377.0852	-1.04	341, 215, 179	chloro adduct of sucrose	SOM
$C_{20}H_{17}O_{11}$	433.0771	433.0771	-0.54	387, 301, 271, 255	quercetin pentoside isomer	SOM
$C_{21}H_{19}O_{11}$	447.0927	447.0932	-0.28	301, 271, 273, 179,	quercetin-3-O-β–rhamnose	SOHE, SOM, SOA
$C_{21}H_{18}O_{12}$	463.0877	463.0880	-0.34	317,217, 273, 211, 179	myricetin-3-rhamnoside	SOHE, SOM, SOA
$C_{21}H_{19}O_{12}.Cl^-$	499.0649	499.0647	-1.32	463, 316, 113	chloro adduct of myricetin-3-rhamnoside	SOM
$C_{30}H_{24}O_{14}$	609.1244	609.1248	-0.26	591, 441, 305	epigallocatechin dimer	SOHE
$C_{45}H_{36}O_{21}$	913.1833	913.1823	1.06	904	epigallocatechin trimer	SOHE

The compound identified via its deprotonated molecule of *m/z* 169 was assigned to gallic acid, which is an endogenous plant phenol, and is abundantly found in Stryphnodrendron species.[29] The ESI(-)-MS/MS data for the ion of *m/z* 169 showed two main fragments. That of *m/z* 151 corresponds to water loss whereas that of *m/z* 125 is formed by the loss of CO_2. Such dissociations are typical for carboxylic acids in their deprotonated forms.[30] The ion of *m/z* 191, identified as

deprotonated quinic acid, which is frequently found in higher plant as a major constituent of the leaves,[31] showed fragments of *m/z* 173 (water loss) as well those of *m/z* 111, 87 and 85 resulting from a retro Diels-Alder (RDA) ring opening mechanism.[32] For deprotonated syringic acid of *m/z* 197, its ESI(-)-MS/MS showed again the typical fragments of *m/z* 135 (CO_2 loss) and *m/z* 179 (water loss).

The ions of *m/z* 289, 301 and 317 were attributed to deprotonated catechin, quercetin, and myricetin, respectively. Note that their ESI(-)MS/MS showed sugar fragments, such as those corresponding to neutral losses of 132 (a pentoside), 146 (a deoxihexoside) and 162 (deoxyhexoside). Deprotonated catechin of m/z 289 also formed the fragment ion of *m/z* 254 likely due to the loss of an acetyl group as described by Perez-Magarino et al.[33] Characteristic fragmentations of deprotonated quercetin of m/z 301 results from cleavage of the B ring after RDA, forming the fragment ion of *m/z* 179 and also by loss of a carbonyl group leading to the fragment ion of *m/z* 151.[34] Deprotonatyed myricetin of *m/z* 317 also showed a characteristic fragment of *m/z* 179 as reported by Sun et al.[35]

Ions of *m/z* 433, 447, 463 and 499 were assigned to deprotonated quercetin and myricetin derivatives. The isomeric ion due to a deprotonated quercetin pentoside of *m/z* 433 produced a characteristic fragment ion of *m/z* 301 due the neutral loss of pentoside. The ion of *m/z* 447 was assigned to deprotonated quercetin-3-O-β–deoxipentose, and its MS/MS showed a aglycone fragment ion of *m/z* 301 due to the loss of a sugar moiety of 146 Da, whereas the fragment ion of *m/z* 273 was also typical for a flavon-3-*O*-monoglycoside.[36] For the ion of *m/z* 463, its MS/MS produced a deprotonated aglycone , that is, the ion of *m/z* 317 (loss of a sugar moiety of 146 units), indicating that such ion is a deprotonated myricetin monohexoside (myricetin 3-O-galactoside or myricetin 3-O-glucoside).[37] Scheme 1 rationalizes a fragmentation route for such deprotonated flavonoids.



Scheme 1- Common fragmentation mechanism proposed for deprotonated quercetin pentoside of *m/z* 433, quercetin-3-O-β–rhamnose of *m/z* 447 and myricetin-3-rhamnoside of *m/z* 463.

The catechin anion of *m/z* 289 dissociated to the fragment ion of *m/z* 245 (CO_2 loss) as well as to that of *m/z* 125, which is considered a diagnostic ion for the presence of two hydroxyl groups on the A-ring of flavan-3-ols.[38] Deprotonated epigallocatechin of *m/z* 305 yielded the fragment ions of *m/z* 125 and 179, which were consistent with a previous report.[39,40] The deprotonated epigallocatechin dimer of *m/z* 609, and its trimer of *m/z* 904, also showed characteristic fragmentation patterns.

The ESI(-)-FT-MS of the hexanic *S. obovatum* extracts detected mainly deprotonated fatty acids with displayed characteristic MS/MS fragmentations.[41] But fatty acids were not the main focus of this work and a detailed description of such components will not be provided. From Table 1 and Fig 4 we can therefore conclude that SOA mostly displayed flavonoids whereas SOM is constituted of both flavonols and phenolic acids (Fig. 2). SOHE also displays phenolic acids and catechins (Fig. 3).

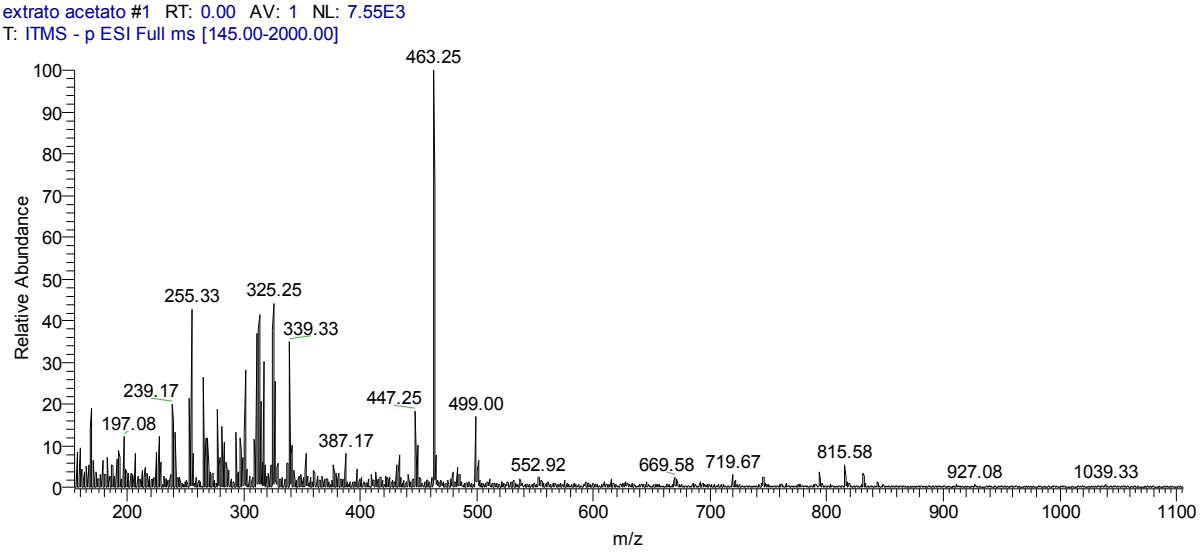


Fig. 1 ESI(-)-MS fingerprints of the *S. obovatum* ethyl acetate leaves extracts (SOA).

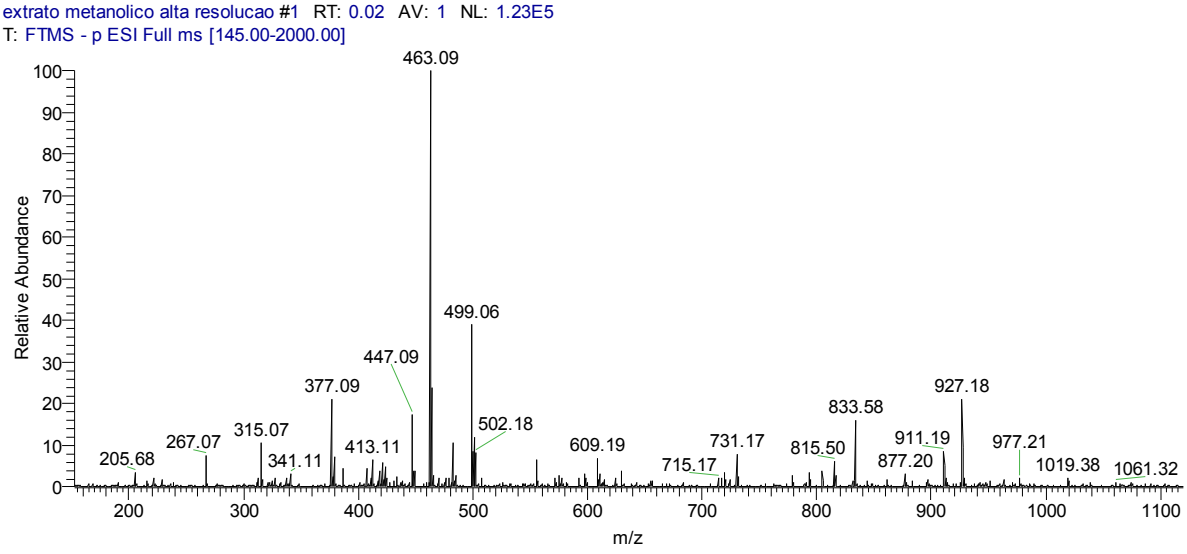


Fig. 2 ESI(-)-MS fingerprints of the *Stryphnodendron obovatum* methanolic leaves extracts (SOM).

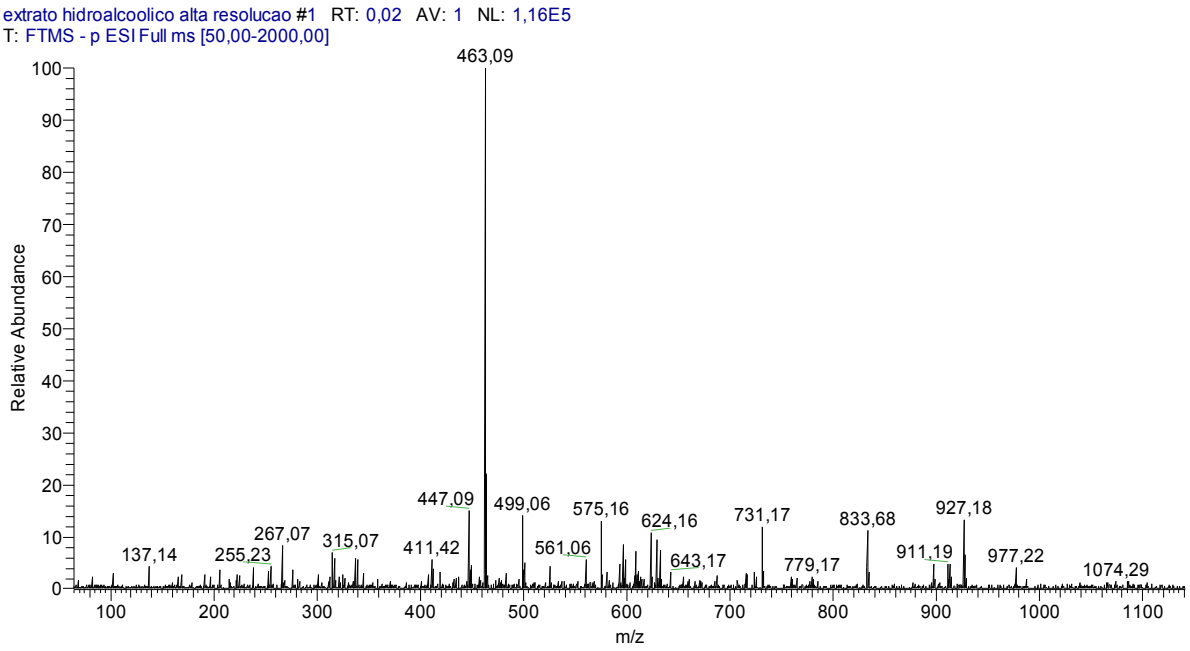


Fig. 3 ESI(-)-MS fingerprints of the *Stryphnodendron obovatum* hidroethanolic leaves extracts (SOHE).

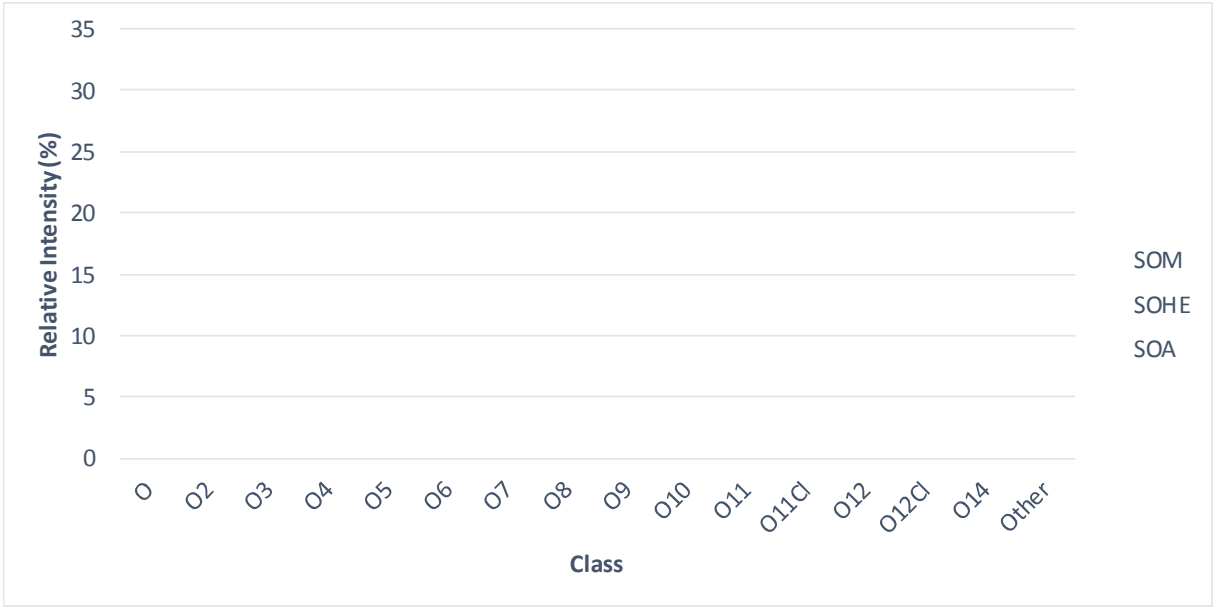


Fig. 4. Class distribution of compounds detect in the *S. obovatum* extracts by ESI FT-ICR MS

Zocoler et al. when studing *S. obovatum* bark, observed that the crude acetonic extract and its ethyl acetate fraction displayed an antioxidant capacity comparable to that of vitamin C and Trolox.[8] The SOM and SOHE extracts were therefore similarly tested, and indeed displayed considerable scavenged DPPH, evidencing their anti-radical properties. The EC_{50} for the SOM extract of EC_{50} 0.86 ± 0.01 μg mL^{-1} showed that it is slightly more active than Trolox (EC_{50} 1.13 ±

0.09μg mL^{-1}, positive control) as well as the SOHE extract (EC_{50} 1.39 ± 0.01 μg mL^{-1}). This antioxidant potential for the SOM extract can be associated with its higher polyphenol content (350.9 ± 12.0 mg gallic acid g^{-1} extract) which was considerably higher than that for the SOHE extract (201.2 ± 4.0 mg gallic acid g^{-1} extract)(Tables 1-3).

Table 2: Antioxidant potential for *Stryphnodendron obovatum* extracts measured by the DPPH and Folin-Ciocalteau (FCR) assays

Extract	DPPH (μg/ml)[a]			FCR($mg_{GAc}/g_{extract}$)
	EC_{50}	TEC_{50}	AE	
Trolox	1.13 ± 0.09	0.1	9.0 ± 0,8	
SOA	418.67 ± 0.01	20	$1.19x10^{-4}$ ± $4.0x10^{-7}$	61.6 ± 1.1
SOM	0.86 ± 0.01	0.1	1.16 ± 0,01	350.9 ± 12.0
SOHE	1.39 ± 0.01	0.1	7.2 ± 0,4	201.2 ± 4.0

[a]Results expressed as mean±S.E.M.; EC_{50}: amount of antioxidant necessary to decrease the initial DPPH concentration by 50%; TEC_{50}: time needed to reach the steady state to EC_{50} (min); AE: antiradical efficiency [$AE=1/(EC_{50} \times TEC_{50})$]; GAc: Gallic acid.

According to Sanchez-Moreno, the DPPH assay is time dependent, hence it can be used to classify samples as fast (TCE_{50} <5 min), intermediate (between 5 and TCE_{50} 30 min) or slow (TCE_{50}> 30 min) antioxidants depending on the time it takes for it to reach EC_{50}. The SOM and SOHE extracts could then be classified as fast antioxidants, whereas SOA display an intermediate degree (Table 1). Antiradical efficiency (AE) is a parameter used to measure the free radicals scavenge efficiency, by correlating potency ($1/EC_{50}$) and time reaction (TEC_{50}).[42] Using AE values, therefore, the SOM and SOHE extracts had more ability for scavenging free radicals than the SOA extract. This difference may be explained by their quite distinct compositions as revealed by the ESI(-) FTMS fingerprinting (Table 2). That is, the MS analysis showed that the SOA extract is free from phenolic acids, but the phenols found in the SOM and SOHE extracts seem to be the main factor leading to enhanced antioxidant activity. The catechins (flavan-3-ol) found in SOHE seems, however, to be exerting an antagonistic effect.

Note that Costa et al. indeed demonstrated that antioxidant activity of aqueous extracts from leaves and barks of *Stryphnodendron rotundifolium* was associated with polyphenol content.[9] Similarly,

Oliveira *et al.* when analyzing extracts of *Baccharis trimera* noted that the phenolic extracts also showed the highest antioxidant potential in relation to the other extracts tested.[43]

To test the antiproliferative hypothesis, the SOM and SOHE extracts were evaluated against a panel of nine human tumor cell lines and a single non-tumoral cell line (VERO). After 48h of treatment, the total growing inhibition (TGI) effective concentration was calculated. According to Fouche,[44] it is possible to classify a sample as inactive (mean TGI > 50 μg mL^{-1}), weakly active (15 μg mL^{-1}< mean TGI < 50 μg mL^{-1}), moderately active (6.25 μg mL^{-1}< mean TGI < 15 μg/mL), and potent active (mean TGI < 6.25 μg mL^{-1}) based on an arithmetical average of TGI values for tumor cell lines. Both the SOM and SOHE extracts should therefore be considered as inactive (mean TGI > 50 μg mL^{-1}), but the SOM extract displayed a selective weak activity against renal (786-0, TGI = 40.6 μg mL^{-1}) and the ovarian expressing resistance phenotype (NCI-ADR/RES, TGI = 42.8μg mL^{-1}) cell lines, whereas the SOHE extract displayed selective weak activity for the melanoma cell line (UACC62, TGI = 44.1 μg mL^{-1}). SOM and SOHE showed low cytotoxic activity against normal cell lines, whereas all the others extracts tested showed to be inactive (Table 4).

Table 3: TGI values, given in μg mL^{-1}, for *S. obovatum* extracts and doxorubicin (DOX) necessary for total inhibition of tumor cell proliferation

	U251	UACC-62	MCF-7	NCI-ADR/RES	786-0	NCI-H460	PC-3	OVCAR-3	HT-29	K562	VERO
DOX	1.70	0.32	>25	>25	0,16	1.5	0.56	4.1	>25	>25	2,0
SOM	162	126	84.5	42.8	40.6	187	>250	106	106	>250	106
SOHE	>250	44,1	250	>250	>250	>250	>250	>250	>250	91.8	>250

Human tumor cell line: U251 (glioma), UACC-62 (melanoma), MCF-7 (breast), NCI-ADR/RES (ovarian expressing multiple drug resistance phenotype), 786-0 (renal), NCI-H460 (lung, non-small cells), PC-3 (prostate), OVCAR-3 (ovarian), HT-29 (colon), K562 (leukemia). Non-tumor cell line: VERO (monkey kidney cells).

For *S. obovatum* therefore, the belief that their antioxidant properties were responsible for its antiproliferative effects has now been found to be apparently an incorrect hypothesis. Note that similarly to the present results, Melo et al reported that tannins obtained from methanolic extract of *Poincianella pyramidalis* (Fabaceae) leaves presented a good antioxidant activity when evaluated on a DPPH (2,2-diphenyl-2-picrylhydrazyl) assay, but just an intermediate antiproliferative activity

against HEp-2 (laryngeal cancer) and NCI-H292 (lung cancer) cell lines using the (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazole) (MTT) method.[45]

Conclusions

Direct infusion ESI(-)FT-MS have provided a quite comprehensive understanding of the chemical composition of *S. obovatum* extracts. Allied to its speed, simplicity, and pre-separation and pre-derivatization free protocols, the method was also able to highlight important differences and similarities on the chemical profiles of the *S.obovatum* extracts. The methanolic (SOM) and hidroethanolic (SOHE) extracts were found to display considerable amounts of polyphenols with excellent antioxidant scavenging activity, which were comparable to Trolox. SOM was rich in flavonoids and flavonols and was a little more active than the SOHE extract, which is rich in catechins. Such constituents are promising for treatment of diseases resulting from oxidative stress. The *S. obovatum* leaves were also shown to display weak antiproliferative effect against renal (786-0), multidrug resistant (NCI-ADR/RES), and melanoma (UACC62) and low toxicity in regard to normal (VERO) cell lines. These findings provide a guide for the use of such extracts in medicine, and further biological studies are underway in our laboratory.

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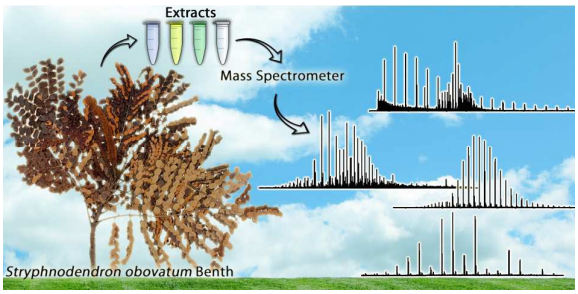
Graphical Abstract (GA)



It was correlated the main phenolic constituents of the *S. obovatum* leaves extracts, analyzed by ultrahigh resolution mass spectrometry (FT-ICR MS), with antioxidant and in vitro antiproliferative activities.