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ORIGINAL ARTICLE

# Chemical Characterization and Evaluation of Antioxidant and Antimicrobial Activities of *Litchi chinensis* Sonn.

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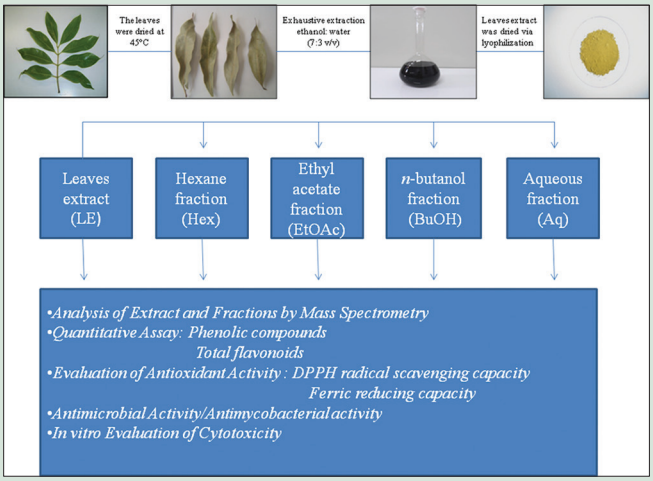
## ABSTRACT

**Background:** *Litchi chinensis* is used in traditional Chinese medicine and by Indian medical system. Ethnopharmacological studies show anti-inflammatory, antidiabetic and analgesic activities, among others. However, there are few studies of antimicrobial activity. This study evaluates antimicrobial, antioxidant, and cytotoxicity properties of the lychee's leaves extract (LE) and fractions. **Materials and Methods:** Extracts were obtained using an exhaustive extraction method with ethanol: Water (7:3 v/v). Subsequently, LE was concentrated in a rotary evaporator. Finally, LE was dried via lyophilization. Fractions were obtained via the partition process. Bioactivity of the LE and fractions (hexane [Hex], ethyl acetate [EtOAc], *n*-butanol [BuOH], and aqueous [Aq]) from *L. chinensis* was evaluated through antimicrobial activity using broth microdilution, antioxidant activity via both 1,1-diphenyl-2-picryl-hidrazila assay and ferric reducing capacity and cytotoxicity through 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction assay. Furthermore, mass spectrometry technique electrospray ionization ion trap mass spectrometry was used to identify the chemical composition of the LE and fractions. **Results:** Phenolic compounds, such as flavonoids and condensed tannins were the main substances found. Total phenolic and flavonoid contents were higher in EtOAc (541.15 ± 2.4 mg/g and 31.06 ± 0.5 mg/g, respectively). This fraction showed the best results for antioxidant activity ($IC_{50}$ = 3.45 mg/mL) and ferric reducing capacity (20.27% ± 0.11). The LE and fractions showed considerable antimicrobial activity, chiefly against *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, and *Proteus mirabilis*, with the minimum inhibitory concentration ranging from 50 to 1560 μg/ml. **Conclusion:** This study revealed that *L. chinensis* is a source of bioactive compounds potentially useful for pharmaceutical and food industries.

**Key words:** Antimicrobial activity, antioxidant activity, medicinal plants, phenolic compounds, phytochemical screening

## SUMMARY

- The greatest values of the total phenolics content and total flavonoid contents were found on ethyl acetate fraction (EtOAc) (541.15 ± 2.4 mg/g sample and 31.06 ± 0.5 mg EQ/g sample, respectively)
- It was observed that 1,1-diphenyl-2-picryl-hidrazila radical scavenging capacity of the EtOAc was as efficient or higher as compared with ascorbic acid, butylated hydroxy toluene , and quercetin standards. The ferric reducing capacity of extracts and fractions indicated that the antioxidant activity of EtOAc was greater when compared with the others fractions
- EtOAc fraction presented the most significant results demonstrated the lowest MIC values (50 μg/mL) against *Bacillus subtilis, Bacillus cereus*, and *Staphylococcus aureus*. The leaves extract (LE) was also significantly active against Gram-positive bacteria *B. cereus* and *S. aureus* and against Gram-negative bacteria *Proteus mirabilis* (50 μg/mL)
- The LE of *Litchi chinensis* and its fractions did not show cytotoxicity at the highest concentration used (160 μg/mL) on murine peritoneal macrophages by 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide method.




**Abbreviations Used:** LE: Leaves extract, Hex: Hexane fraction, EtOAc: Ethyl acetate fraction, BuOH: n-butanol fraction, Aq: Aqueous fraction, DPPH: 1,1-diphenyl-2-picryl-hidrazila, MTT: 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide, ESI-IT-$MS^n$: Electrospray ionization ion trap mass spectrometry, $IC_{50}$: Median Inhibition Concentration (concentration that reduces the effect by 50%), MIC: Minimum inhibitory concentration, MS/MS: Mass spectrometry, $MS^n$: Tandem mass spectrometry, GAE: Equivalents of gallic acid, QE: Equivalents of quercetin, BHT: Butylated Hydroxy Toluene, UV: Ultraviolet, ATCC: American Type Culture Collection, MHB: Mueller Hinton broth, DMSO: Dimethyl sulfoxide, ANOVA: Analysis of variance, $CC_{50}$: Cytotoxic concentration 50.

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## INTRODUCTION

*Litchi chinensis* Sonn. is a native species from southern China that belongs to the Sapindaceae family. Its fruits are very popular in South and Southeast Asia. The exotic culture of this species occurs in the tropical and sub-tropical regions, also occurring in North and South America.[1]



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*L. chinensis* is used in traditional Chinese medicine[2] and is also used by the medical system of the Indian subcontinent, where ethnopharmacological studies have shown that the most popular uses found for litchi are as anti-inflammatory, antidiabetic, anti-obesity, analgesic, antitussive, and diuretic, as well as to treat neuralgic disorders, scurvy, orchitis, and sore throats.[1,3-5] The tea from the bark is used to treat diarrhea, and the seed is ground into a powder to be used for intestinal problems. Pharmacological studies have demonstrated its strong antioxidant activity[6-9] and anticancer[10-13] effects, mainly due to the presence of phenolic compounds and flavonoids.[14-18]

The phenolic compounds present in fruits and vegetables have important bioactive properties and are well-known for their health benefits. These compounds are commonly found in parts of both edible and inedible plants and have been associated with multiple biological effects including antioxidant, antibacterial, antiviral, anti-inflammatory, anti-thrombotic, and vasodilatory activities.[19]

Multi-drug resistance of micro-organism has led to serious public health problems, resulting in high rates of morbidity and mortality from infectious diseases.[20-23] Thus, it is extremely important the discovery of new compounds from natural sources that may support new therapeutic options. In this context, plants play a determinant role in the search for compounds with antimicrobial activity.[24-26]

In this study, the *in vitro* antimicrobial, antioxidant, and cytotoxic activities of the leaves and fractions from *L. chinensis* were investigated. In addition, the quantification of the total phenolics and total flavonoids contents and the identification of the chemical constituents by mass spectrometry electrospray ionization ion trap mass spectrometry (ESI-IT-MS$^n$) were carried out.

## MATERIALS AND METHODS

### Plant material

The plant material from *L. chinensis* Sonn. was collected in the city of Alfenas-MG (geographical data: Altitude of 808.0 m, latitude 21°20'27,3"S and longitude 45°53'29,2"). The plant was identified in the botany laboratory of the Federal University of Alfenas and a reference specimen (nº 2276) was deposited in the herbarium at UNIFAL-MG.

### Preparation of hydroethanolic extract and fractions

The leaves were dried at 45°C and were then pulverized. The extracts were obtained using an exhaustive extraction method with a liquid mixture of ethanol: Water (7:3 v/v). Subsequently, leaves extract (LE) was concentrated in a rotary evaporator. Finally, the LE was dried via lyophilization. Fractions were obtained via a liquid–liquid partition process, using as solvents hexane (Hex), ethyl acetate (EtOAc) and n-butanol (BuOH) yielding the fractions Hex, EtOAc, and BuOH. The residual was named as an aqueous fraction (Aq).

### Analysis of extract and fractions by mass spectrometry

The analysis of the extract and fractions was performed using a tandem mass spectrometry method (MS/MS or MS$^n$) by direct insertion into the ESI-IT-MS$^n$ system. The samples were submitted to an electrospray ionization mode (ESI) and fragmentation was performed in an ion trap-type interface (IT). Negative mode was selected for generation of the first-order MS and MS$^n$. The analysis range was between 50–2000 m/z. Mass spectra were obtained on a Thermo Scientific Linear 2D LTQ XL mass spectrometer. The Xcalibur software version 2.2 (Thermo Scientific®) was used for the acquisition and processing of spectral data.

### Quantitative assay

#### *Phenolic compounds*

The content of phenolic compounds in the extract and fractions was determined based on the Folin-Ciocalteau colorimetric method[27] using equivalents of gallic acid (GAE) as a standard. The absorbance of the samples was measured at 750 nm and the results were expressed in mg equivalents of GAE per gram of extract.

#### *Total flavonoids*

Determination of flavonoids was performed using the colorimetric spectrometric method of chelation with aluminum chloride in ultraviolet[28] using quercetin as the standard. Absorbance of the samples was measured at 425 nm corresponding to the absorption peak of quercetin aluminum chelate, and the results were expressed in mg equivalents of quercetin (QE) per gram of extract.

### Evaluation of antioxidant activity

#### *1,1-diphenyl-2-picryl-hidrazila radical scavenging capacity*

Evaluation of scavenging activity of 1,1-diphenyl-2-picryl-hidrazila (DPPH) was performed according to the methodology previously described,[29] using solutions of the samples and standards (quercetin, ascorbic acid and butylated hydroxy toluene [BHT]), which were prepared at concentrations between 12.5 μg/ml and 400 μg/ml. An aliquot of 0.5 ml of DPPH solution (0.5 mM) was added in 0.3 ml of samples and standards and was then diluted in 3 ml of ethanol. The mixture was stirred vigorously and was kept away from light exposure for 30 min. The readings were made at 517 nm. The $IC_{50}$ value was determined for each sample. $IC_{50}$ value is the concentration of the sample required to scavenge 50% of the free radicals present in the system. All experiments were performed in triplicate.

### Ferric reducing capacity

The reduction capacity of the samples was calculated as previously described.[30] Samples and standards (ascorbic acid and BHT) were prepared in triplicate at concentrations between 12.5 μg/ml and 400 μg/ml. An aliquot of 1.0 ml was added to a solution of 2.5 ml a phosphate buffer 0.2 M (pH 6.6) and 2.5 mL of $K_3[Fe(CN)_6]$ (1%) to each tube. The mixture was kept at 50ºC for 30 min. Subsequently, 2.5 mL of trichloroacetic acid (10%) was added, and the mixture was stirred. Finally, 2.5 mL of the mixture was transferred to a solution of 2.5 ml of distilled water and 0.5 mL $FeCl_3$ (0.1%). The readings were done at 700 nm, and the results were expressed as a percentage of $Fe^{2+}$ chelating activity.

### *In vitro* evaluation of antimicrobial activity

#### *Microbial strains*

The strains of micro-organism used were from the American type culture collection (ATCC) and Microbiology and Immunology Laboratory (LMI) at UNIFAL-MG. These micro-organism are representative of the main groups of mycobacteria, bacteria, and fungi and demonstrate medical and environmental importance. The fungi included *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 2601). The Gram-positive bacteria included *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (ATCC 11778), *Micrococcus luteus* (ATCC 9341), *Enterococcus faecalis* (ATCC 51299), and *Staphylococcus aureus* (ATCC 6538). The Gram-negative bacteria included *Escherichia coli* (ATCC 25922), *Serratia marcescens* (LMI-UNIFAL), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028), and *Enterobacter aerogenes* (LMI-UNIFAL). The mycobacteria included *Mycobacterium bovis* (BCG strain, ATCC 27289) and *Mycobacterium tuberculosis* (H37Ra strain, ATCC 27294).

### *Broth microdilution method*

The minimum inhibitory concentration (MIC) of *L. chinensis* extract and fractions was determined through broth microdilution against bacteria and fungi, according to methodologies established in the M7A6 (Clinical and Laboratory Standards Institute [CLSI], 2003)[31] and M27A3 (CLSI, 2008)[32] documents. Tests were performed on 96-well microplates. At first, the turbidity of microbial suspensions in sodium chloride 0.9%, cultured overnight at 35°C for 18 h, was adjusted according to a McFarland standard (0.5 tube). Next, 100 µl of Mueller Hinton broth (MHB) was added to the wells and after that, 100 µl of the LE and each fraction tested were added. Serial dilutions were made with the final concentrations of the LE or fractions ranging between 25 µg/mL to 1,250 µg/mL. Finally, 10 µl of micro-organism was added to each well. The reading was performed visually as previously determined (CLSI, 2003),[31] wherein the presence of turbidity in the wells after incubation for 24 h at 37°C was considered indicative of bacterial growth. The $MIC_{99.9}$ was established as the lowest concentration of the extract or fraction in which no turbidity had occurred. The growth control was comprised 100 µl of MHB and 10 µl of inoculum. The extract control was comprised 100 µl of MHB and 100 µl of the LE or fraction and the sterility control contained only 100 µl of MHB. Chlorhexidine 0.12% was also used as a positive control.

### *Antimycobacterial activity*

The LE, at a concentration of 50,000 µg/mL, was evaluated against both the *M. bovis* and *M. tuberculosis* via an agar diffusion assay, according to the M24A2 (CLSI, 2008) document.[33,34] Regarding controls, Rifampicin 30 µg was used as a positive control and distilled water was used as a negative control.

### *Quantitative evaluation of antimicrobial activity*

The antimicrobial activity of plant extracts can be expressed in different ways. According to previous studies,[35,36] one can express antimicrobial effectiveness through numerical values beyond the MIC (µg/ml). In this study, two calculations were used to demonstrate the activity of the extracts tested: total activity and the values of the percentage activity (%). The use of the total activity values allows for comparing the bioactivity of different parts of the plant or between different plants on a more rational and standardized basis, allowing for the best interpretation of the results.[37] These values would indicate the largest volume to which biologically active compounds in 1 g of plant material can be diluted and still inhibit microbial growth. The percentage of activity evaluates the antimicrobial potential of the tested *L. chinensis* extracts and represents the percentage of micro-organism that do not grow in the presence of these extracts. This index allows for a comparison between the statements that presented MIC of ≤100 µg/ml and determines the most active. These values were calculated as follows:

$$\text{Percent activity}\,(\%) = \frac{\text{Number of susceptible strains to a specific extract}}{\text{Total number of tested microbial strains}} \times 100$$

$$\text{Total activity} = \frac{\text{Quantity of material extracted from 1g of plant material}}{\text{MIC}}$$

### *In vitro evaluation of cytotoxicity*

Cytotoxicity was evaluated using the 3-(4,5-dimethyl-thiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT)[38] method. Murine peritoneal macrophages were used in RPMI 1640 medium and were kept at 37°C and 5% $CO_2$. They were arranged in 24-well plates at the rate of $8 \times 10^5$ cells per well. The extract and fractions were added at concentrations ranging from 0.1 µg/ml to 160 µg/ml and were incubated for 72 h. After the incubation period, 50 µL of MTT was added to each well, with further incubation for 4 h. The cells were treated with dimethyl sulfoxide and evaluated at 570 nm to determine the cytotoxic concentration for 50% of the cells ($CC_{50}$).

## Statistical analysis

To evaluate the correlation between the amounts of total phenols and flavonoids with antioxidant and antimicrobial activities, the Spearman correlation coefficient (r) was employed using the BioEstat Software version 5.0. All of the results were submitted to analysis of variance followed by Tukey test ($P < 0.05$).

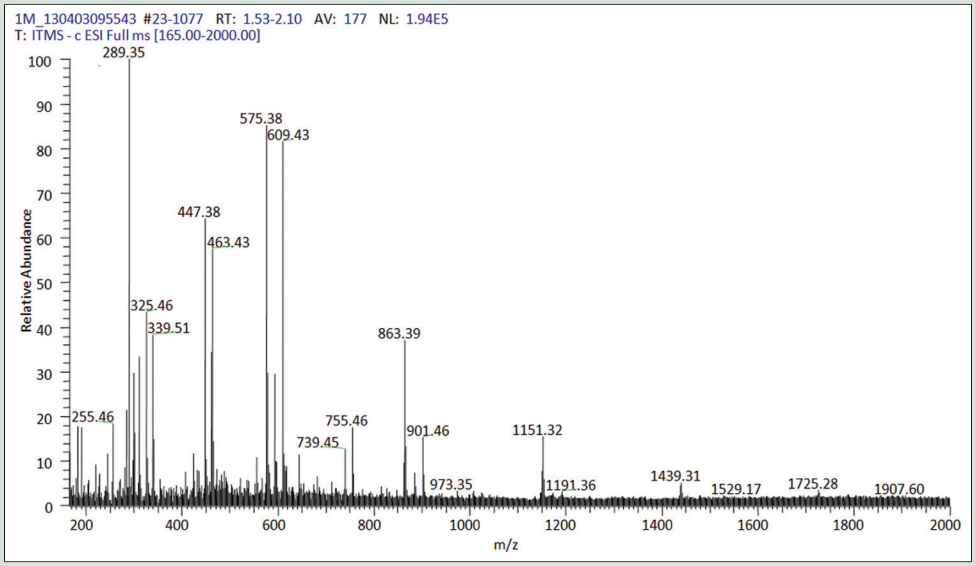


**Figure 1:** Mass spectrum, in full scan mode, obtained via direct injection of leaves extract of *Litchi chinensis*

## RESULTS AND DISCUSSION

The mass spectra ESI-MS obtained from the LE [Figure 1] from 200 to 2000 m/z showed some major peaks, which were recognized as known phenolic compounds (catechin and quercetin), along with some tannins, which were confirmed via second order MS. The peaks of 863 and 1151 m/z corresponded to the polimeric condensed tannins known as procianidines, present in this species.[2,15,17,39] The $MS^2$ spectrum of the 447 m/z ion [Supplementary Figure 1] showed a peak of 285 m/z, due to a loss of a 162 Da fragment of hexose, generating the aglycone form of catechin ($C_{15}H_9O_6$). The ion 289 m/z represented the flavonoid epicatechin ($C_{15}H_{14}O_6$), based on previous data.[18,40] The $MS^2$ spectrum of the 609 m/z ion [Supplementary Figure 2] showed a peak of 301 m/z, which represents a loss of two fragments from hexose and desoxi-hexose, with 162 Da and 146 Da, respectively, resulting in the aglycone form of quercetin ($C_{15}H_{10}O_7$). The outstanding presence of the peaks of 289, 447, and 609 m/z in EtOAc indicates that this fraction exhibited a greater amount of phenolic compounds when compared with the other fractions [Supplementary Figure 3].

In the present study, we found significant levels of total phenols in the extract and fractions of *L. chinensis* [Table 1]. The greatest value of total phenolics content was found on EtOAc (541.15 ± 2.4 mg/g sample). Similarly, the greatest value of total flavonoid contents was found in the EtOAc (31.06 ± 0.5 mg EQ/g sample). Similar studies indicate the presence of polyphenolics, flavonoids, condensed tannins, and proanthocyanidins in this species, especially in the fruit, flowers and seeds.[7,14,18,19,41-43] This study confirmed the presence of these compounds in significant concentrations in the *L. chinensis* leaves, which have the advantage of being a renewable source and are available throughout the entire year. Phenolic compounds, especially flavonoids, have aroused great interest since recent discoveries have linked these compounds and their properties to the ability to scavenge oxidants, preventing chronic diseases such as cardiovascular disease, diabetes, kidney diseases, and cancer.[38,44-47]

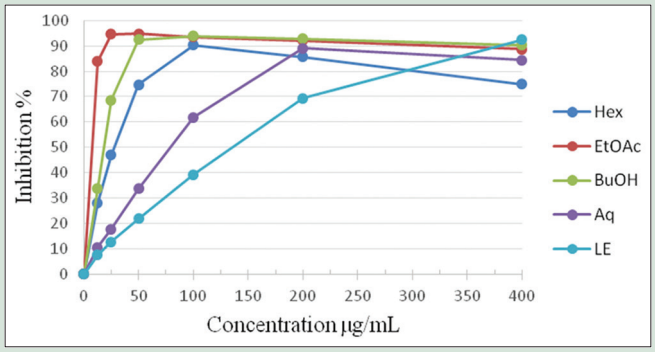


**Figure 2:** DPPH radical scavenging activity of leaves extract and fractions of *Litchi chinensis* Sonn.

The DPPH radical scavenging activity of LE and fractions of *L. chinensis* is shown in [Figure 2]. The antioxidant activity by DPPH method ($IC_{50}$) of the extract and fractions showed the following profile: EtOAc > BuOH > Hex > Aq > LE [Table 1]. The highest antioxidant potential was shown on the EtOAc, which had the lowest concentration able to sequestering 50% of DPPH free radicals, with $IC_{50}$ = 3.45 ± 0.12 µg/mL. It was observed that DPPH radical scavenging capacity of the EtOAc was as efficient or higher as compared with ascorbic acid (6.49 ± 0.15 µg/mL), BHT (70.11 ± 0.10 µg/mL) and quercetin standards (4.57 ± 0.20 µg/mL). The antioxidant activity of EtOAc was greater than the observed by Yang *et al.*, 2012[7] and Shahwar *et al.*, 2010,[48] in EtOAc fraction of flower acetone extract (16.73 ± 2.25 µg/mL) and in EtOAc fraction of stem-bark methanolic extract (15.30 µg/mL), respectively.

The ferric reducing capacity of extract and fractions at 100 µg/mL concentration [Table 1], indicated that the antioxidant activity of EtOAc was greater (20.27% ± 0.11) when compared against the LE and others fractions (Hex = 12.30% ± 0.08, BuOH = 10.80% ± 0.31, LE = 8.74% ± 0.03, Aq = 7.85% ± 0.15). The EtOAc result was higher when compared with BHT standard (11.50% ± 0.17). However, the EtOAc result was found to be lower than that of ascorbic acid standard (36.85% ± 0.14) at the same concentration.

Total phenolics content and total flavonoids were correlated with antioxidant activity, and the Spearman coefficients were calculated [Table 2]. The Spearman correlation coefficient is a measure of the degree of linear relationship between two quantitative variables. This coefficient varies between the values-1 and 1, wherein value of 0 (zero) means that there is no linear relationship, a value of 1 indicates perfect linear relationship and the value of-1 indicates a perfect inverse linear relationship. The closer the Spearman coefficient is to 1 or-1, the stronger the linear association between the two variables. Based on the Spearman coefficient, the results of total phenols and flavonoids indicated a positive correlation between these compounds and antioxidant activity. The results showed the highest correlation between the concentration of flavonoids with ferric reducing capacity ($r$ = 0.9). The Spearman coefficient was 0.8 when correlated the DPPH scavenging capacity with flavonoids.

It is known that the number of phenolic hydroxyl groups present in the substance is directly associated with the antioxidant capacity, as observed mainly in dimeric substances. Flavonoids such as catechin, epicatechin, and their dimmers, as well as procyanidins, exhibit strong

**Table 1:** Antioxidant activity by 1,1-diphenyl-2-picryl-hidrazila method (median inhibition concentration) and ferric reducing capacity of leaves extract and fractions of *Litchi chinensis* Sonn., total phenolics content (values at milligram equivalent of gallic acid), and total flavonoids content (values at milligram equivalent of quercetin)

| Extract and fractions | DPPH radical scavenging $IC_{50}$ values (µg/mL) 100 µg/mL | Ferric reducing capacity (%) 100 µg/mL | Total phenolics (mg/g) | Total flavonoids (mg/g) |
|---|---|---|---|---|
| LE | 102.51±0.60$^h$ | 8.74±0.03$^b$ | 393.14±6.60$^d$ | 19.99±0.72$^c$ |
| Hex | 23.80±0.10$^e$ | 12.30±0.08$^d$ | 239.37±0.30$^b$ | 21.98±0.70$^d$ |
| EtOAc | 3.45±0.12$^a$ | 20.27±0.11$^e$ | 541.15±2.40$^e$ | 31.06±0.50$^e$ |
| BuOH | 16.28±0.23$^d$ | 10.80±0.31$^c$ | 269.97±4.10$^c$ | 16.71±0.35$^b$ |
| Aq | 64.71±0.15$^f$ | 7.85±0.15$^a$ | 119.63±0.60$^a$ | 3.00±0.10$^a$ |
| Ascorbic acid | 6.49±0.15$^c$ | 36.85±0.14$^f$ | NA | NA |
| BHT | 70.11±0.10$^g$ | 11.50±0.17$^d$ | NA | NA |
| Quercetin | 4.57±0.20$^b$ | NA | NA | NA |

The results are expressed as mean±SEM. Values with different letters are statistically differents (Tukey test, $P$<0.05). SEM: Standard error of the mean; LE: Leaves extract; Hex: Hexane fraction; EtOAc: Ethyl acetate fraction; BuOH: n-butanol fraction; Aq: aqueous fraction; $IC_{50}$: Median inhibition concentration (concentration that reduces the effect by 50%); NA: Not applicable; DPPH: 1,1-diphenyl-2-picryl-hidrazila

antioxidant activity and can be cardioprotective, antimutagenic, and anticarcinogenics agents.[49]

The presence of substances such as catechin, epicatechin, their dimers and polymers in *L. chinensis* have been previously reported[4,7,15-18,39] and it is possible to infer that flavonoids and condensed tannins may be partly responsible for the antioxidant activity of EtOAc, considering many studies that describe these different classes of compounds as potent antioxidants[50-52] and their use in the food and pharmaceutical industries.[53] Even though *in vitro* assays on antioxidant activity are not able to predict *in vivo* activity, the *in vitro* tests are a convenient, fast, and stable means of screening for future *in vivo* trials.

In the evaluation of antimicrobial activity, many authors consider the antimicrobial activity of extracts to be significant if the MIC value is 100 μg/ml or lower, moderate if 100< MIC ≤625 μg/ml and weak if MIC >625 μg/ml.[37,54] Thus, the EtOAc fraction that showed the most significant results demonstrated the lowest MIC values (50 μg/mL) against *B. subtilis, B. cereus*, and *S. aureus* [Table 3]. The LE was also significantly active against Gram-positive bacteria *B. cereus* and *S. aureus* and against Gram-negative bacteria *P. mirabilis* (50 μg/mL). The LE was inactive against mycobacteria (*M. tuberculosis* and *M. bovis*) and fungi (*C. albicans* and *S. cerevisiae*). Due to this, the fractions were not tested against these micro-organism.

Other studies have investigated the antimicrobial activity of stem bark and seed of this species[49,55,56] with positive results. Singh *et al*., 2013,[55] and Bath and al-Daihan, 2014,[56] reported that the *L. chinensis* seeds exhibited moderate growth inhibition against *Proteus vulgaris*, *Klebsiella pneumoniae*, *S. aureus*, *Streptococcus pyogenes*, *Bacilllus subtillis*, *E. coli*, and *P. aeruginosa*. Luteolin, (–)-epicatechin, procyanidin $A_2$, and quercetin-3-*O*-rutinoside were identified from the EtOAc-soluble extract of litchi leaves.[57] These compounds possessed strong antimicrobial activity towards *S. aureus*, *E. coli*, *S. dysenteriae*, *Salmonella*, and *B. thuringiensis*.

The MS analysis of the extract and fractions allowed for the indication of catechin and quercetin [Supplementary Figures 1-3], as well as polymeric proanthocyanidins. The presence of these compounds justifies the satisfactory MIC values found for antimicrobial activity and is supported by previous studies.[23,57] Moreover, in quantitative terms, total phenolics and flavonoids compounds showed a positive correlation with MIC results of $r = 0.75$ and $r = 0.5$, respectively [Table 2].

The search for new antibiotics to treat infectious diseases caused by multiresistant micro-organism is necessary. Thus, plants are presented as a viable source of new herbal substances or as an alternative to this problem. In this study, we observed excellent MIC values (<100 μg/mL) against *S. aureus* and *B. cereus* for LE and EtOAc fractions. *S. aureus* is one of the most important agents of opportunistic and nosocomial infections and is considered a significant causative agent for infections in the community, in addition to multi-drug resistance to currently used antibiotics. This results in increased morbidity and mortality rates.[58]

The LE and the EtOAc showed the percentage of activity of 40% and 75%, respectively, against the different micro-organism used. These results demonstrate that the partitioning process can increase the antimicrobial activity. However, when one takes into account the yield factor in the calculation of the total activity, it has been observed that the LE displays better results in terms of total activity [Table 4] compared to the EtOAc (5384 and 2576, respectively). This fact can be explained by the synergy between the constituent compounds, increasing the antimicrobial action. Hex, BuOH, and Aq fractions did not show satisfactory activity against micro-organism in the evaluation using the microdilution broth method (MIC >100 μg/ml).

Of great relevance, the LE of *L. chinensis* and its fractions did not show cytotoxicity at the highest concentration used (160 μg/mL) on murine peritoneal macrophages by MTT method.

**Table 2:** Spearman coefficients for the correlation between total phenolics content, total flavonoids and 1,1-diphenyl-2-picryl-hidrazila, ferric reducing capacity and minimum inhibitory concentration

| Quantitative determinations | Correlation coefficient (*r*) | | |
|---|---|---|---|
| | DPPH | Ferric reducing | MIC |
| Total phenolics | 0.40* | 0.60* | 0.75** |
| Total flavonoids | 0.80** | 0.90** | 0.50* |

*Correlation is significant at α<0.05, **Correlation is significant at α<0.0. DPPH: 1,1-diphenyl-2-picryl-hidrazila; MIC: Minimum inhibitory concentration

**Table 3:** Values of minimum inhibitory concentration (μg/mL) of leaves extract and fractions of *Litchi chinensis* across different micro-organism

| micro-organism | Extract/fraction | | | | |
|---|---|---|---|---|---|
| | LE | Hex | EtOAc | BuOH | Aq |
| Gram-positive | | | | | |
| *Bacillus subtilis* | 400 | N | 50 | 1560 | 1560 |
| *Bacillus cereus* | 50 | N | 50 | 200 | 800 |
| *Staphylococcus aureus* | 50 | N | 50 | 800 | 1560 |
| Gram-negative | | | | | |
| *Proteus mirabilis* | 50 | N | 400 | 1560 | 800 |
| *Escherichia coli* | N | NA | NA | NA | NA |
| *Serratia marcescens* | N | NA | NA | NA | NA |
| *Pseudomonas aeruginosa* | N | NA | NA | NA | NA |
| *Salmonella typhimurium* | N | NA | NA | NA | NA |
| Mycobacteria* | | | | | |
| *Mycobacterium tuberculosis* | N | NA | NA | NA | NA |
| *Mycobacterium bovis* | N | NA | NA | NA | NA |
| Fungi | | | | | |
| *Candida albicans* | N | NA | NA | NA | NA |
| *Saccharomyces cerevisiae* | N | NA | NA | NA | NA |

*Antimycobacterial activity was assessed through agar diffusion screening with LE. LE: Leaves extract; Hex: Hexane fraction; EtOAc: Ethyl acetate fraction; BuOH: n-butanol fraction; Aq: Aqueous fraction; N: No growth inhibition the maximum concentration used in the experiments (50,000 μg/ml against mycobacteria and 12,500 μg/ml against other micro-organisms); NA: Not applicable

**Table 4:** Total activity of the extract and fractions from the leaves extract of *Litchi chinensis* with minimum inhibitory concentration ≤100 μg/ml

| Extract/fraction | Yield (w/w)* | micro-organism | MIC (μg/mL) | Total activity (mL/g) |
|---|---|---|---|---|
| LE | 26.92 | *Bacillus cereus* | 50 | 5384 |
| LE | 26.92 | *Staphylococcus aureus* | 50 | 5384 |
| LE | 26.92 | *Proteus mirabilis* | 50 | 5384 |
| LE | 26.92 | *Enterococcus faecalis* | 100 | 2692 |
| EtOAc | 12.88 | *Bacillus subtilis* | 50 | 2576 |
| EtOAc | 12.88 | *Bacillus cereus* | 50 | 2576 |
| EtOAc | 12.88 | *Staphylococcus aureus* | 50 | 2576 |

*Related with plant drug. LE: Leaves extract; EtOAc: Ethyl acetate fraction; MIC: Minimum inhibitory concentration

## CONCLUSION

The phytochemical profile of the extract and fractions of *L. chinensis* Sonn. presented phenolic compounds as the main chemical constituents, such as flavonoids (quercetin and catechin) and condensed tannins. The EtOAc showed high levels of total phenolics and flavonoids and the best results of antioxidant tests, while LE showed the best result for antimicrobial activity, suggesting the possibility of the compounds act synergistically to this biological activity. Therefore, we demonstrated the feasibility of the leaves from

this species as a source of bioactive compounds, being it renewable and available throughout the entire year. However, additional studies should be conducted aimed at the isolation of new compounds from leaves of *L. chinensis* and further evaluation of their potential use as an antimicrobial and/or an antioxidant agent that may be useful for the pharmaceutical and food industries.

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## Conflicts of interest

There are no conflicts of interest.

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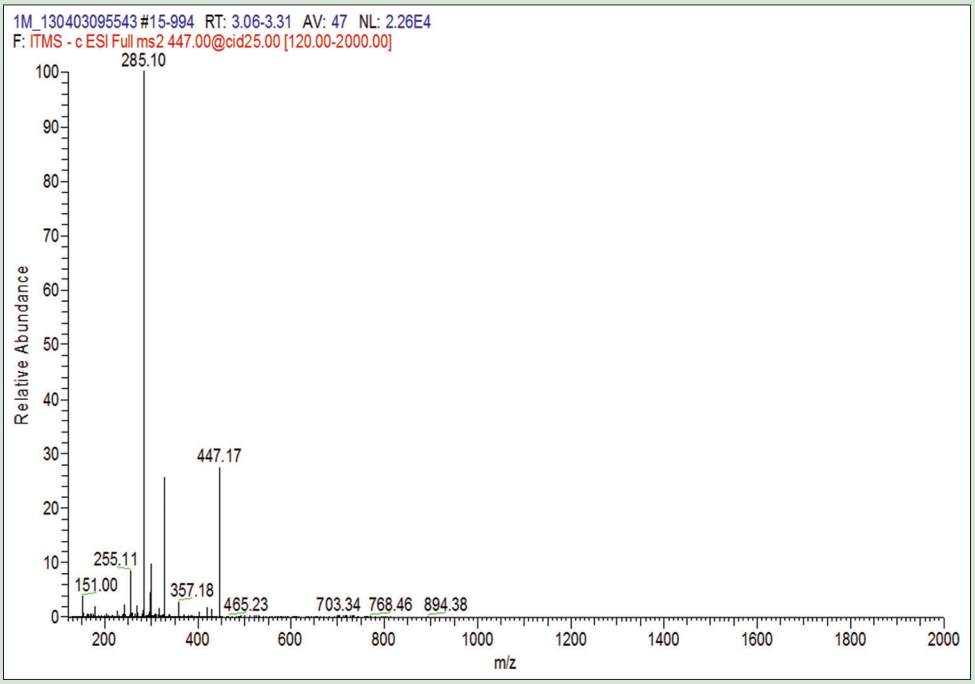
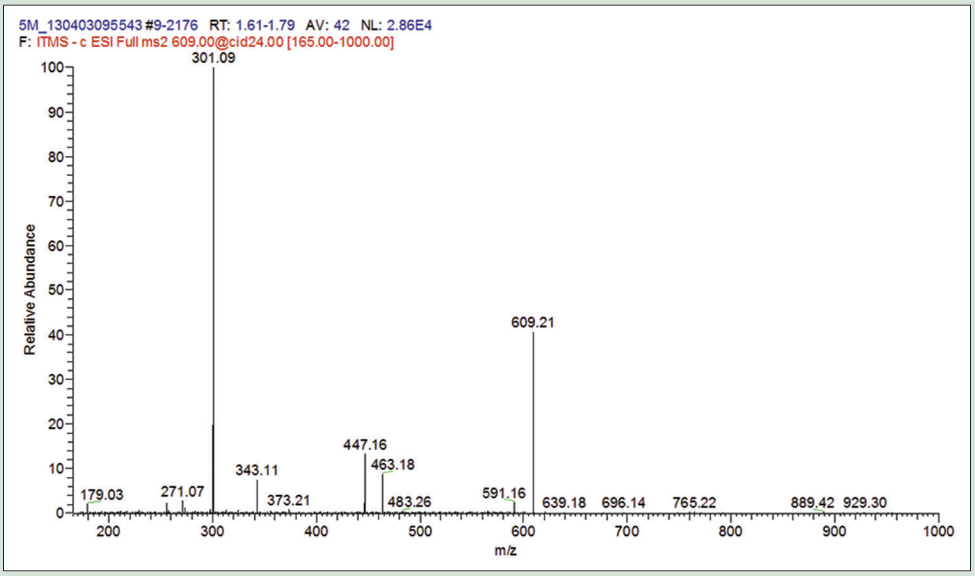
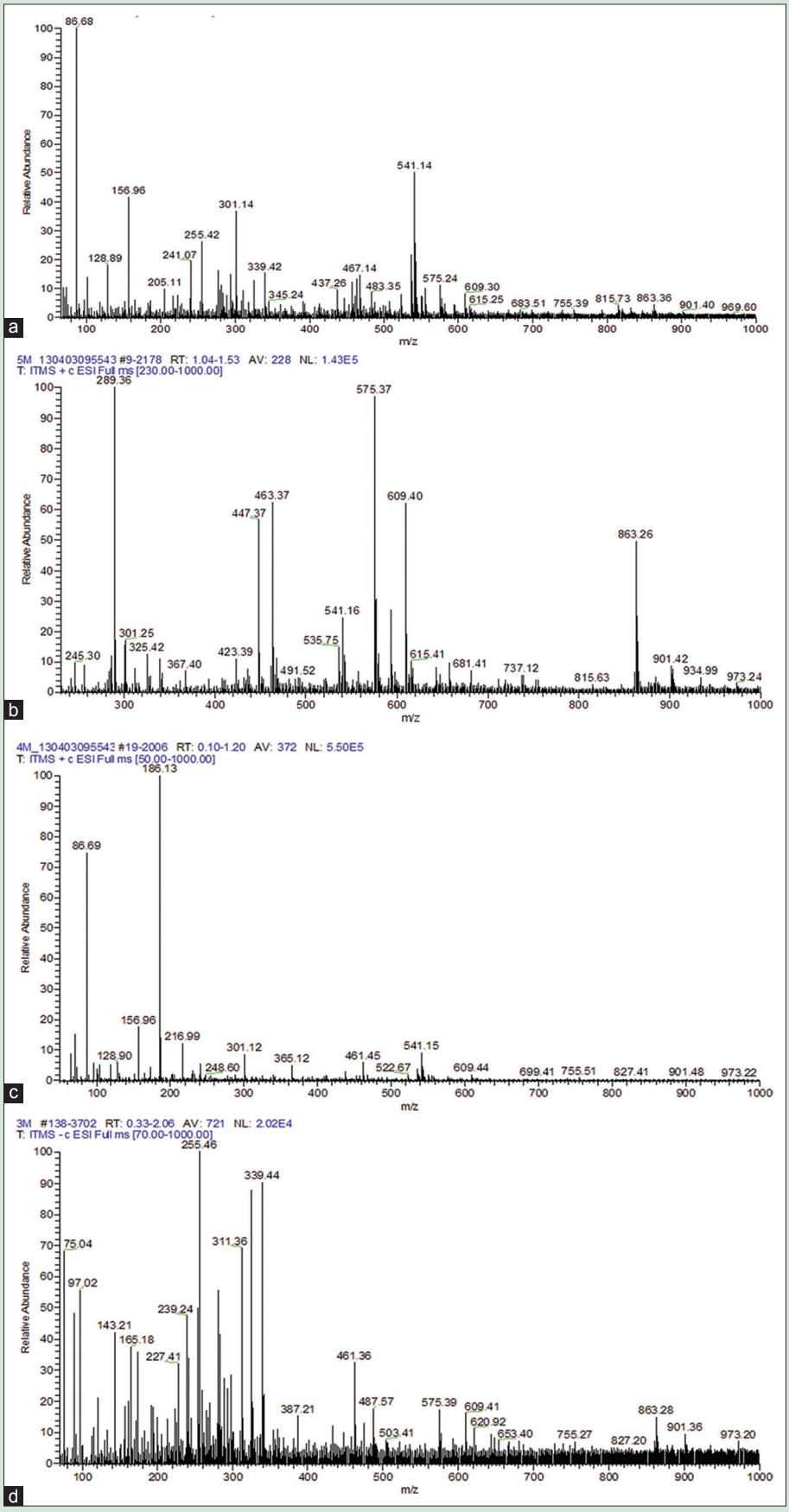



**Supplementary Figure 1:** MS$^2$ spectrum of the ion *m/z* 447 from the leaves extract (LE) of *Litchi chinensis*




**Supplementary Figure 2:** MS$^2$ spectrum of the ion *m/z* 609 from the ethyl acetate fraction




**Supplementary Figure 3:** Mass spectrum, in full- scan mode of the fractions from the leaves extract of *Litchi chinensis*. (a) Hexane fraction. (b) Ethyl acetate fraction. (c) *n*-butanol fraction. (d) Aqueous fraction