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SHORT COMMUNICATION

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Detection of caffeic and chlorogenic acids from methanolic extract of *Annona squamosa* bark by LC-ESI-MS/MS

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ABSTRACT

Aim: The aim of the present study was to determine the metabolite profile of *Annona squamosa* bark using high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS).

Methods: The plant material of *A. squamosa* bark was collected and processed during the month of February. The bark material was extracted by Soxhlet apparatus using methanol as a solvent. The metabolite profile of the plant extract was determined by using LC-ESI-MS/MS.

Results: Caffeic and chlorogenic acids were detected from the methanolic extract of *A. squamosa* bark. To the best of our knowledge, this is the first study to report the presence of caffeic and chlorogenic acids in *A. squamosa* bark.

Conclusion: The study suggested further investigations to be carried out to evaluate these compounds *in vitro* and *in vivo* to develop the pharmaceutical products.

ARTICLE HISTORY

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KEYWORDS

Plant extract; anticariogenic; metabolite profile; pharmaceutical; pharmacology

Introduction

Annona squamosa belongs to the family Annoanaceae, cultivated in India and other tropical countries. Commonly known as Custard apple in English, Sharifa in Hindi, Seema Atha in Tamil, Seetha Phala in Kannada, and Seetha Pandu in Telugu [1,2].

A. squamosa was traditionally used in medicine to treat epilepsy, constipation, diarrhea, hemorrhage, fever, dryness, and ulcers [3]. The extract of different parts of *A. squamosa* was reported to have anticancer, antioxidant, anti-inflammatory, and antimicrobial activity [4–7].

In the previous study, we tested the antibacterial activity of the methanolic extracts of *A. squamosa* and *A. reticulata* (leaves and bark) against *Streptococcus mutans* and *Streptococcus sobrinus*, and among the tested plant materials, *A. squamosa* bark showed antibacterial activity [8]. In this background, the present study was aimed to study the metabolite profile of *A. squamosa* bark using high-performance liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS).

Materials and Methods

Collection and processing of test plant material

The bark material of *A. squamosa* L was collected and authenticated by Dr. Vasundhara M, professor, Horticulture Department, UAS University, GKVK Bangalore, India. The bark was collected and processed during the month of February 2015. The plant material was cleaned and rinsed at least three times in sterile distilled water and dried in the hot air oven. The dried plant material was squashed by blender and then stored in an airtight bottle for further uses.

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Preparation of plant extract

The extraction of *A. squamosa* bark was performed by soxhlet apparatus. The soxhlet apparatus was filled with 600 ml of 100% v/v methanol (high-performance liquid chromatography [HPLC] grade) and 40 g of the dried material of *A. squamosa* bark. The extraction was carried out at a temperature of 25°C for 30 h. The extract was filtered using two sheets of Whatman paper. The rotary vacuum evaporator was used to concentrate the extract and the yield was transferred to a screw cap bottle and stored at 4°C for further uses [8].

LC-ESI/MS and data analysis

The photochemical from the methanolic extract of *A. squamosa* bark were analyzed by direct injection to auto-sampler in LC-ESI/MS. The LC-ESI/MS was performed using a Perkin Elmer Sciex API-3000 triple quadruple mass spectrometer equipped with an Agilent 1100 series HPLC. A 150 × 3.9 mm C18, symmetry column (Waters, India) was used at a flow rate of 0.5 ml/min. Separation based on gradient chromatography was performed for the solvent extract of the samples by a mobile phase of solvent A: 0.01% formic acid in acetonitrile and solvent B: 0.01% of MilliQ water with a constant flow rate of 0.5 ml/min. The gradient program started with 100% A: 5 min, followed by 85% A: 10 min, 80% A: 20 min, 75% A: 25 min, 73% A: 27 min, 60% A: 30 min, 50% A: 35 min, 10% A: 40 min, 10% A, then returned to 100% A for 50 min and maintained for 60 min at 100% A. HPLC of the extract was measured by MS/MS. All the analyses were performed using the electrospray ionization in both positive ion and negative ion modes with the following settings: ion spray voltage 4200 V for positive, and −4200 V for negative; nebulizer gas (N_2) 7 units, curtain gas (N_2) 12 units, collision gas (N_2) 6 units, declustering potential (DP) between 50 and 80 V for positive and between −50and −80 V for negative, focusing potential 300 V for positive and −300 V for negative, entrance potential 10 V for positive and −10 V for negative, collision energy (CE) 50 V for positive and −50 V for negative, and collision cell exit potential (CXP) 5 V for positive and −5 V for negative. The drying gas (N_2) was heated to 550°C and established at a flow rate of 6,000 cm^3/min. The full scan data acquirement was executed by scanning from *m*/*z* 100 to 1,500 in profile mode with a cycle time of 2 second with a step size of *m*/*z* 0.1 and an interscan pause of 2 μs. The metabolites were identified by comparing the precursor and fragment ions *m*/*z* with METLIN database.

Results

In order to obtain metabolite profile of the methanolic extract of *A. squamosa* bark, an analytical method based on LC-ESI-MS/MS was used. The LC-ESI-MS/MS profile highlighted the existence of a large group of compounds related to the protonated molecular ions of various polyphenols. The *m/z* obtained in both positive mode (Fig. 1) and negative mode (Fig. 2) was subjected to METLIN metabolite search. The search for the respective positive or negative charges was depended on the ionization with an accuracy of 50 ppm tolerance to find the possible metabolites. Among the possible metabolites, caffeic acid and chlorogenic acid were found to be present in the methanolic extract of *A. squamosa* bark. The caffeic acid and chlorogenic acid were subjected to further fragmentation. The metabolites were confirmed by direct infusion method of MS/MS. LC/ESI-MS/MS spectra of caffeic acid (*m/z* 179.0) and chlorogenic acid (*m/z* 353.0) are shown in Figs. 3 and 4, respectively.

Discussion

Among the metabolites present in the methanolic extract of *A. squamosa* bark, caffeic acid (Fig. 3) and chlorogenic acid (Fig. 4) were detected. To the best of our knowledge, this report is the first to detect caffeic acid and chlorogenic acid in *A. squamosa* bark.

Caffeic acid with precursor ion of 179.0 was subjected to fragmentation in positive mode by varying the DP from −101 to −1 volt, fixed potential (FP) from −350 to −50 volts, CE from −130 to −5.0, CXP from −55 to 0. The MS/MS fragments of caffeic acid included 135.0, 134.0, 106.0, and 65.0 which was as reported earlier [9]. The chlorogenic acid was subjected to fragmentation in negative mode (precursor ion of 353.0) by varying the DP from −101 to −1 volt, FP from −350 to −50 volts, CE from −130 to −5.0, CXP from −55 to 0. The MS/MS fragments of chlorogenic acid included 191.5, 161.0, and 111.0 which was as previously reported [10–12].

A. squamosa bark was reported to contain anticariogenic activity against *S. mutans* and *S. sobrinus* [8]. Chlorogenic acid is a polyphenolic

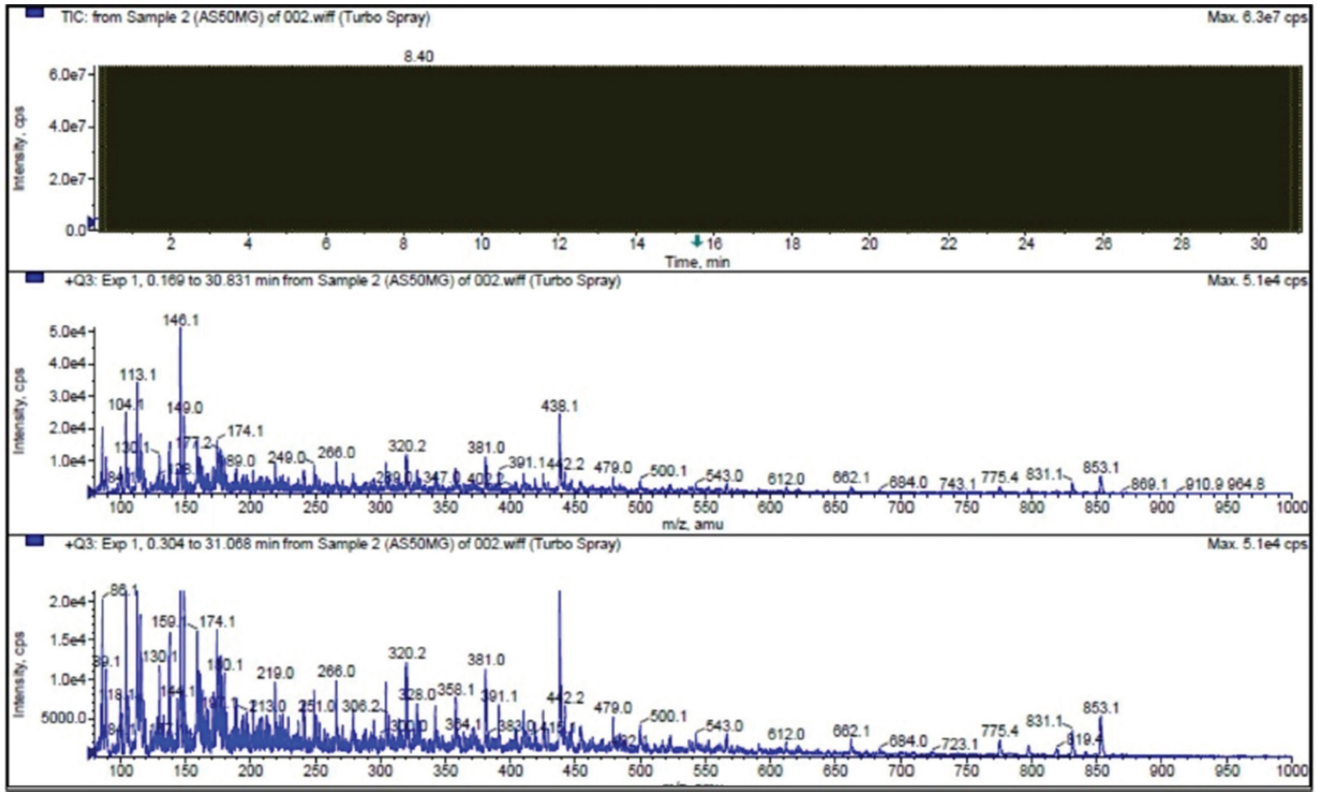

Figure 1. LC/ESI-MS/MS spectra of positive mode of *A. squamosa* bark.

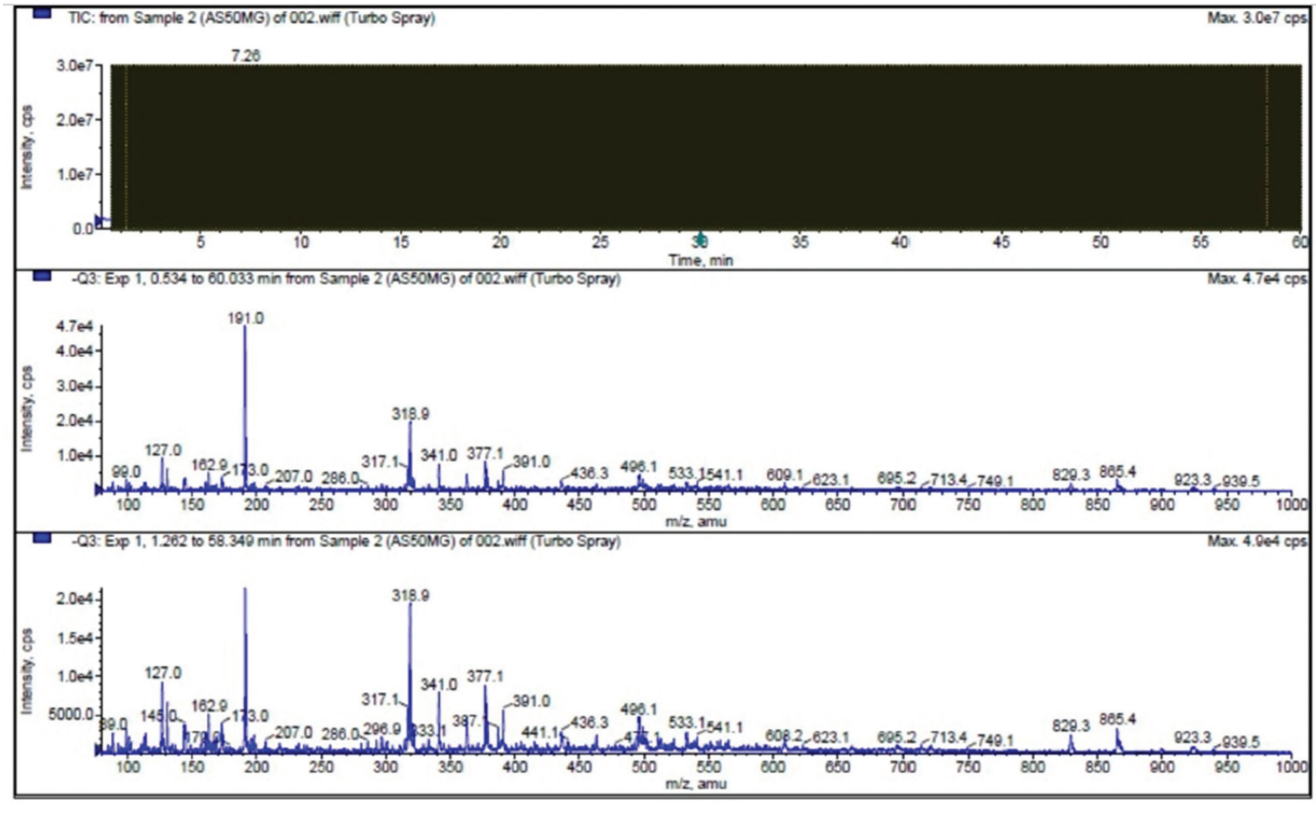

Figure 2. LC/ESI-MS/MS spectra of negative mode of *A. squamosa* bark.

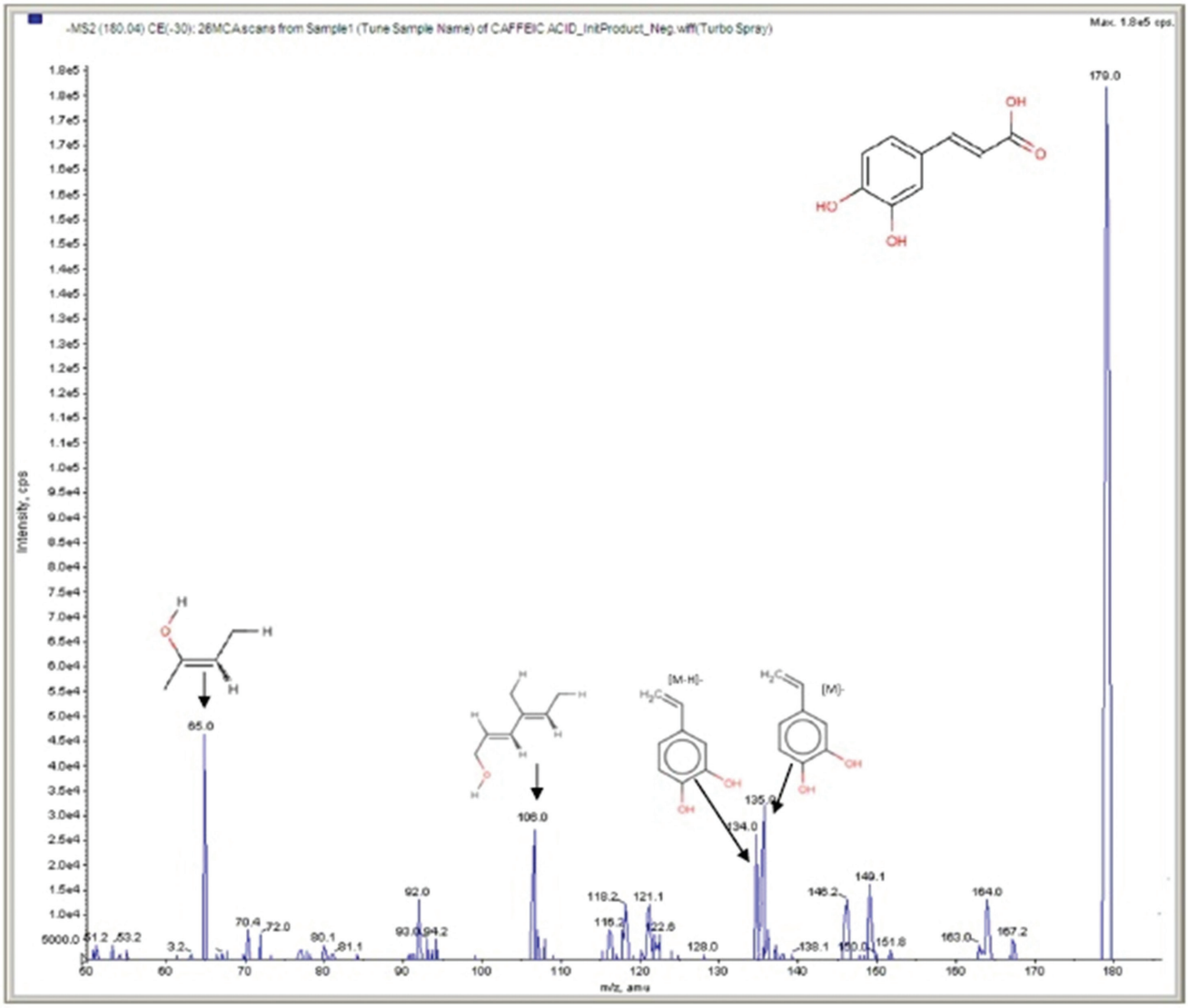

Figure 3. LC/ESI-MS/MS spectra of caffeic acid (*m/z* 179.0) fraction of *A. squamosa* bark.

compound that forms an ester with caffeic acid with the 3-hydroxyl group of a quinic acid. It was demonstrated to have various health benefits including antiviral, antioxidant, antibacterial, antifungal, and other biological activities [13,14]. Furthermore, caffeic acid and chlorogenic acid were also found to be anticariogenic compounds [15,16]. Chlorogenic acid and caffeic acid are nonvolatile organic acids present in coffee, investigators reported the effectiveness of coffee extracts to reduce the adherence of *S. mutans* on the glass surface [17]. Caffeic acid was approved to inhibit the growth of *S. mutans* and *S. sobrinus* [18].

Moreover, Caffeic and chlorogenic acids reduce the ability to inhibit α-amylase and α-glucosidase activities and therefore lead to anti-diabetic effects [19]. Researchers also considered caffeic and chlorogenic acids as promising agents for treating human breast cancer, head and neck squamous, lung and cervical carcinoma cells [20–23].

Conclusion

The study concluded the presence of caffeic acid and chlorogenic acid in *A. squamosa* bark. The current investigation suggested that the combination of caffeic acid and chlorogenic acid may potentially increase the antibacterial activity of *A. squamosa* bark. Further investigations are warranted to understand the possibility of incorporation of these compounds into pharmaceuticals.

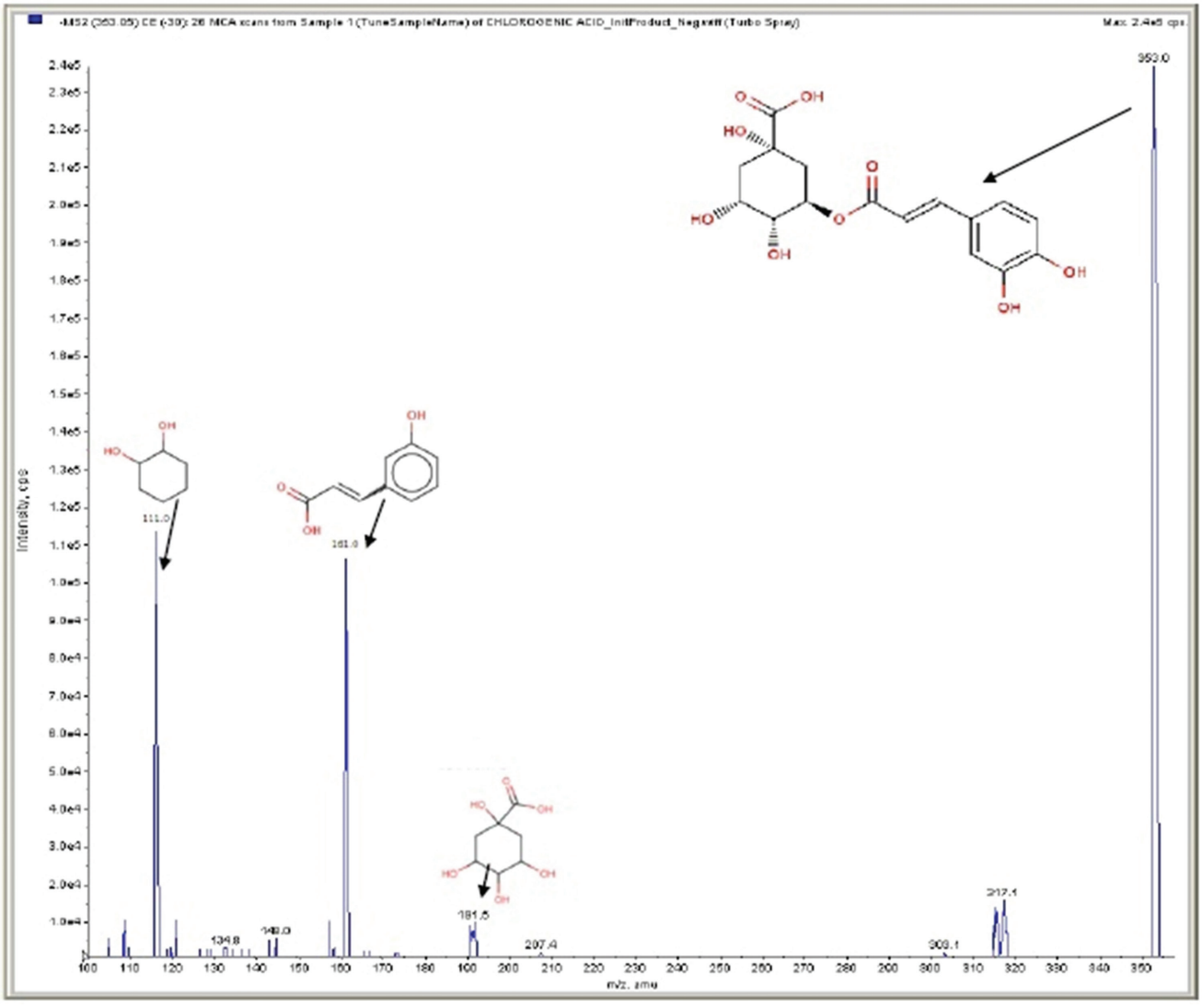

Figure 4. LC/ESI-MS/MS spectra of chlorogenic acid (*m/z* 353.0) fraction of *A. squamosa.*

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