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Screening of antioxidants present in unripe *Manilkara zapota* fruit of Indian origin using LC-MS/MS

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Introduction

Antioxidants are compounds that act as radical scavengers, prevent radical chain reactions of oxidation, delay or inhibit the oxidation process and increase shelf life by retarding the process of lipid peroxidation[1]. Antioxidants found in fruits and vegetables play an important role via their protective effects against the onset of aging-related chronic diseases[1]. The objectives of this study is to screen the antioxidants present in unripe *Manilkara zapota* fruit (shown in Fig. 1), which is commonly available in India, and to indicate that it can become a new source of natural antioxidants for food, nutraceutical and pharmaceutical industries. LC-MS/MS is used to identify the presence of antioxidants from this plant.

Fig. 1 *Manilkara zapota* fruit

Catechin

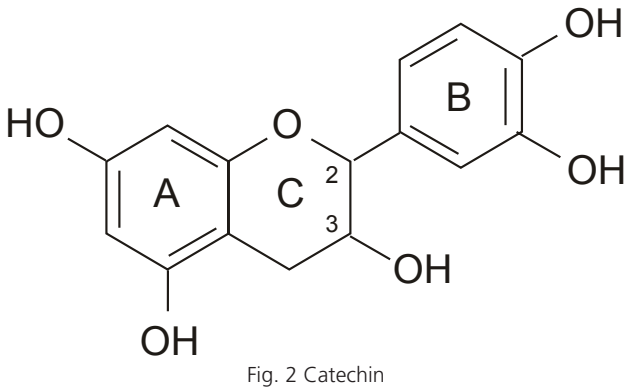

Fig. 2 Catechin

Catechin (shown in Fig. 2) possesses two benzene rings (called the A- and B-ring) and a dihydropyran heterocycle (the C-ring) with a hydroxyl group on carbon 3. The A-ring is similar to Resorcinol moiety while the B-ring is similar to Catechol moiety. There are two chiral centers on the molecule on carbons 2 and 3. Therefore, it has four diastereoisomers[2].

Two of the isomers are in trans configuration and are called 'Catechin' and the other two are in cis configuration and are called 'Epicatechin'. The most common Catechin isomer is the '(+)-Catechin'. The other stereoisomer is '(-)-Catechin'. The most common Epicatechin isomer is '(-)-Epicatechin'. Regarding the antioxidant activity, (+)-Catechin has been found to be the most powerful scavenger between different members of the different classes of flavonoids. The ability to quench singlet oxygen seems to be in relation with the chemical structure of Catechin, with the presence of the Catechol moiety on ring B and the presence of a hydroxyl group activating the double bond on C-ring.

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Method of Analysis

Manilkara zapota fruits were collected from Dahanu area from Maharashtra, India. 100 g of the pulp in 100 mL of HPLC grade methanol was ground finely using a mixer. This mixture was heated for 2 Hrs to produce an aqueous solution. The solution was cooled and filtered using muslin cloth to remove the residual solid material. The solution was heated to evaporate to 1/10th of volume and centrifuged at 3000 rpm for 10 min. Supernatant was further subjected to Solid Phase Extraction (SPE) technique to extract catechins, as described in the following flowchart.

Condition C-18 SPE cartridges (6cc) with 12 mL of methanol and water.

↓

Pass the supernatant collected after centrifugation through the catridge.

↓

Elute with 6 mL of distilled water adjusted to pH 7.0 to eliminate phenolic acids. Dry the cartridge with nitrogen gas.

↓

Pass 6 mL of ethyl acetate to elute catechins.

↓

Evaporate the ethyl acetate fraction to dryness using nitrogen gas and reconstitute in water.

↓

Again pass this through the C-18 cartridges. Dry the cartridges using nitrogen gas.

↓

Elute first with 6 mL of diethyl ether to give catechins.

↓

Elute with 2 mL of IPA (2-propanol) to give Oligomeric Proanthocyanidins (OPCs). Evaporate and reconstitute in 1 mL HPLC grade water.

Table 1 Mass spectrometric analytical conditions

Interface	:	ESI
Mode of Ionization	:	Positive
Temperature	:	Desolvation line 250°C ; Heat block 400°C
Nitrogen Gas flow	:	Nebulizing gas 3 L/min; Drying gas 15 L/min

Fig. 3 LCMS-8030 triple quadrupole mass spectrometer by Shimadzu

Results

Catechins Product Ion Scan results

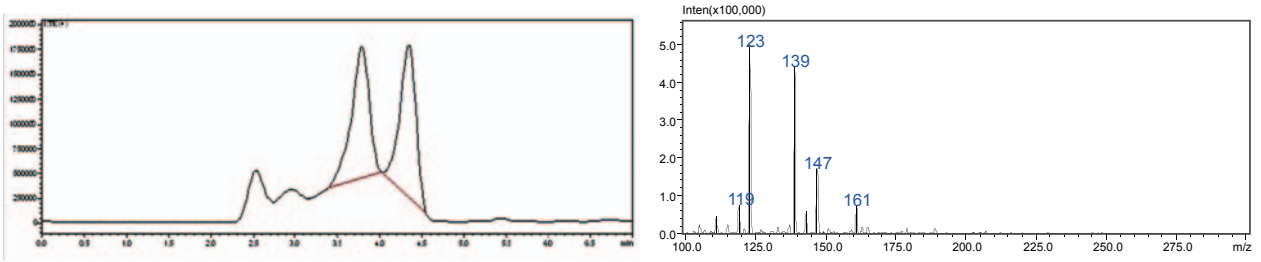

Fig. 4a Catechin and Epicatechin positive product ion scan results

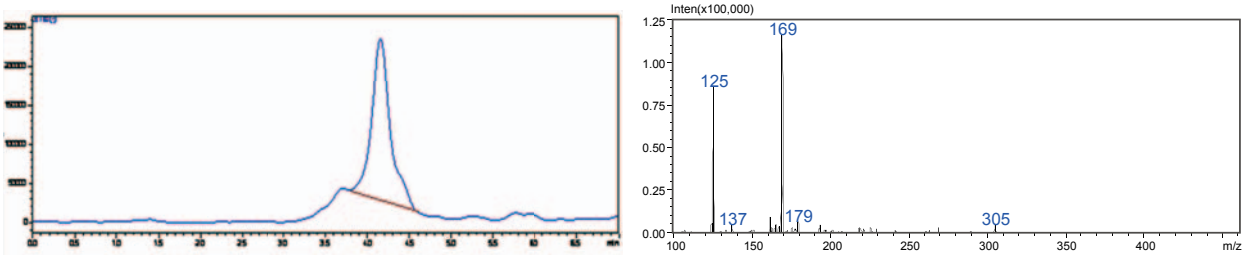

Fig. 4b Gallocatechin gallate and Epigallocatechin gallate negative product ion scan results

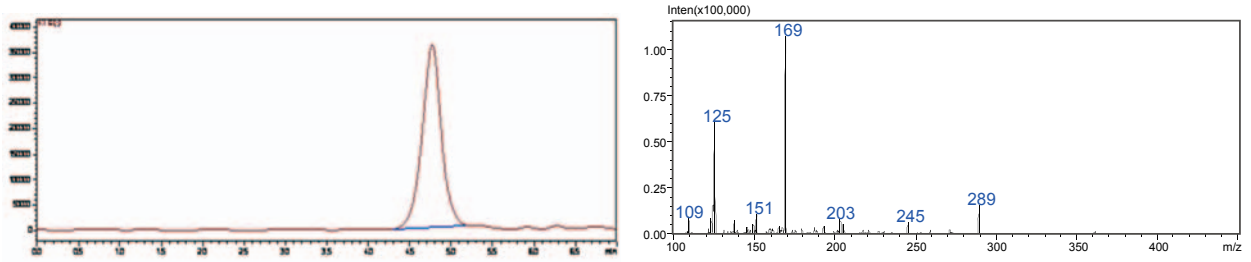

Fig. 4c Epicatechin gallate negative product ion scan results

Conclusions

Antioxidants were extracted from unripe Manilkara zapota fruit and UHPLC method was developed for the analysis of the same. The extract was also subjected to mass spectrometric analysis using triple quadrupole LCMS-8030 system (shown in Fig. 3). Polyphenol antioxidants like Catechin, Epicatechin, Gallocatechin gallate, Epigallocatechin gallate and Epicatechin gallate (shown in Fig. 4a, 4b and 4c respectively) were observed in the extract. Their presence was confirmed by comparing *m/z* values obtained with those cited in the literature. Product Ion Scan spectra for selected precursor ions gave further confirmation of their presence.

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References

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