INTRODUCTION

About 2% of all carbon photosynthesized by plants are converted into flavonoids or its closely related analogues. They occur abundantly in flowers, fruits and leaves of plants. Flavonoids may occur in various forms which can be obtained by hydroxylation, methylation and glycosylation. Aromatic and aliphatic acids, sulfate, prenyl, methylenedioxy, or isoprenyl groups get attached to the flavonoid nucleus while aglycones can be studied based on their comparison with standard bio-markers and the new creations are licensed under the identical terms. Flavonoids are commonly found as mixtures in various plants extracts. However, similarities in their structures and polarities have made identification of each compound difficult. However, emerging powerful advanced techniques of liquid chromatography-mass spectroscopy (LC-MS) has paved the way for identification of flavonoids quiet easily.

Flavonoids are produced by a series of condensation reactions between hydroxycinnamic acid and malonyl residues (B-ring and carbon atoms 2, 3 and 4 of the C-ring) resulting in formation of C_6-C_6-C_6 basic structure. In addition, the three-carbon bridges between the phenyl rings are commonly cyclized to form a third ring (C-ring). Cyclization with the degree of unsaturation and oxidation of the three-carbon segment allows them to be put into different classes.

Based on the C-ring, flavonoids can be subdivided as flavonols (with 2, 3-double bond, 3-OH and 4-keto groups), flavones (with 2, 3-double bond and 4-keto group), dihydroflavonols or flavanonols (with 3-OH and 4-keto group), dihydroflavonols or flavanonols (with 3-OH and 4-keto groups), flavones (with 2, 3-double bond and 4-keto groups), flavonols (with 3-OH and 4-keto groups), flavonols (with 3-OH and 4-keto groups).

In flavonoids, precise characteristic fragments of A- and B-rings have been interpreted while additional identification of unknown flavonoids can be studied based on their comparison with standard bio-markers and ultraviolet-visible (UV-Vis) spectra. Two common absorbance bands are A and B (rutin and quercetin) that lies in the range of 310–350 nm (flavones) and 350–385 nm (flavonols). Band B with range of 250–290 nm are almost identical in all the above mentioned flavonoid subgroups. In case of flavanones and dihydroflavonols, Band A is frequently observed with little reduction more than a shoulder at 300–330 nm and Band B with range of the 277–295 nm range, is the characteristic peak.

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Cite this article as: Doshi GM, Nalawade VV, Mukadam AS, Chaskar PK, Zine SP, Somani RR, et al. Elucidation of Flavonoids from Carissa congesta, Polyalthia longifolia, and Benincasa hispida Plant Extracts by Hyphenated Technique of Liquid Chromatography-mass Spectroscopy. Phcog Res 2016;8:281-6.
It is reported that plants containing polyhydroxy substituted flavonoids have highest antioxidant activity. O-di-OH substitution of the B-ring has appeared to be the most favorable structural characteristic in these compounds. Inactive quinines are formed due to free radicals abstraction of the two hydroxyl hydrogens of B-ring. C-ring plays an important role in antioxidant potential. Entirely substituted C-ring (catecholic flavonoids) represent greater antioxidant than corresponding members of the group.\[3,4\] UV-Vis spectra provide informative as well as interpretative tool for the characterization of C-ring, but MS could provide much accurate information ion the molecular mass (parent ion) as well as and on structural analogues.\[10\]

Flavonoids act as scavengers of nitric oxide synthesis, inhibitors of xanthine oxidase, growth regulators, photosensitizers and chemotaxonomic markers.\[3,11,12\] They inhibit cyclooxygenase as well as lipoxygenase enzymes resulting in reduced platelet activation and aggregation, offers protection against cardiovascular diseases and inflammation, anticancer, anti-viral, anti-microbial, anti-hepatotoxic, anti-osteoporotic, anti-allergic, anti-spasmodic, and antiulcer agents.\[2,10,13-18\]

Researchers have interpreted the results based on mass elucidation with structural identification of different classes of flavonoids. However, fragmentation data was not satisfactory enough to analyze subgroups of these class of compounds. There are losses of smaller molecules or radicals from the (M + H) + ion e. g. 18 a.m.u. (H₂O), 28 a.m.u. (CO), and 42 a.m.u. (CH₂CO₂). The depiction of C-ring structures is commonly based on the UV-Vis spectra or spectral information libraries which are not the trusted sources of information.\[10\]

Flavonoids have 3-hydroxy substitution and are bounded with sugar moiety attached to carbon-carbon bond which makes them unique since they are resistant to acid hydrolysis.\[10\] Though there are hundreds of flavonal aglycones known, kaempferol, quercetin, and myricetin are commonly elucidated by MS. In quercetin, different O-substituted compounds are observed. In addition, more than 200 quercetin glycosides have been noticed of which quercetin-3-rutinoside is locally known as rutin. In current research studies, a logical positive ionization mode LC-MS analysis of flavonoids was attempted. The research paper illustrates fragmentation patterns of rutin and quercetin from Carissa congesta (CC), Benincasa hispida (BH), and Polyalthia longifolia (PL) plant extracts as depicted in graphical abstract. The studies provide researchers with a view that the selected plant extracts elucidated good amount of flavonoids.

**MATERIALS AND METHODS**

Fresh selected parts of CC, PL and BH were collected, dried, authenticated, and extracted and preliminary phytochemical constituents were identified as reported by us. In addition, we have identified rutin as a constituent from PL leaves extract by high-performance thin layer chromatography and high-performance LC.\[5,10-21\]

The instrument used in the experimentation was as follows:

- LC-MS instrument: Varian Inc, USA
- Model: 410 Prostar Binary LC with 500 MS IT PDA Detectors
- Column: ZORBAX ECLIPSE XDB-C18 with Marrow Bore 2.1 × 150 mm 5 micron
- Mobile phase: The plant extracts were dissolved in mobile phase as mentioned below:
  - CC and PL – 0.1% formic acid + H₂O (60: 40)
  - BH - Acetonitrile.

**RESULTS**

LC-MS studies revealed the presence of the components from the CC, PL, and BH extracts as mentioned in Tables 1–3. Quercetin [Figure 1] was found in roots of CC (Apocynaceae) having a characteristic peak at m/z 301.9 (M) [Figure 1] confirmed with available references in the literature.\[3\] MS spectrum showing m/z 480.2 (M + glucoside) consisted of glucoside m/z 178 suggested the addition of glucoside molecule and m/z at 578.6 (M + glucosyl glucoside) consisted of glucosyl glucoside m/z 276 suggesting addition of glucosyl glucoside.

Rutin [Figure 2] was present in CC extract showed molecular ion at m/z 609.7 (M). Further peaks obtained at m/z 643.9 (M + 2H₂O) refers to addition of formate ion. m/z 430.2 (M – O - glucosyl) refers to loss of O-glucosyl as a fragment. (M – O-glucosyl – CO₂) refers to loss of O-glucosyl with CO₂ while (M – O-glucosyl – HCOO⁻) having m/z 344.9 refers to loss of O-glucosyl with three molecules of CO₂. Other expected compounds detected from the CC extract were quercetin-O-hexoside [Figure 3] having m/z 463.0 and its fragment ion observed at m/z 544.5, indicating loss of formic acid which was seen at m/z 416.0 and m/z 344.9 (M – HCOOH). Isomers quercetin-3-O-xylloside/arabinoside [Figure 4] has shown a characteristic peak at m/z 433.2. Fragments at m/z 594.7 (M + glucosyl) referred addition of glucose and m/z 653.0 (M + glucosylacetate) showed addition of glucosylacetate.

Quercetin [Figure 5] was found in the leaves of PL at m/z 301.9 (M). Fragments were observed at m/z 678.7 which were assumed to be diacyl prenyl quercetin-3-O-glucoside. Fragments ions observed at m/z 592.8 were expected to be derivative of prenyl-3-O-glucoside. Another fragment was observed at m/z 430.1 shows [prenyl quercetin + CO₂].

Ions at m/z 283.5 shows (M-H₂O) removal of water molecule while ions at m/z 239.4 indicate elimination of formic acid with water molecule (M – HCOOH – H₂O) or elimination of water molecule with CO₂ (M-H₂O-CO₂).

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**Table 1:** Various components and their fragments in Carissa congesta extract

| Retention time | Identity | Molecular formula | m/z | Fragments m/z | Figure number |
|---------------|---------|-------------------|-----|--------------|---------------|
| 8.90          | 2       | C₁₀H₁₀O₄          | 301.9 | 578.6, 480.2, 301.9 | I             |
| 10.25         | 3       | C₁₀H₁₀O₄          | 609.7 | 643.9, 609.7, 430.2, 388.9, 344.9, 300.6 | II            |
| 14.29         | 4       | C₁₀H₁₀O₄          | 430.3 | 544.5, 463, 416, 344.9 | III           |
| 11.61         | 5       | C₁₀H₁₀O₄          | 433.2 | 653, 594.7, 433.2 | IV            |

**Table 2:** Various components and their fragments in Polyalthia longifolia extract

| Retention time | Identity | Molecular formula | m/z | Fragments m/z | Figure number |
|---------------|---------|-------------------|-----|--------------|---------------|
| 7.22          | 2       | C₁₀H₁₀O₄          | 301.8 | 678.7, 592.8, 430.1, 283.5, 239.4 | V              |
| 3.45          | 3       | C₁₀H₁₀O₄          | 610.8 | 699.4, 610.8, 594.7, 556.8, 245.5 | VI             |
| 4.99          | 6       | C₁₀H₁₀O₄          | 594.8 | 549.2, 611.7, 594.8 | VII            |
| 4.99          | 7       | C₁₀H₁₀O₄          | 464.4 | 464.4 | VII            |
| 11.05         | 8       | C₁₀H₁₀O₄          | 613.9 | 671.2, 661.6, 569.5, 613.9, 388.8 | VIII           |

**Table 3:** Various components and their fragments in Benincasa hispida extract

| Retention time | Identity | Molecular formula | m/z | Fragments m/z | Figure number |
|---------------|---------|-------------------|-----|--------------|---------------|
| 8.14          | 2       | C₁₁H₁₀O₇          | 301.8 | 688.9, 594.6, 432.2,301.8, 239.2, 689, 683.7, 667.6, 608.1, 544.1, 500.5 | IX             |
| 11.20         | 3       | C₁₁H₁₀O₇          | 608.1 | 683.7, 667.6, 608.1, 544.1, 500.5 | X              |
Rutin [Figure 6] was spotted at m/z 610.8 (M). Fragment ions at m/z 699.4 (M + 2CO\textsubscript{2}) while 594.7 (M – O\textsuperscript{+}) consisting oxygen ion, m/z 556.8 (M – 3H\textsubscript{2}O) indicates elimination of three water molecules and m/z 245.5 (M – CH\textsubscript{3}COOH) indicates elimination of acetic acid.

Quercetin derivative vicenin 2 [Figure 7] was seen at m/z 594.8 (M) with molecular peaks at m/z 611.7 (M + O\textsuperscript{+}) which shows addition of oxygen ion and m/z 649.2 (M + 3H\textsubscript{2}O) shows addition of three water molecules.

Quercetin-3-O-glucoside [Figure 7] with m/z at 464.4 (M) and quercetin-3-(O-galloyl) hexoside [Figure 8] m/z 613.9 (M) were identified. The other fragments obtained were m/z 661.6 (M + HCOOH) referred as addition of formic acid, m/z 569.5 (M – CO\textsubscript{2}) referred as elimination of CO\textsubscript{2}, m/z 388.8 (M – CO\textsubscript{2} – hexoside) refers to elimination of hexoside with CO\textsubscript{2} and m/z 671.2 (M + CH\textsubscript{3}COO\textsuperscript{+}) shows addition of acetate ion.

Quercetin [Figure 9] was probably identified in the seeds of BH which showed molecular ion peak at m/z 301.8. The fragment ion obtained at m/z 432.2 (M + 3-O-arabinoside) or (M + 3-O-xyloside) showed addition of O-arabinoside or O-xyloside, respectively. Another fragment having m/z 594.6 (M + 3-O-glucosylarabinoside/xyloside) suggests addition of O-glucosylarabinoside/xyloside while m/z 634.2 (M + 3-O-glucosyl arabinoside/xyloside + 2H\textsubscript{2}O) suggests addition of O-glucosylarabinoside/xyloside with two water molecules. Two more fragments with m/z 688.9 (M + 3-O-glucosylarabinoside/xyloside) and 239.2 (M + CH\textsubscript{3}COOH) indicates addition of O-glucosylarabinoside/ xyloside with five molecules of water and two molecules of acetic acid.
Rutin [Figure 10] was characterised at m/z 608.1 (M) along with fragments at m/z 667.6 (M + CH₃COOH) showing addition of acetic acid. Other peaks at m/z 683.7 (M + CH₃CH₂COOH) showing addition of propionic acid and m/z 544.1 (M – CH₃COOH-4) suggesting the elimination of acetic acid with four hydrogen ions. Another fragment having m/z 500.5 (M – CH₃COOH-48) showed elimination of acetic acid, CO₂ and four hydrogen ions. The m/z at 689 indicates presence of rutin sulfate.

**DISCUSSION**

In the current study, flavonols (rutin and quercetin) and their analogues were characterized from plant. LC-MS analysis revealed that both compounds and their analogues possess fragmentation behavior with protonation which was concerned with dehydration, loss of CO and C-ring fission. Any unknown flavanol aglycone can be directly interpreted according to C-ring identification, dehydration pattern and CO losses of protonated molecule as well as produced ions. Fragmentation pattern was associated with the applied collision energy. If the collision energy is less than main fragment in the MS, the spectra produced was (M + H)+. However, by enhancing collision energy, a complete fragmentation of the protonated molecule can be obtained.[23-27]

**CONCLUSIONS**

Thus, CC, BH and PL extracts revealed the presence of flavonoids belonging such as rutin and quercetin as parent ions and close analogues
such as quercetin-6-O-hexoside, Vicenin 2, quercetin-3-O-xyloside/ arabinoside, and quercetin-3-O-glucoside were identified as fragments.

Acknowledgment
We acknowledge the management of Vivekanand Education Society’s College of Pharmacy, Mumbai for providing all facilities to carry out this work. We would also like to acknowledge Indian Institute of Technology, Mumbai, for their help in LC-MS analysis.

Financial support and sponsorship
Nil.
Figure 10: Mass spectrum showing presence of quercetin (2) and rutin (3) in *Benincasa hispida* extract

Conflicts of interest

There are no conflicts of interest.

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