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# Preliminary screening of potential flavonoid-subclasses in *Myristica fragrans* and *Cordyline terminalis* by LC-ESI-MS

**B Chandrasekhar Reddy, Ayesha Noor, Varatharajan Sabareesh and MA Vijayalakshmi**

**Abstract**
Although extensive literature exists on antioxidant properties of medicinal plants, very few studies have focused on their polyphenolic composition. Here, preliminary phytochemical screening and total flavonoid content in extracts of seeds of *Myristica fragrans* and leaves of *Cordyline terminalis* have been investigated. Screening of potential flavonoid-subclasses was done by LC-ESI-MS, whose data were interpreted using database of Lipid Metabolites and Pathways Strategy Consortium. 'Flavones and Flavonols' and 'Anthocyanidins' appear to be more abundant in *C. terminalis* than in *M. fragrans*. Higher content of isoflavonoids, chalcones and dihydrochalcones and some isoprenoids were observed in *M. fragrans* than in *C. terminalis*. The strategy and results of this study will help to choose appropriate standards prior to their identification and confirmation in natural extracts. These results may also be useful to assess antioxidant activity of only the subclass alone and accordingly appropriate subclasses can be selected to prepare formulations having nutraceutical applications.

**Keywords:** Flavonoids, polyphenols, phytochemical screening, LC-ESI-MS, *Myristica fragrans*, *Cordyline terminalis*

## Introduction
*Cordyline terminalis* (L.) Kunth (*C. terminalis*; also called Ti plant) (Liliaceae) is mostly used as an ornamental plant, whereas *Myristica fragrans* Houtt. (*M. fragrans*) (Myristicaceae) is very commonly employed in day-to-day culinary purposes. Previous studies on *C. terminalis* have demonstrated its antipyretic, analgesic and antibacterial activities, while antidiarrhoeal, stomachic stimulant and carminative properties have been reported from *M. fragrans* [1, 2]. Both these plant extracts are rich in polyphenols and have good antioxidant potential [3]. Further knowledge of the polyphenolic constituents, particularly 'flavonoids', could be useful in understanding their role and maximizing their usage for dietary purposes. Based on the variation in the type of heterocycle involved, flavonoids are classified into different sub-classes such as flavonols, flavones, flavanones, flavanols, anthocyanins and isoflavones. Flavonoids have been reported to possess many useful properties, such as anti-inflammatory, antimicrobial, anti-allergic, vascular, cytotoxicity and antitumor activities [4], however, their antioxidant activity is the most studied one. It is well established that antioxidant activity of flavonoids are responsible for other biological activities, in which the prevention of oxidative stress is beneficial [5]. But, so far, there are no reports on the identification of different flavonoid type of polyphenolic compounds from these two plants. Only steroid-class of compounds: steroidal sapogenins, steroidal saponins and new cholestane glycosides are known from the leaves of *C. terminalis* [6-8], whereas lignan class of polyphenolic compounds and terpenes were identified from *M. fragrans* [9-11]. For flavonoid identification and quantification, high performance liquid chromatography-diode array detector (HPLC-DAD) and/or liquid chromatography-mass spectrometry (LC-MS), are commonly used. In case of non-availability of commercial standards' techniques that can screen and provide structural information would useful for flavonoids identification e.g. LC-MS technique [5]. Therefore, we sought to look at the distribution of different flavonoid-subclasses from the crude extracts of these two plants, prior to identification of distinct flavonoid compounds. Herein, we report the details on screening of potential flavonoids in these two plant extracts, by following LC- electrospray ionization (ESI)-MS approach. Database of Lipid Metabolites and Pathways Strategy consortium (LIPID MAPS; www.lipidmaps.org) enabled interpretation of mass spectral data, which aided in obtaining an overview of potential flavonoid-subclasses. Additionally, preliminary results of phytochemical analysis and total flavonoid content estimated from each of the two extracts are presented.

## Materials and Methods

### Plant Material

Seeds of *M. fragrans* Houtt and healthy leaves of *C. terminalis* (L.) Kunth were collected during December-January from Vellore Institute of Technology (VIT) University, Vellore, Tamil Nadu, Southern India. The voucher specimen (CBST-1102) is deposited in Centre for Bio Separation Technology (CBST).

### Extraction

The extract preparation was carried out according to Chandrasekhar *et al*., 2011 [3]. *C. terminalis* and *M. fragrans* were collected from Vellore (Tamil Nadu, India) and used for extraction on the same day. *C. terminalis* leaves and *M. fragrans* seeds (100 gm) were grounded. The extraction was carried out using two different solvent systems: methanol-water (70:30; v/v) as already reported [3] and only water, viz., 100% water. The filtrate was centrifuged at 7,000 rpm for 10 min. and the supernatant was collected. It was subjected to evaporation in Buchi Rota vapor apparatus (R-215, Switzerland) and the extract was lyophilized. The dried powder was stored at 4ºC in dark until subsequent analyses. These extracts were then characterized by reverse phase LC-ESI-MS.

### Preliminary Phytochemical analysis

The obtained extracts were subjected to phytochemical tests to determine the presence of secondary metabolites like alkaloids, saponins, flavonoids, tannins, glycosides and phenols were carried out qualitatively [12].

### Determination of total flavonoids

Aluminum chloride - colorimetric method [13] with some modifications was used to determine total flavonoid content. 0.5 ml of the respective extract was mixed separately with 1.5ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml potassium acetate and 2.8 ml Milli Q water. Each reaction mixture was incubated at room temperature for 30 min. and the absorbance of mixture was measured at 415 nm with UV-VIS spectroscopy (Beckman). Blank was set along with the working standard. For blank, aluminum chloride was substituted by the same quantity of water. Quercetin was used as standard. Flavonoid content is expressed in terms of quercetin equivalent (mg/g of extracted compound).

### LC-ESI-MS data analysis

As part of LC-ESI-MS, LC was carried out on Hypersil gold AQ (150 mm × 0.5 mm, 3 µm) employing water (solvent A) and acetonitrile (solvent B), each containing 0.1% acetic acid; a gradient elution was followed. Flow rate was maintained at 12 µl/min. and the eluents were monitored by online UV detection (λ 255 nm and 290 nm). Subsequent to UV detection, the eluents were directed to an ESI mass spectrometer (*Quattro Premier* (*Waters*)) and total ion chromatograms (TICs) were acquired. The mass spectral data acquisitions were done in positive ion polarity in the range, *m/z*: 200 - 650. The capillary voltage was kept at about 3-3.5 kilo Volt (kV) and cone voltage was set at 30 V.

## Results and Discussion

Qualitative analysis of seeds of *M. fragrans* and leaves of *C. terminalis* were carried out for Alkaloids, Flavonoids, Phenols, Saponins, Glycosides, cardiac glycosides, Carbohydrates, Proteins, Phytosterols, Tannins, Saponins and gum mucilage. The qualitative phytochemical analysis of both the extracts revealed the presence of all the phytochemicals except Saponins, Phytosterols and Gum Mucilage, which were found to be absent in *M. fragrans* extract (Table1). All these constituents have been reported to impart medicinal benefits like antioxidant, hyperglycemia, anticancer, anti-inflammatory antiepileptic, antipyretic, analgesic, antimicrobial and immunomodulatory activities [14].

In continuation of our studies [3] on these two plants, the total content of flavones, flavonols and isoflavones in methanol-water extracts of *M. fragrans* and *C. terminalis* were analyzed by aluminum chloride ($AlCl_3$) method and was found to be 38.5 ±0.5 and 49.0 ± 0.2 mg/g, respectively. Consumption of certain specific subclasses of flavonoids might be more beneficial for human health rather than intake of total polyphenols [15]. There are emerging evidences that flavonoids in tea, cocoa and chocolate are beneficial for cardiovascular health, particularly flavanol-rich cocoa can be a cardioprotective nutraceutical [16]. Anthocyanins have the potential to suppress the increase of post-prandial glucose levels [17]. Procyanidins in oligomeric form have the ability to prevent weight gain, insulin resistance and impaired glucose tolerance, as studied in a high-fat fed mice model [18]. Further, an investigation by Hui *et al*. [19] demonstrate that intake of flavonols and flavones and not total flavonoid or other subclasses, is associated with reduced risk of breast cancer in post-menopausal women. However, more work has been devoted to the antioxidant activity of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals. The antioxidant activity of both the extracts was reported in our previous work [3]. All these studies prompted testing the biological activity of each of the subclass of flavonoids rather than total flavonoids. Therefore, we decided to investigate these crude extracts by reverse phase (LC) coupled to ESI-MS (LC-ESI-MS) with the objective to identify the potential subclasses of flavonoids in these two plants.

**Table 1:** Phytochemical analysis of *M. fragrans* and *C. terminalis* extracts

| Qualitative Analysis | Test | *M. fragrans* extract | *C. terminalis* extract |
|---|---|---|---|
| Carbohydrates | Molisch's test/Benedict's test | ✓ | ✓ |
| Protein | Millon's test/ Biuret test | ✓ | ✓ |
| Fixed oils & fats | saponification test | ✓ | ✓ |
| Saponins | Frothing test | × | ✓ |
| Tannins | Ferric chloride test/lead acetate test | ✓ | ✓ |
| Alkaloids | Mayer's reagent test/Dragendorff's test | ✓ | ✓ |
| Flavonoids | Shinoda's test/FeCl3 test | ✓ | ✓ |
| Glycosides | Borntrager's test | ✓ | ✓ |
| Cardiac Glycosides | Keller-Killiani test | ✓ | ✓ |
| Phytosterols | Liebermann-burchard test | × | ✓ |

| | | | |
|---|---|---|---|
| Gum Mucilage | Gum Mucilage test | × | ✓ |
| Total Flavonoid Content | Aluminum Chloride Test | 38.5 ±0.5mg/g | 49.0 ± 0.2 mg/g |

✓indicates present and × indicates absent.

For the interpretation of mass spectral data, among different databases available (USDA: http://fnic.nal.usda.gov/food-composition/phytonutrients; Phenol-Explorer: http://phenol-explorer.eu/downloads; LIPID MAPS: www.lipidmaps.org), the database of LIPID MAPS Consortium (LIPID MAPS) was chosen. The observed *m/z* values are considered to be singly protonated adducts ($[M+H]^+$) and were searched in the database of LIPID MAPS (www.lipidmaps.org) [20]. According to the classification scheme proposed by LIPID MAPS, various flavonoid-subclasses are within the main class of 'Flavonoids', which are placed in the category of "polyketides (PK)" (Table 2).

**Table 2:** Flavonoids: a main class of polyketide (PK) category[a]

| Eight Category Classification of Lipids | Main Classes of Polyketides (PKs) | Subclasses of Flavonoids |
|---|---|---|
| 1. Fatty Acyls (FA) | 1. Linear Polyketides | 1. Anthocyanidins (AC) |
| 2. Glycerolipids (GL) | 2. Halogenated Acetogenins | 2. Flavans, Flavanols & Leucoanthocyanidins (FFL) |
| 3. Glycerophospholipids (GP) | 3. Annonaceae Acetogenins | 3. Proanthocyanidins (PAC) |
| 4. Polyketides (PK) | 4. Macrolides & Lactone Polyketides | 4. Biflavonoids & Polyflavonoids (BPF) |
| 5. Prenol Lipids (PR) | 5. Ansamycins & Related Polyketides | 5. Isoflavonoids |
| 6. Saccharo Lipids (SL) | 6. Polyenes | 6. Rotenoid Flavonoids (RF) |
| 7. Sphingolipids (SP) | 7. Linear Tetracyclines | 7. Pterocarpans (PC) |
| 8. Sterol Lipids (ST) | 8. Angucyclines | 8. Isoflavans (IF) |
| | 9. Polyether Polyketides | 9. Coumestan Flavonoids (CF) |
| | 10. Aflatoxins & Related Substances | 10. Neoflavonoids (NF) |
| | 11. Cytochalasins | 11. Flavones & Flavonols |
| | 12. Flavonoids | 12. Chalcones & Dihydrochalcones |
| | 13. Aromatic Polyketides | 13. Aurone Flavonoids (AF) |
| | 14. Non-ribosomal peptide/polyketide hybrids | 14. Flavanones |
| | 15. Other Polyketides | 15. Dihydroflavonols (DHF) |
| | | 16. Other Flavonoids (OF) |

[a] www.lipidmaps.org (LIPID MAPS Consortium) [20]

Table 3 summarizes the *m/z* values observed from the mass spectral data of two different extracts from the two plants. It can be noted in Table 3 that some *m/z* values are observed only from water extract, while some are detected from methanol-water extract only; and there are ions detected in both these extracts. Depending on the relative polar and apolar nature, different flavonoid compounds extract into different solvents. Taking these m/z values, the search was carried out using a standalone program, Mass Spectrometry based Lipid(ome) Analyzer and Molecular Platform (MS-LAMP), in which LIPID MAPS' database has been integrated [21]. Window range for these searches was either set at *m/z* 0.05 or 0.1 or 0.25. The search was actually pointed to all eight categories of lipids in the database. For most of the *m/z* values submitted to MS-LAMP, many of the output hits were from PK category only. And these hits in PK category mainly belonged to 'Flavonoids' main class and a few were from the main class of 'Other Polyketides'. The resulting searched hits were organized in ascending order of the molecular masses and according to the population distribution of different subclasses of flavonoids, in particular 'flavones & flavonols', 'flavanones', 'isoflavonoids' and 'chalcones and dihydrochalcones'.

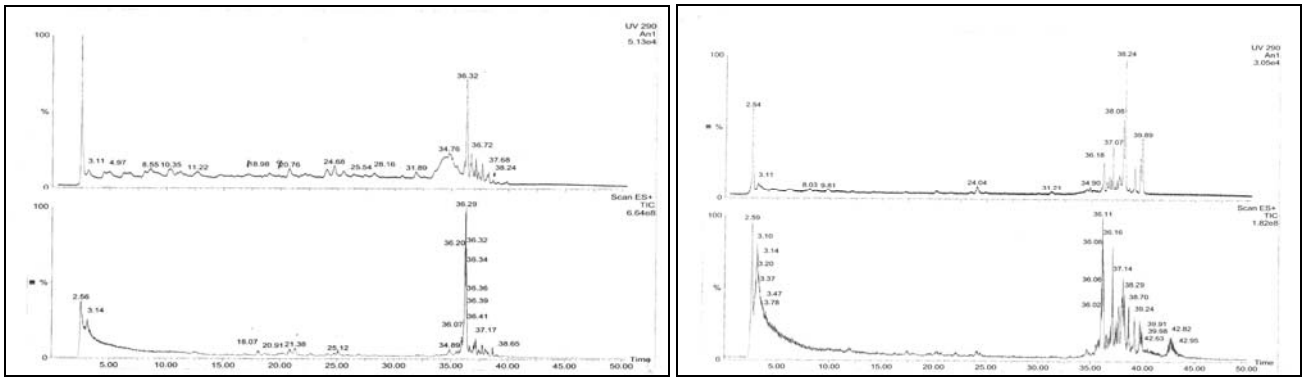
**Table 3:** *m/z* values from LC-ESI-MS data of Water and Methanol-Water extracts of *M. fragrans*[a] and *C. terminalis*[b]

| S. No | Solvent Extracts | Observed *m/z* values *of M. fragrans*[c] | Observed *m/z* values *of C. terminalis*[d] |
|---|---|---|---|
| 1. | Water | 271.3, 273.4, 293.3, 305.2, **309.4**, 323.4, 343.2, 345.4, 353.4, 383.4, 401.3, 413.3, **417.4**, 419.4, 455.3, 471.5, 519.5, 617.4 | **309.2**, **417.3**, 563.3 |
| 2. | Water & Methanol-Water | 223.3, 325.3, 327.3, 341.4, 357.3, 371.3, 373.3, 387.3 | 287.3, 303.2, 433.3, 449.2, 465.2, 579.3, 595.3, 611.2 |
| 3. | Methanol-Water | 217.3, 233.3, 247.4, 265.4, 403.3, 427.3, 355.4 | 317.2, 479.3, 549.4 |

[a] Figures 1 - 7; [b] Figures 8 - 12; [c] Table 4 shows search results of *M. fragrans*; [d] Table 5 shows search results of *C. terminalis*.

***LC-ESI-MS of M. fragrans:*** The search results of MS-LAMP from the LC-ESI-MS data of *M. fragrans*' extracts are enlisted in Table 4 and the total ion chromatograms acquired from extracts of *M. fragrans* are shown in Figure 1. In the case of anthocyanidins, experimentally observed *m/z* values directly correspond to their molecular mass, since they are inherently singly positively charged; hence, the peaks at *m/z* 271.3 (Figure 2a) and *m/z* 519.5 (Figure 3a) can be directly assigned to pelargonidin (271.06065 Da, C15 H11 O5: italicized in Table 4) and pelargonidin 3-(6"-malonylglucoside) (C24 H23 O13; 519.11387 Da: italicized in Table 4), respectively. The signal at *m/z* 343.2 (Figure 4) can be ascribed to $[M+H]^+$ of seventeen flavonoid compounds (C18 H14 O7, mol. mass 342.073955 Da; Table 4), as found in LIPID MAPS' database. It can also be interpreted due to $[M+H]^+$ of C21 H26 O4 (malabaricone B, mol. mass 342.18311 Da), which is an acylphenol [22-26]. LIPID MAPS' database does not have any detail about acylphenols, thus far and hence, the search results from this database (i.e., Tables 4 and 5) also do not contain any acylphenol. Acylphenols

(including malabaricones) have been found in *M. fragrans* Houtt (nutmeg) [22], seeds of *M. dactyloides* [23], dried fruits of *M. maingayi* [24], dried fruit rinds of *M. malabarica* [25] and leaves/fruits of *M. crassa* [26]. Acetyl cholinesterase-inhibitory activity has been noted from the extracts of *M. crassa* and *M. fragrans* [26, 27].



**(a)** **(b)**

**Fig 1:** LC-UV-MS chromatograms of (a) Water extract and (b) Methanol-Water extract of *Myristica fragrans* (*M. fragrans*)

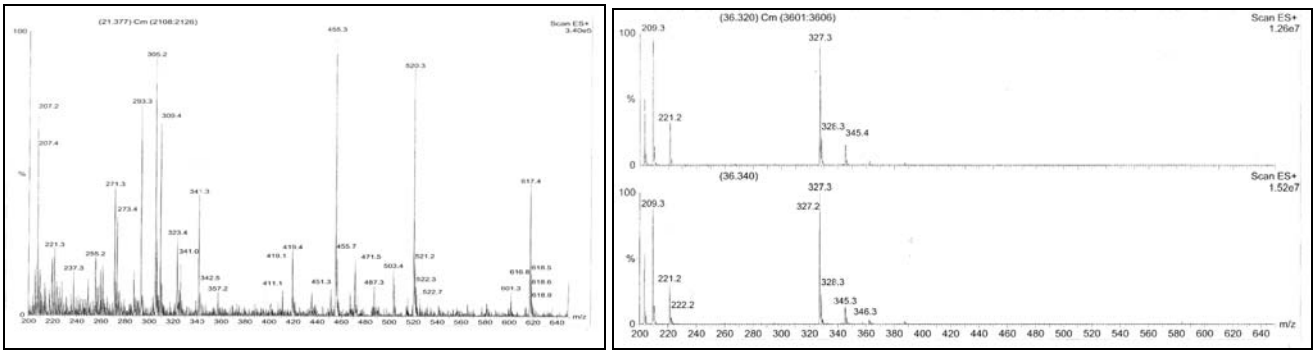
**Table 4:** Flavonoids and other metabolites identified from LC-ESI-MS data of Water extract and Methanol-Water extract of *M. fragrans* (Table 3) using LIPID MAPS' database and MS-LAMP

| Molecular Mass (based on observed m/z) | Molecular Formula | Flavones & Flavonols | Flavanones | Isoflavonoids | Chalcones & Dihydro chalcones | Other Flavonoid subclasses or Metabolites[a] |
|---|---|---|---|---|---|---|
| 215.842156 | C2H2Br2O2 | --- | --- | --- | --- | 1 HFA[b] |
| 216.1878 | C16H24 | --- | --- | --- | --- | 1 SQT[c] |
| 216.11503 | C14H16O2 | --- | --- | --- | --- | 1 UFA[d] |
| 216.13616 | C11H20O4 | --- | --- | --- | --- | 1 DCA[e] |
| 216.151415 | C15H20O | --- | --- | --- | --- | 6 SQT |
| 216.172545 | C12H24O3 | --- | --- | --- | --- | 14 Hyd. FA[f] |
| *222.06808* | *C15H10O2* | *1* | --- | --- | --- | --- |
| 222.08921 | C12H14O4 | --- | --- | --- | --- | 1 DCA |
| 232.14633 | C15H20O2 | --- | --- | --- | --- | 1 SQT |
| 246.005827 | C5H12O7P2 | --- | --- | --- | --- | 2 HT[g] |
| *264.078645* | *C17H12O3* | --- | --- | --- | *1* | --- |
| 270.052825 | C15H10O5 | 9 | --- | 6 | --- | 2 AF, 1 OF, 3 A&P[h] |
| 270.064058 | C14H10N2O4 | --- | --- | --- | --- | 1 Ar.P[i] |
| 270.08921 | C16H14O4 | --- | 7 | 1 | 10 | 1 OF, 1 NF, 1 IF, 2 PC, 1 FFL |
| *271.06065* | *C15H11O5* | --- | --- | --- | --- | *1 AC* |
| 272.068475 | C15H12O5 | --- | 9 | 1 | 6 | 2 PC |
| 292.07356 | C18H12O4 | 10 | -- | -- | -- | 2 AF |
| 304.013852 | C15H9ClO5 | 1 | -- | -- | -- | 1 A&P |
| 308.068475 | C18H12O5 | --- | 1 | --- | --- | 1 PC |
| 322.06887 | C15H14O8 | --- | --- | --- | --- | 2 FFL |
| 322.084125 | C19H14O5 | 2 | --- | --- | 1 | --- |
| 324.06339 | C18H12O6 | --- | ---- | --- | --- | 1 PC |
| 324.099775 | C19H16O5 | --- | 1 | 1 | --- | 1 PC |
| 326.042655 | C17H10O7 | --- | --- | --- | --- | 3 CF |
| 340.058305 | C18H12O7 | 2 | --- | --- | --- | --- |
| 340.09469 | C19H16O6 | --- | --- | --- | --- | 1 IF |
| 342.073955[j] | C18H14O7[j] | 4 | --- | 9 | --- | 2 NF, 1 PC, 1 FFL |
| 344.05322 | C17H12O8 | 5 | --- | --- | --- | --- |
| 344.089605 | C18H16O7 | 49 | 2 | 5 | 1 | 1 AF,1 RF, 1 NF, 3 PC |
| 352.02529 | C15H12O8S | --- | 1 | --- | --- | --- |
| 352.058305 | C19H12O7 | --- | --- | --- | --- | 1 RF |
| 352.09469 | C20H16O6 | 6 | --- | 7 | --- | 2 RF, 3 CF, 2 OF |
| 354.073955 | C19H14O7 | --- | 1 | 1 | --- | --- |
| 356.05322 | C18H12O8 | 1 | --- | --- | --- | 1 CF |
| 356.089605 | C19H16O7 | 5 | --- | 8 | --- | 1 OF |
| 370.06887 | C19H14O8 | 3 | --- | --- | --- | 1 RF |
| 372.048135 | C18H12O9 | --- | --- | --- | --- | 1 RF |
| 372.08452 | C19H16O8 | 8 | --- | --- | --- | 1 RF |
| 372.099775 | C23H16O5 | --- | 1 | --- | 1 | --- |
| 381.99947 | C15H10O10S | 7 | --- | --- | --- | --- |
| 386.10017 | C20 H18 O8 | 7 | --- | 2 | --- | --- |

| | | | | | | |
|---|---|---|---|---|---|---|
| 386.115425 | C24H18O5 | --- | 1 | --- | 1 | --- |
| 386.136555 | C21H22O7 | 2 | 3 | 2 | 1 | --- |
| 386.15181 | C25H22O4 | 1 | --- | --- | --- | --- |
| 386.17294 | C22H26O6 | --- | --- | --- | --- | 2 FFL |
| 400.079435 | C20H16O9 | 3 | --- | --- | --- | --- |
| 400.09469 | C24H16O6 | 1 | --- | --- | 1 | --- |
| 402.095085 | C20H18O9 | 9 | --- | --- | --- | --- |
| 412.010035 | C16H12O11S | 3 | --- | --- | --- | --- |
| 416.089605 | C24H16O7 | 1 | --- | --- | --- | --- |
| 417.11856 | C21H21O9 | --- | --- | --- | --- | 1 AC |
| 418.090000 | C20H18O10 | 13 | --- | --- | --- | --- |
| 426.025685 | C17H14O11S | 3 | --- | --- | --- | --- |
| 426.095085 | C22H18O9 | --- | --- | --- | --- | 2 FFL |
| 453.996313 | C17H14N2O7S3 | --- | --- | --- | --- | 1Ar.P |
| 470.084915 | C23H18O11 | 1 | --- | --- | --- | --- |
| 470.1213 | C24H22O10 | 2 | --- | --- | --- | --- |
| 470.136555 | C28H22O7 | --- | --- | --- | 1 | --- |
| *470.157685* | *C25H26O9* | *1* | --- | --- | --- | --- |
| *519.11387* | *C24H23O13* | --- | --- | --- | --- | *1 AC* |
| 616.10644 | C28H24O16 | 9 | --- | --- | --- | --- |

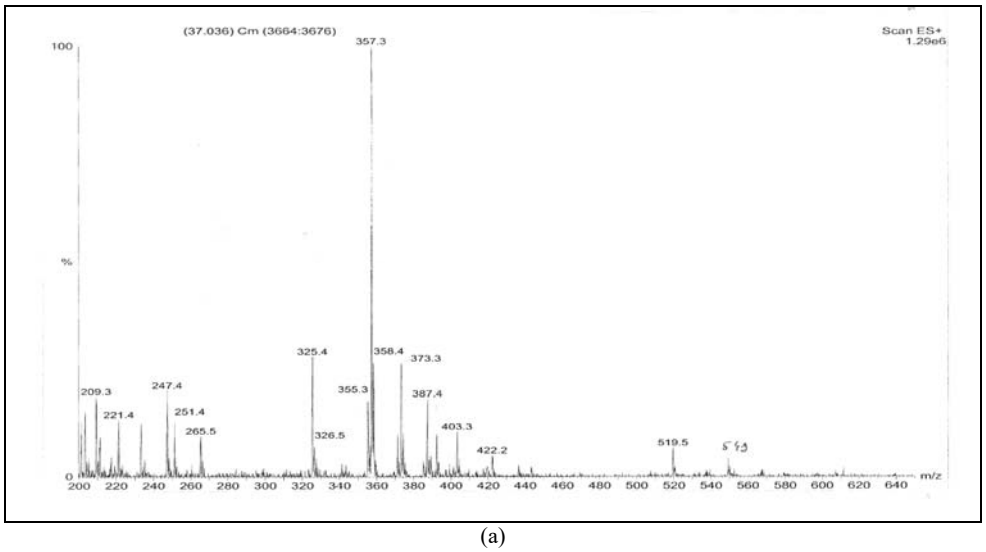
[a]See Table 2 for expansions of abbreviations of flavonoid-subclasses; [b]HFA: Halogenated Fatty Acids; [c]SQT: Sesquiterpenes (Isoprenoids: Prenol Lipids); [d]UFA: Unsaturated Fatty Acids; [e]DCA: Dicarboxylic Acids; [f] Hyd. FA: Hydroxylated Fatty Acids; [g]HT: Hemi Terpenes (Isoprenoids); [h]A&P: Anthracenes & Phenanthrenes; [i]Ar.P: Aromatic Polyketides: Diphenyl ethers, biphenyls, dibenzyls and stilbenes; [j]342.18311 Da: C21 H26 O4: Malabaricone B - an acylphenol.

Hence, the extracts of *M. fragrans* that we have, might also possess acetyl cholinesterase-inhibitory activity, besides antioxidant capacity. The presence of a few flavonoid compounds can be ascertained in these extracts from LC-ESI-MS data only. Each of the signals at *m/z* 223.3 (Figure 4), *m/z* 265.4 (Figure 6a) and *m/z* 471.5 (Figure 2a) correspond to proton adduct of only one flavonoid molecule and hence, the three compounds: C15 H10 O2 (Flavone & Flavonol: 222.06808 Da), C17 H12 O3 (Chalcone & Dihydrochalcone: 264.078645 Da) and C25 H26 O9 (Flavone & Flavonol: 470.157685 Da), can be confirmed in the extracts of *M. fragrans* (shown italicized in Table 4). It needs to be noted that the number of flavonoids screened herein (Tables 4 & 5) can be an overestimate, when compared to the number of identifications that would result from a high resolution mass spectrometer, as the data here are from a triple quadrupole mass spectrometer, which offers only unit resolution. For example, the signal at *m/z* 345.4 in the mass spectrum obtained from water extract of *M. fragrans* (Figure 2b) can be attributed to all sixty three compounds (C18 H16 O7, 344.089605 Da) or any one or some of these sixty three.

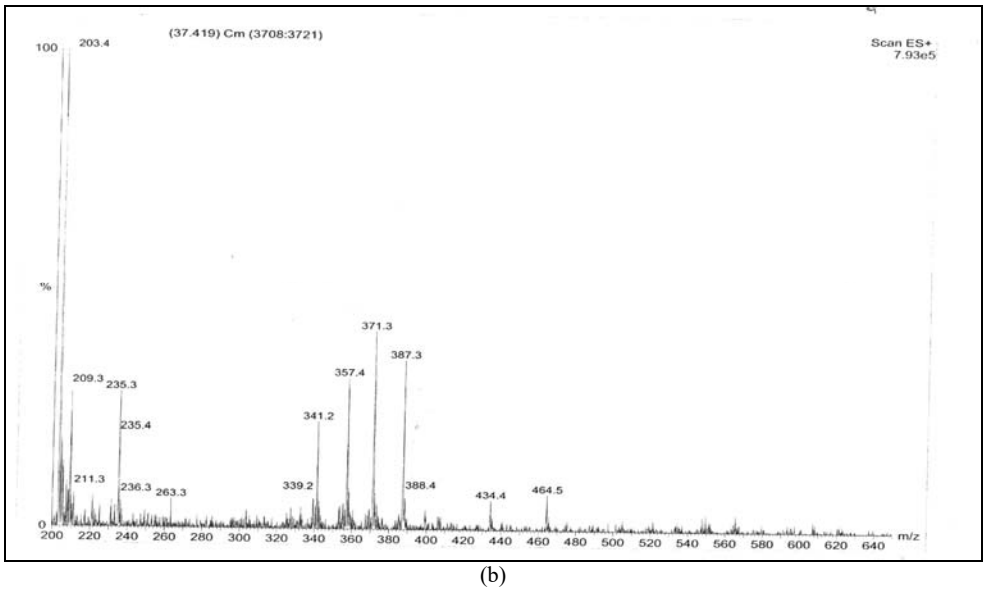



(a) (b)

**Fig 2:** Mass spectra corresponding to (a) $t_R$: 21.377 min. and (b) $t_R$: 36.32 & 36.34 min. processed from LC-MS chromatogram (see Figure 1a) of Water extract of *M. fragrans*.




(a)




(b)

**Fig 3:** Mass spectra corresponding to (a) $t_R$: 37.036 min. and (b) $t_R$: 37.419 min. processed from LC-MS chromatogram (Figure 1a) of Water extract of *M. fragrans*.

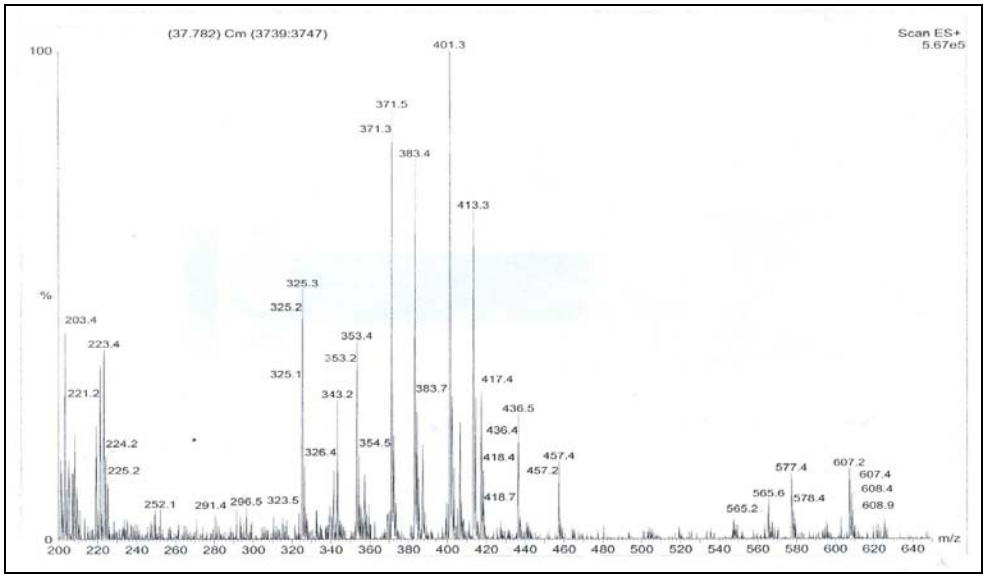
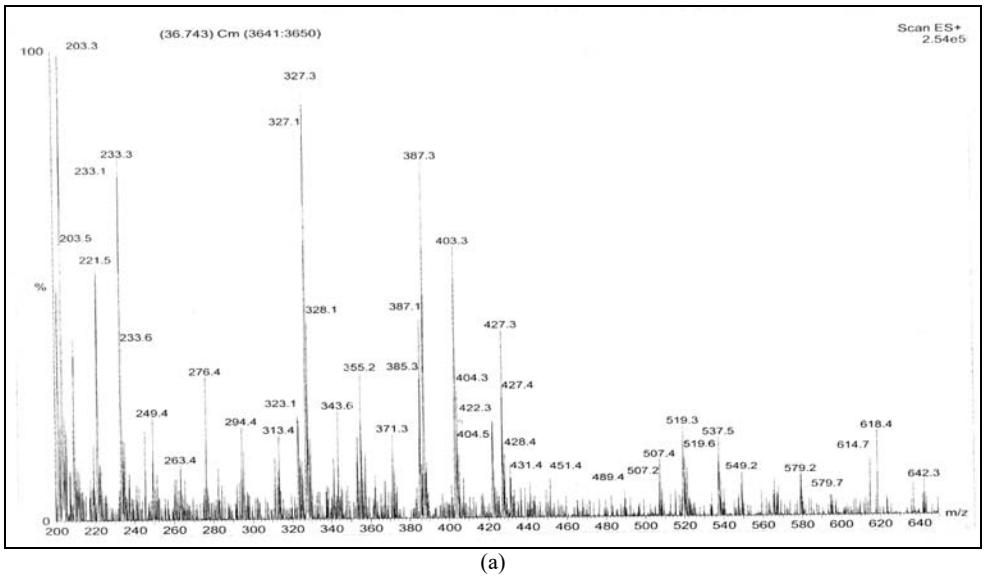
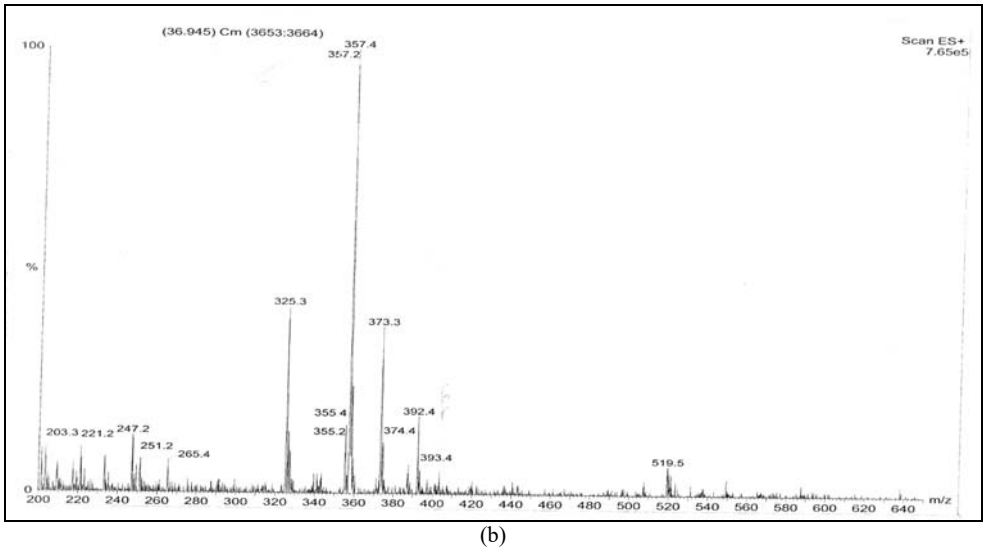



**Fig 4:** Mass spectrum corresponding to $t_R$: 37.782 min. processed from LC-MS chromatogram of Water extract (Figure 1a) of *M. fragrans*.

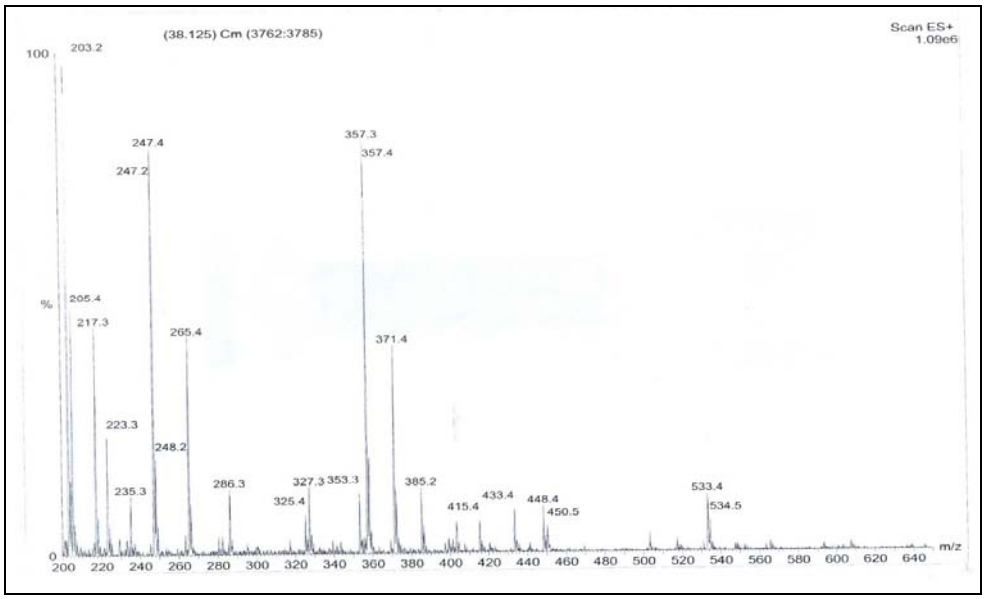



(a)

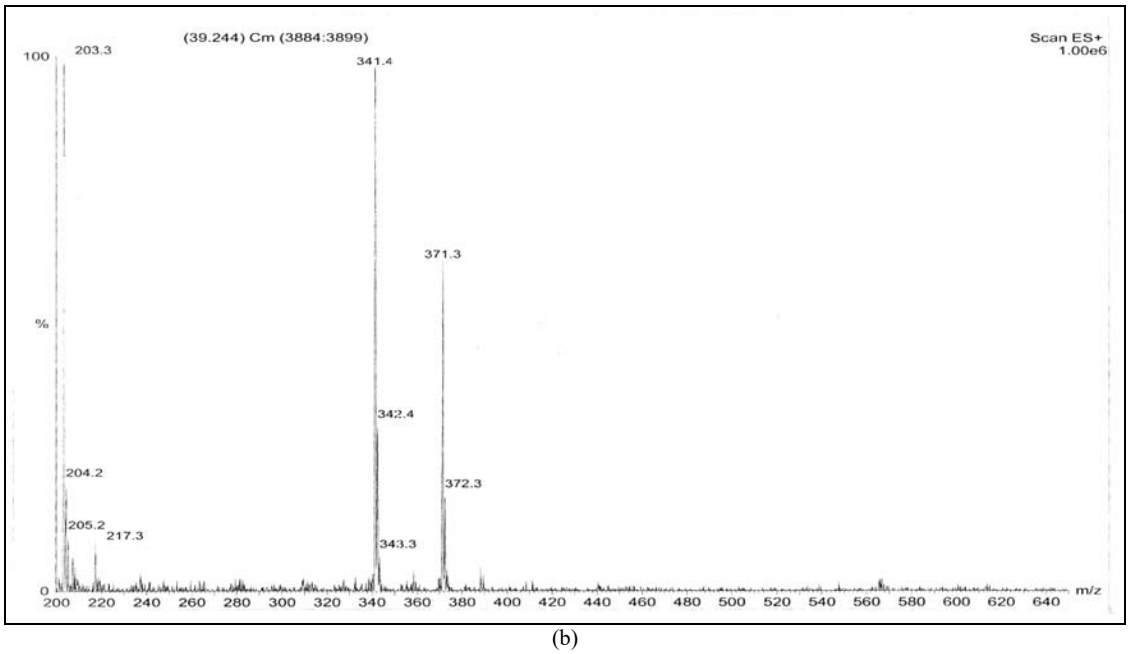



(b)

**Fig 5:** Mass spectra corresponding to (a) $t_R$: 36.74 min. and (b) $t_R$: 36.94 min. processed from LC-MS chromatogram (Figure 1b) of Methanol-Water extract of *M. fragrans*.




(a)




(b)

**Fig 6:** Mass spectra corresponding to (a) $t_R$: 38.125 min. and (b) $t_R$: 39.24 min. processed from LC-MS chromatogram (Figure 1b) of Methanol-Water extract of *M. fragrans*.

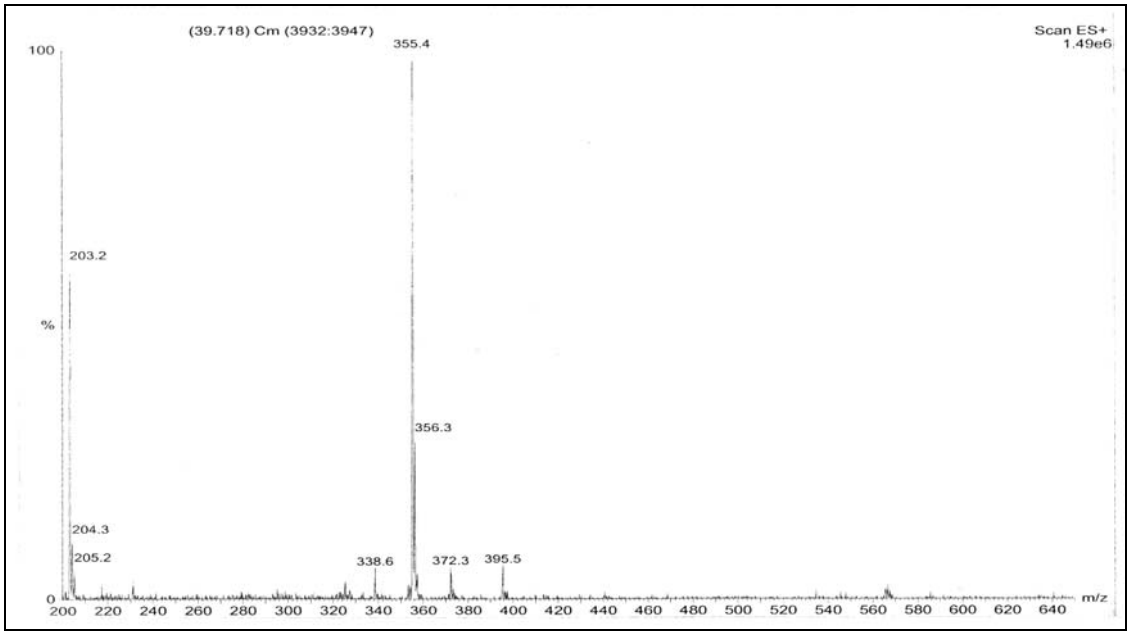


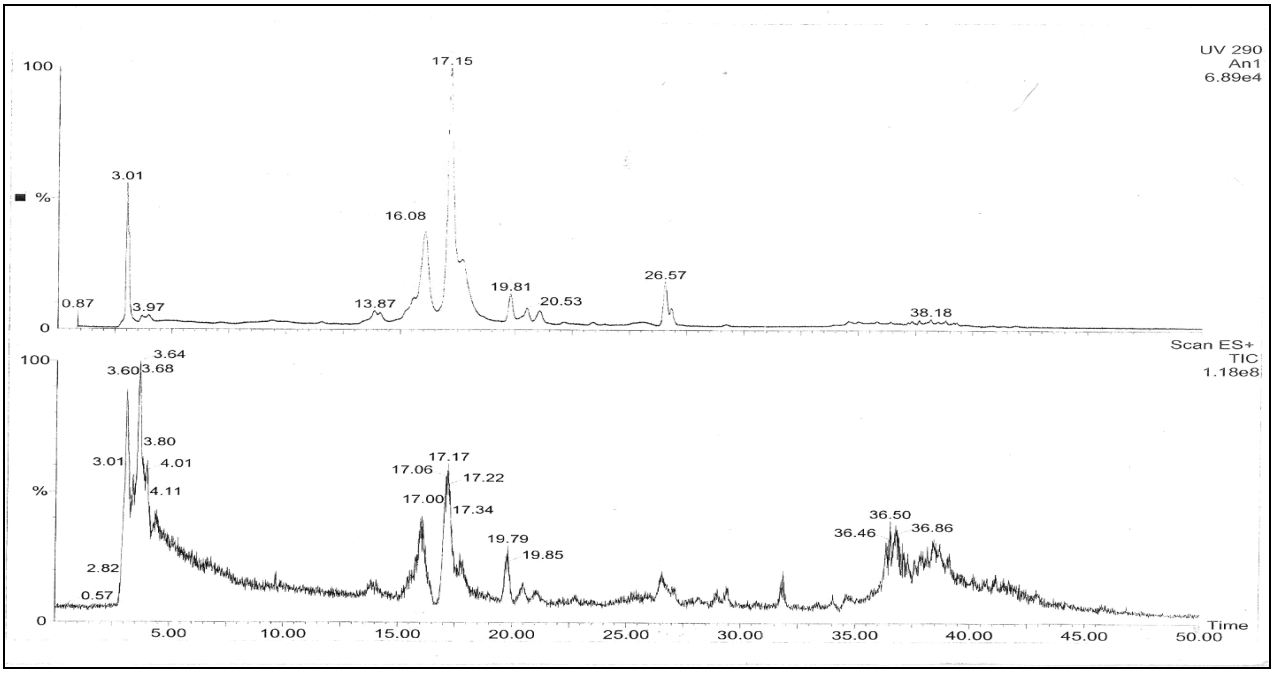

**Fig 7:** Mass spectrum corresponding to $t_R$: 39.71 min. processed from LC-MS chromatogram (Figure 1b) of Methanol-Water extract of *M. fragrans*.

***LC-ESI-MS of C. terminalis:*** In Table 5, distribution of flavonoid-subclasses in the extracts of *C. terminalis* as interpreted from MS-LAMP is presented and LC-UV-MS chromatograms of *C. terminalis* are shown in Figure 8. Four anthocyanidins of molecular formula C21 H21 O10 of mol. mass 433.113475 Da (Table 5) can correspond to the peak at *m/z* 433.3 (Figure 9a); it may be recalled that molecular mass directly corresponds to the observed *m/z* value in the case of anthocyanidins, as they are inherently charged (*vide supra*). Likewise, the peak at *m/z* 595.3 (Figure 10) can be ascribed to 13 anthocyanidins having the formula C27 H31 O15 of molecular mass 595.1663 Da. Further, in Table 5, there are 14 'flavones & flavonols', 5 'isoflavonoids' and 3 'aurone flavonoids', whose molecular mass is 286.04774 Da corresponding to the formula C15 H10 O6; a well-known compound among the 14 'flavones & flavonols' is 'kaempferol'. This suggests that the peak at *m/z* 287.3 (Figure 10) can be due to all these 22 compounds or any one or some of these 22 compounds. Likewise, the signal at *m/z* 303.2 (Figure 9b) can be interpreted due to 15 flavonoid molecules, which consists of 13 'flavones & flavonols', 1 isoflavonoid and 1 aurone flavonoid. The well-known 'quercetin' is one of the 13 flavones &flavonols. In *C. terminalis*, there are fifty six compounds having the same molecular formula C21 H20 O11 (Mol. mass 448.100565 Da), forty six molecules have C21 H20 O12 (Mol. mass 464.09548 Da) and fifty compounds have C27 H30 O14 (Mol. mass 578.16356 Da).

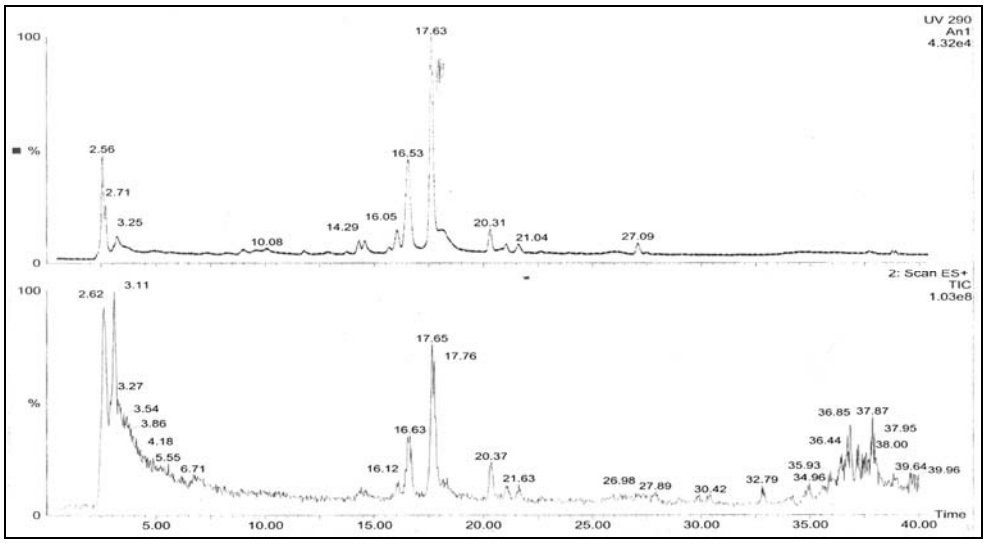
**Table 5:** Flavonoids and other metabolites identified from LC-ESI-MS data of Water extract and Methanol-Water extract of *C. terminalis* (Table 3) using LIPID MAPS' database and MS-LAMP

| Molecular Mass (based on observed *m/z*) | Molecular Formula | Flavones & Flavonols | Flavanones | Isoflavonoids | Chalcones & Dihydro chalcones | Other Flavonoid Subclasses[a] |
|---|---|---|---|---|---|---|
| 286.04774 | C15H10O6 | 14 | --- | 5 | --- | 3 AF |
| 302.042655 | C15H10O7 | 13 | --- | 1 | --- | 1 AF |
| 308.068475 | C18H12O5 | --- | 1 | --- | --- | 1 PC |
| 316.058305 | C16H12O7 | 30 | 1 | 3 | --- | 1 PC, 1 RF |
| 316.09469 | C17H16O6 | --- | 15 | 7 | 3 | 4 IF, 6 PC, 1 FFL |
| 416.089605 | C24H16O7 | 1 | --- | --- | --- | --- |
| 417.11856 | C21H21O9 | --- | --- | --- | --- | 1 AC |
| 433.113475 | C21H21O10 | --- | --- | --- | --- | 4 AC |
| 448.100565 | C21H20O11 | 45 | 2 | 3 | 1 | 4 AF, 1 NF |
| 448.11582 | C25H20O8 | 1 | --- | --- | --- | --- |
| 448.13695 | C22H24O10 | 3 | 11 | --- | 6 | --- |
| 448.152205 | C26H24O7 | 4 | --- | --- | --- | --- |
| 448.173335 | C23H28O9 | --- | --- | ---- | 1 | 1 FFL |
| 448.18859 | C27H28O6 | --- | --- | --- | --- | 1 OF |
| 464.09548 | C21H20O12 | 42 | 2 | --- | --- | 2 AF |
| 477.93344 | C15H10O12S3 | 1 | --- | --- | --- | --- |
| 478.074745 | C21H18O13 | 10 | --- | --- | --- | --- |
| 548.11661 | C25H24O14 | 5 | --- | 1 | --- | --- |
| 548.152995 | C26H28O13 | 8 | --- | 4 | --- | --- |
| 548.16825 | C30H28O10 | --- | --- | --- | 1 | --- |
| 548.18938 | C27H32O12 | --- | --- | --- | --- | 1 AF |
| 548.225765 | C28H36O11 | --- | --- | --- | --- | 1 FFL |
| 562.147515 | C30H26O11 | --- | --- | --- | --- | 1 PAC |
| 562.168645 | C27H30O13 | 9 | --- | 2 | --- | 1 AF |
| 562.20503 | C28H34O12 | 1 | --- | --- | --- | --- |
| 578.14243 | C30H26O12 | 13 | --- | 1 | --- | 4 PAC |
| 578.16356 | C27H30O14 | 42 | --- | 6 | --- | 2 AF |
| 595.14517 | C30H27O13 | --- | --- | --- | --- | 2 AC |
| 595.1663 | C27H31O15 | --- | --- | ---- | --- | 13 AC |
| 610.095875 | C29H22O15 | --- | --- | --- | --- | 6 FFL |
| 548.152995 | C26H28O13 | 8 | --- | 4 | --- | --- |
| 548.16825 | C30H28O10 | --- | --- | --- | 1 | --- |

[a]See Table 2 for expansion of abbreviations of flavonoid-subclasses.

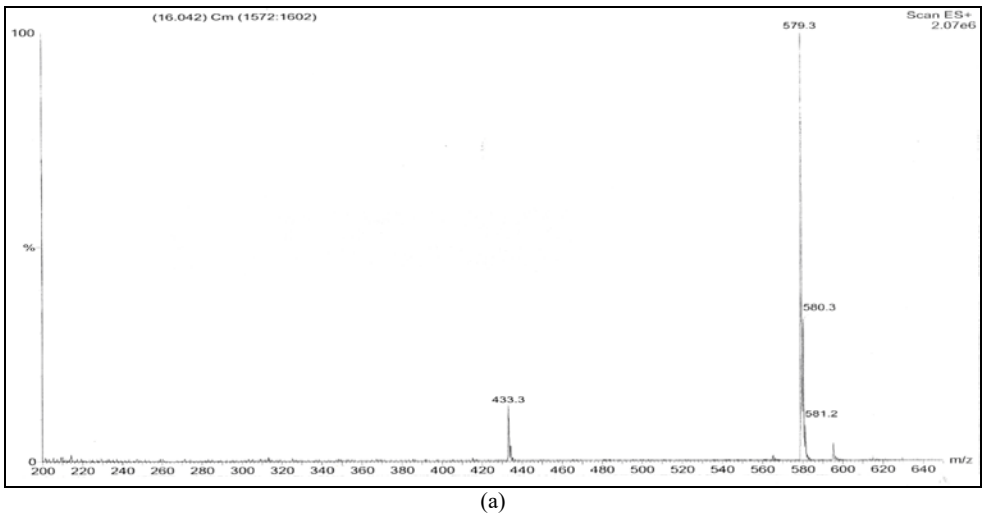



(a)

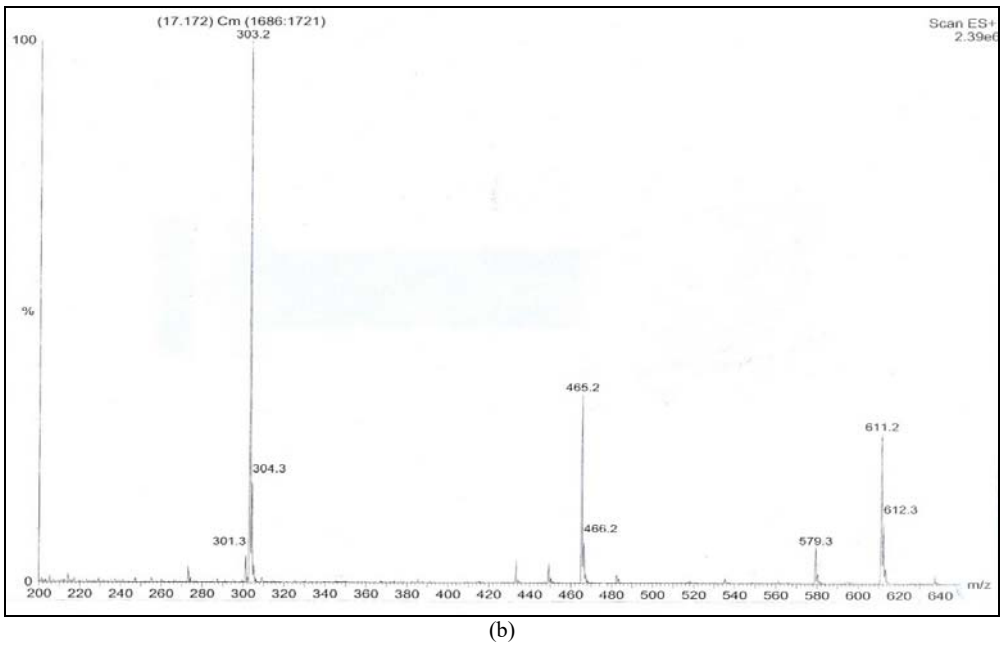



(b)

**Fig 8:** LC-UV-MS chromatograms of (a) Water extract and (b) Methanol-Water extract of *Cordyline terminalis* (*C. terminalis*)




(a)




(b)

**Fig 9:** Mass spectra corresponding to (a) $t_R$: 16.04 min. and (b) $t_R$: 17.17 min. processed from LC-MS chromatogram (see Figure 8a) of Water extract of *C. terminalis*.

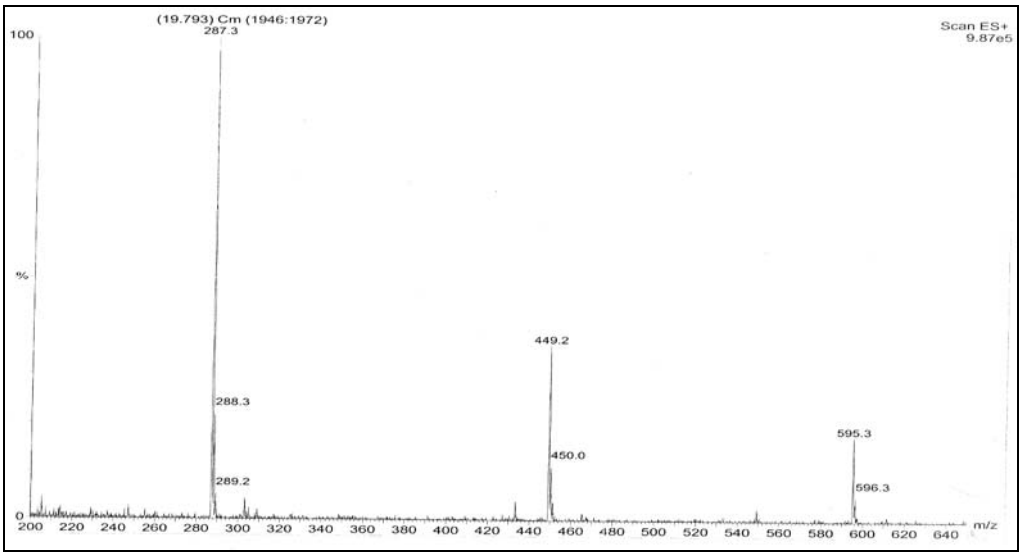
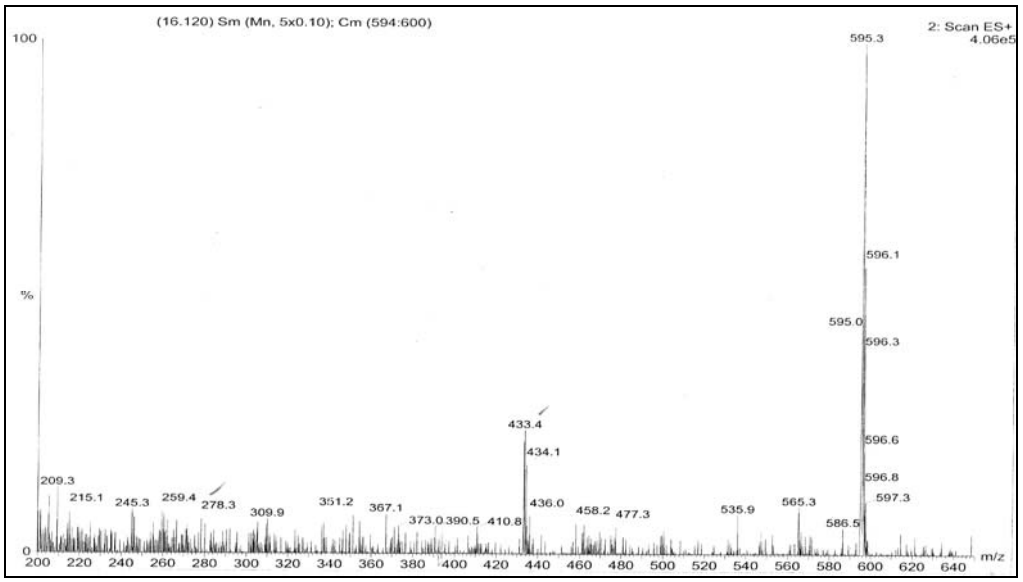
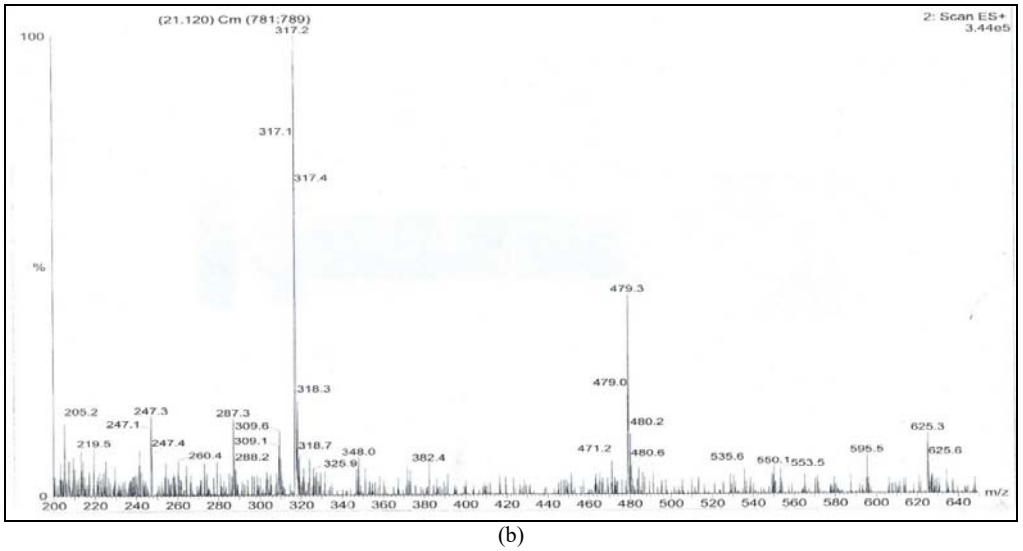



**Fig 10:** Mass spectrum corresponding to $t_R$: 19.79 min. processed from LC-MS chromatogram (Figure 8a) of Water extract of *C. terminalis*.




(a)




(b)

**Fig 11:** Mass spectra corresponding to (a) $t_R$: 16.12 min. and (b) $t_R$: 21.12 min. processed from LC-MS chromatogram (see Figure 8b) of Methanol-Water extract of *C. terminalis*.

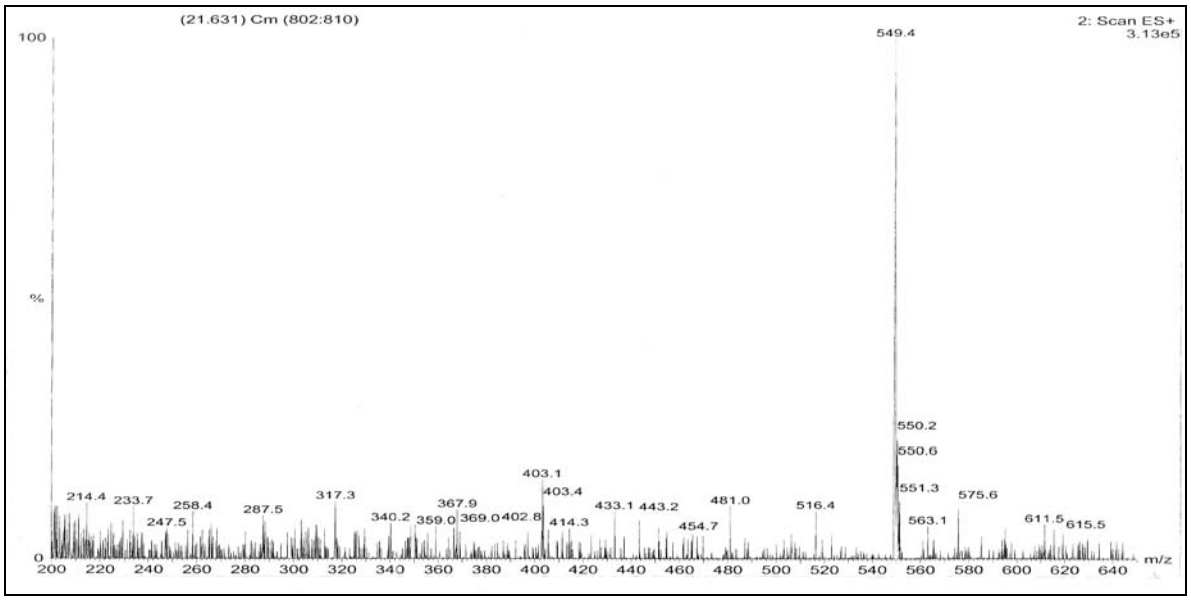



**Fig 12:** Mass spectrum corresponding to $t_R$: 21.63 min. processed from LC-MS chromatogram (Figure 8b) of Methanol-Water extract of *C. terminalis*.

***Comparative Analyses in both the extracts:*** It can be noticed in Table 3 that same flavonoids in both the plant extracts have been detected from their respective water extracts (*m/z* 309.3, 309.4, 417.3, 417.4; bold-faced in Table 2). *m/z* 309.3 (or 309.4) can be interpreted as $[M+H]^+$ of C18 H12 O5, which corresponds to 1 flavanone and/or 1 pterocarpan (bold-faced in Tables 4 & 5). Either an anthocyanidin C21 H21 O9 or $[M+H]^+$ of C24 H16 O7 can contribute for the signal at *m/z* 417.3 (or 417.4) (bold-faced in Tables 4 & 5). Rest of the other observed signals (Table 3) are unique to each of the plant extract. Interestingly, in the mass spectral data of the extracts of *M. fragrans*, there were no peaks at *m/z* 287.3 and 303.2 (see Figures 2-7), suggesting that the extracts of *M. fragrans* lack those compounds that contribute for these two signals. In other words, kaempferol and quercetin are absent in the extracts of *M. fragrans*, but present in *C. terminalis*.

***Influence of solvent extraction:*** Higher abundance of 'flavones & flavonols' and 'isoflavonoids' were observed in the water extract of *M. fragrans* than in water extract of *C. terminalis*. In case of *C. terminalis*, more 'flavones & flavonols' are noticed in both water as well as methanol-water (70:30; v/v) extracts. Altogether, extraction by water gives the highest yield of total flavonoids from the seeds of *M. fragrans*, indicating the presence of large content of polar compounds; whereas in *C. terminalis*, most flavonoids are common to both water and methanol-water extracts, suggesting that the leaves of *C. terminalis* might contain both polar and moderately polar/non-polar flavonoids. With reference to extraction by methanol-water only, *C. terminalis* extract possess higher content of total flavonoids than that of *M. fragrans*. The results from both the solvent extracts of each plant are summed-up and shown in Figure 13, which illustrates the comparison of population distribution of compounds in different flavonoid-subclasses between *M. fragrans* and *C. terminalis*. It appears that *C. terminalis* has a higher content of 'flavones & flavonols' than *M. fragrans*, which corroborates with the data obtained by $AlCl_3$ method [3]. *M. fragrans* could possess relatively high content of 'isoflavonoids' and 'chalcones and dihydrochalcones'. The subclass other flavonoids is relatively predominant in *M. fragrans*. Intriguingly, in *M. fragrans*, it seems that there are also some prenol lipids (sesquiterpenes), hydroxylated fatty acids, 'anthracenes and phenanthrenes', which are absent in *C. terminalis*.

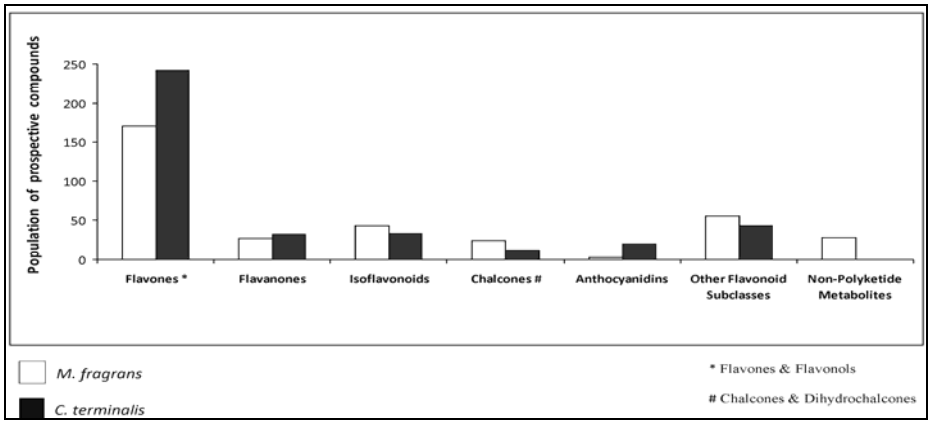



**Fig 13:** Total population distribution of potential polyphenols in *M. fragrans* and *C. terminalis*; screened using LC-ESI-MS data and LIPID MAPS' database

## Conclusion

This is the first report on the elucidation of polyphenolic composition, particularly of flavonoid-subclasses from *C. terminalis* and *M. fragrans*. Also, to the best of our knowledge, this is the first investigation demonstrating the use of LIPID MAPS' database for interpreting LC-MS data to identify subclasses of flavonoids in plant extracts. The advantage of using LIPID MAPS database permitted in knowing some new flavonoid subclasses, for example aurone flavonoids, rotenoid flavonoids, pterocarpanas, chalcones, etc. Though the mass spectral data acquired herein, are not sufficient to identify compounds distinctly within a subclass, they were adequately useful to distinguish different flavonoid-subclasses. The results of this LC-MS based profiling study prompt designing of interesting formulations, wherein different subclasses of flavonoids from these plants can be blended in various proportions, for preparation of dietary supplements and nutraceuticals. Towards this, it will be interesting to determine antioxidant activity of each of the subclass alone and examine contribution of every subclass to the total antioxidant activity. Moreover, very recently, a study has shown that polyphenols inhibit cyclic diadenylatecyclase (DisA), which emphasized the importance of polyphenols in potentiating the effects of cell wall-targeting antibiotics [28]. Hence, it seems that the role of polyphenols is gradually expanding, in addition to their usual beneficial applications in the context of nutrition.

## Acknowledgements

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