**Determination of Bioactive components from the Ethanolic Peel extract of *Citrus reticulata* by Gas chromatography – Mass Spectrometry**

Rane Zab Anish Kumar P, Anusha Bhaskar*
Department of Biochemistry, PRIST Univeristy, Vallam, Thanjavur 614 403.

**Abstract**
The present study was carried out for identification of the bioactive components present in the *Citrus reticulata* (Mandarin orange) is one of the medicinally important plants belonging to the Rutaceae family. In the present study the ethanolic peel extract of *Citrus reticulata* has been subjected to GC-MS analysis. This analysis revealed that Ethanolic peel extract of *Citrus reticulata* contains Maltol, 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one, Glycerol, 5-Hydroxymethyl furural, Nitroisobutylglycerol, heptamethoxyflavone etc., justifying the use of this plant to treat many ailments in folk and herbal medicine.

*Corresponding author, Mailing address: Anusha Bhaskar Email: dranushaparthiban@gmail.com

**Key words:**
*Citrus reticulata*, GC-MS Technique, Ethanol extract, phytochemicals, Herbal medicine.

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**reticulata** one of the commercially important species, is grown and traditionally used by different ethnic groups and local people in North East India [3].

In traditional Chinese medicine, the dried peel of the fruit is used to treat abdominal distension, to enhance digestion, and to reduce phlegm, and its various parts are used to cure cutaneous complaints, hemiplegia, snake bite, fever, loss of taste, chronic rheumatism, stomach ache, menorrhagia, splenomegaly, edema and cardiac diseases, bronchitis and asthma.[4-5]. In the traditional Chinese medicine the dried mature fruit peels of *Citrus reticulata* and their varieties, have been widely used for centuries as remedies to treat indigestion and to improve inflammatory syndromes of the respiratory tract [6]. Three types of flavonoids occur in *Citrus* species are flavanones, flavones and flavonols. Amongst them, polymethoxyflavones (PMFs) show chemopreventive potential in antimutagenic and antitumor properties [7]. *Citrus* peels contain more bioactive compounds, such as phenolic acids, flavonoids, limonoids, and fibre [8].

Experimental studies have demonstrated its analgesic, antibacterial, antimicrobial, antiviral, antifungal, antiinflammatory, uricosuric activity, antimutagenic, antispasmodic, antiatherogenic, antiperoxidative activity, anticarcinogenic activity, and radical scavenging activity [9-10].

Since there is no report on the phytoconstituents of Ethanolic peel extract of *Citrus reticulata* was chosen as the subject of this study. The aim of this paper is to determine the organic compounds present in the active fraction of *Citrus reticulata* peel extract with the aid of GC-MS Technique, which may provide an insight in its use in folklore medicine.

**MATERIALS AND METHODS**

**Preparation of plant extract**

Peels of *Citrus reticulata* was collected in Tiruchirapalli market. The ethanolic extracts of *Citrus reticulata* peel was prepared according to the method of Hossain et al. [11].

**Column chromatography**

Ten grams of the crude extract was subjected to column chromatography over silica gel (100-200 mesh) and eluted with n-hexane, chloroform, ethanol and methanol respectively. n-Hexane and Chloroform did not elute much of the compounds. The ethanol fraction of the *Citrus reticulata* peel was taken for GC-MS analysis.

**Gas Chromatography- Mass Spectrum Analysis (GC-MS)**

GC-MS technique was used in this study to identify the phytocomponents present in the extract. GC-MS analysis of this extract was performed using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column (Length : 30.0 m, Diameter : 0.25 mm, Film thickness : 0.25 µm Composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.5ml/min and an injection volume of 1µl was employed (split ratio: 10). Injector temperature 240°C; Ion-source temperature 200°C. The oven temperature was programmed from 70°C (isothermal for 3 min.), with an increase of 300°C for 10 min. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds with scan range of 40 – 1000 m/z. Total GC running time was 35 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a GC MS solution ver .2.53.

**Identification of components**

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST08s), WILEY8 and FAME having more patterns. The spectrum of the
unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library. The Name, Molecular weight, Molecular formula and Structure of the component of the test material was ascertained.

Table 1: Phytocomponents identified in the ethanolic extracts of Citrus reticulate peel by GC-MS.

<table>
<thead>
<tr>
<th>R.time</th>
<th>Name of the compound</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Peak area %</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.183</td>
<td>maltol</td>
<td>C₆H₁₀O₃</td>
<td>126</td>
<td>2.04</td>
<td>Anticonvulsant, Antifatigue, Antioxidant, Antitumour activity Cancer-Preventive.</td>
</tr>
<tr>
<td>6.182</td>
<td>3,5-dihydroxy-6-methyl-2,3-dihydro-4h-pyran-4-one</td>
<td>C₆H₈O₄</td>
<td>144</td>
<td>6.03</td>
<td>Antimicrobial, Anti-inflammatory Antiproliferative.</td>
</tr>
<tr>
<td>6.332</td>
<td>glycerol</td>
<td>C₃H₆O₂</td>
<td>134</td>
<td>1.73</td>
<td>Anticataract, Antineuralgic, Arrhythmigeni, Hyperglycemic.</td>
</tr>
<tr>
<td>7.509</td>
<td>5-hydroxymethylfurfural</td>
<td>C₆H₁₀O₃</td>
<td>126</td>
<td>1.76</td>
<td>Antimicrobial, Antibacterial.</td>
</tr>
<tr>
<td>8.619</td>
<td>2-methoxy-4vinylphenol</td>
<td>C₆H₁₀O₂</td>
<td>150</td>
<td>2.19</td>
<td>Antimicrobial, Antibacterial. Antiviral.</td>
</tr>
<tr>
<td>9.795</td>
<td>3-[n’-(3h-indol-3-ylmethylene)-hydrazino]-5-methyl-[1,2,4]triazol-4-ylamine</td>
<td>C₁₂H₁₃N₇</td>
<td>255</td>
<td>2.41</td>
<td>Antimicrobial, Antibacterial. Antiviral.</td>
</tr>
<tr>
<td>10.677</td>
<td>nitroisobutylglycerol</td>
<td>C₄H₁₀NO₃</td>
<td>151</td>
<td>12.76</td>
<td>Oxytocin-induced activity, Antioxidant, Antistaphylococcal Activity.</td>
</tr>
<tr>
<td>12.829</td>
<td>1,6-anhydro-3beta-d-glucopyranose</td>
<td>C₆H₁₀O₅</td>
<td>162</td>
<td>42.89</td>
<td>Anti-inflammatory, Thrombolytic activity</td>
</tr>
<tr>
<td>27.047</td>
<td>3,3',4',5,5',7,8-heptamethoxyflavone</td>
<td>C₁₃H₁₄O₈</td>
<td>432</td>
<td>7.50</td>
<td>Cancer chemopreventive activity, Anti-inflammatory</td>
</tr>
<tr>
<td>27.275</td>
<td>butylphosphonic acid, pentyl 4-(2-phenylprop-2-yl)phenyl ester</td>
<td>C₂₃H₃₆O₃P</td>
<td>402</td>
<td>9.37</td>
<td>Antioxidant, Antitumour</td>
</tr>
<tr>
<td>27.356</td>
<td>4h-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy-</td>
<td>C₂₅H₂₀O₇</td>
<td>372</td>
<td>4.83</td>
<td>Antimalarial, Antitumour, Antioxidant Antihyperglycemic</td>
</tr>
</tbody>
</table>

Figure 1. GC-MS Chromatogram of ethanolic extract of Citrus reticulata peel
Figure 2: Mass spectra of maltol

Figure 3: Mass spectra of 3,5-dihydroxy-6-methyl-2,3-dihydro-4h-pyran-4-one

Figure 4: Mass spectra of glycerol
Figure 5: Mass spectra of 5-hydroxymethylfurfural

Figure 6: Mass spectra of 2-methoxy-4-vinylphenol

Figure 7: Mass spectra of 3-[n’-(3h-indol-3-ylmethylene)-hydrazino]-5-methyl-[1,2,4]triazol-4-ylamine
Figure: 8 Mass spectra of nitroisobutylglycerol

![Mass spectra of nitroisobutylglycerol](image)

Figure: 9 Mass spectra of 1,6-anhydro-beta-d-glucopyranose

![Mass spectra of 1,6-anhydro-beta-d-glucopyranose](image)

Figure: 10 Mass spectra of 3,3',4',5,5',7,8-heptamethoxyflavone

![Mass spectra of 3,3',4',5,5',7,8-heptamethoxyflavone](image)
RESULTS AND DISCUSSION

The results show that these compounds found in *Citrus reticulata* possess various therapeutically and medicinal values. Gas Chromatography – Mass Spectrometry is the convenient way to study the phytochemicals found in plant samples. In the current study we characterized the phytochemical nature of *Citrus reticulata* using GC-MS. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving
rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. This report is the first of its kind to analyze the chemical constituents of *Citrus reticulata* using GC-MS. The results of the present study were tabulated in Table 1.

The compound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases. The results revealed that the presence of Maltol (RT: 5.183), 3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one (RT: 6.182), Glycerol (RT: 6.332), 5-Hydroxyethylfurfural (RT: 7.509), 2-methoxy-4-vinylphenol (RT: 8.619), 3-[N-(3H-Indol-3-ylmethylene)-hydrazino]-5-methyl-[1,2,4]triazol-4-ylamine (RT: 9.795), Nitroisobutylglycerol (RT: 10.677), 1,6-anhydro-β-d-glucopyranose (RT: 12.829), and heptamethoxyflavone (RT: 27.047). The other components like butylphosphonic acid, pentyl 4-(2-phenylprop-2-yl)phenyl ester (RT: 27.275) and 4H-1-Benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-5,6,7-trimethoxy- (RT: 27.356). The spectrum profile of Gas Chromatography-mass spectrometry confirmed the presence of eleven major components with the peak area % 2.04, 6.03, 1.73, 1.76, 2.19, 2.41, 12.76, 42.89, 7.50, 9.37 and 4.83 respectively (Table 1). The individual fragmentation patterns of the components were illustrated in Figure 1-11. The mass spectrum of the compound with retention time 5.183 (2.04%) gave 12 major peaks (m/z) at 42, 43, 44, 45, 55, 56, 70, 83, 97, 102, 126 and 127 (Figure 2). The mass spectrum of the compound with retention time 6.182 (6.03%) gave 12 major peaks (m/z) at 42, 43, 44, 45, 55, 56, 58, 72, 73, 101, 115, 144 and 145 (Figure 3). The mass spectrum of the compound with retention time 6.332 (1.73%) gave 9 major peaks (m/z) at 42, 43, 44, 45, 60, 61, 62, 72 and 84 (Figure 4). The mass spectrum of the compound with retention time 7.509 (1.76%) gave 10 major peaks (m/z) at 41, 50, 51, 53, 69, 81, 90, 109, 125 and 126 (Figure 5). The mass spectrum of the compound with retention time 8.619 (2.19%) gave 18 major peaks (m/z) at 50, 51, 52, 53, 55, 62, 63, 65, 67, 78, 79, 89, 91, 107, 135, 136, 142, 143, 144 and 154 (Figure 6). The mass spectrum of the compound with retention time 9.795 (2.41%) gave 25 major peaks (m/z) at 43, 44, 45, 47, 59, 61, 65, 75, 77, 93, 99, 101, 103, 116, 117, 121, 122, 127, 129, 130, 131, 142, 143, 144 and 154 (Figure 7). The mass spectrum of the compound with retention time 10.677 (12.76%) gave 21 major peaks (m/z) at 41, 42, 43, 44, 45, 47, 49, 55, 56, 57, 58, 60, 68, 69, 71, 73, 74, 77, 85, 86 and 87 (Figure 8). The mass spectrum of the compound with retention time 12.829 (42.89%) gave 33 major peaks (m/z) at 41, 42, 43, 44, 45, 47, 55, 56, 57, 60, 61, 69, 70, 71, 73, 74, 75, 76, 84, 85, 86, 87, 88, 86, 97, 100, 102, 110, 112, 118, 131, 144 and 159 (Figure 9). The mass spectrum of the compound with retention time 27.047 (7.50%) gave 25 major peaks (m/z) at 41, 53, 69, 83, 92, 109, 137, 165, 173, 187, 216, 241, 257, 273, 303, 329, 343, 359, 373, 387, 401, 417, 418, 432 and 434 (Figure 10). The mass spectrum of the compound with retention time 27.275 (9.37%) gave 25 major peaks (m/z) at 40, 65, 83, 109, 139, 153, 172, 182, 197, 210, 225, 239, 255, 273, 298, 313, 326, 344, 359, 387, 388, 402, 403, 415 and 429 (Figure 11). The mass spectrum of the compound with retention time 27.356 (4.83%) gave 22 major peaks (m/z) at 41, 53, 69, 91, 104, 119, 139, 167, 178, 195, 210, 237, 257, 271, 296, 313, 326, 341, 357, 358, 372 and 417 (Figure 12). In addition to this, the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant. The result of the present study supported and supplemented the previous observations. GC-MS analysis showed the existence of various compounds with different chemical structures. The presence of various bioactive compounds confirms the application of *Citrus reticulata* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.
CONCLUSION
The present study has been found useful, where a variety of active compounds have been found in ethanolic extract of *Citrus reticulata* instead going for essential oils. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners. It could be concluded that *Citrus reticulata* ethanolic peel extract is of phytopharmaceutical importance.

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