GC-MS Analysis of Papaya Leaf Extract (Carica Papaya L.)

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Abstract

The current study aimed to determine the phytochemicals present in the leaf extract of two papaya varieties grown in southern Iraq. The phytochemicals present in the ethanolic extract of papaya leaves were identified using the GC-MS detection system. The results showed the presence of more than thirty phytochemicals in the ethanolic extract of papaya leaves. The main phytochemicals present in papaya leaf extract in terms of their relative abundance are Oleic Acid, Tocopherol, Sitosterol, Neophytadiene, Butyl 9,12,15-octadecatrienoate, n-Hexadecanoic acid, Phytol, Tetramethyl-2-hexadecen, Dasycarpidan-1-methanol, acetate (ester), Campesterol, Squalene, Octadecenoic acid, Stigmasterol and D-Limonene. The present study revealed that the papaya leaf extract was composed of a variety of metabolites and therapeutic active substances, in addition to novel substances. These substances can be isolated and evaluated experimentally to confirm their biological and medicinal activities as well as verify their mechanism of action.

Keywords: Papaya, Phytochemicals, GC-MS analysis, Plant extract, Red lady.

1. Introduction

Papaya (Carica papaya L.) belongs to family Caricaceae. It is one of the fastest growing tropical or subtropical fruit plants. The original home of this plant is the tropics of America, possibly southern Mexico, Costa Rica or Central America, and spread around the sixteenth century to the tropics, Papaya is an evergreen dicotyledonous plant [1]. Papaya is rich in nutrients and antioxidants and has a high medicinal value, it is the source of many powerful and effective medicines [2]. All parts of the plant, leaves, fruits and seeds have been used traditionally to treat many different diseases including malaria, blood pressure, dengue fever, jaundice, sinus and eczema, anti-inflammatory, indigestion, anti-hypertensive activities, and tumors. These medicinal and nutritional properties are due to the presence of many phytochemicals such as vitamins, glycosides, alkaloids, steroids, flavonoids and phenols [1,3]. Papaya leaves have many uses, juice of the leaves helps to increase white blood cells and platelets. It is also used as a treatment for diseases of Urogenital diseases [4]. Dried leaves are best as a tonic and blood purifier [5]. Papaya leaf extract in an unrevealed composition is shown to possess anticancer activity and inhibition of cell proliferation in a variety of cancer cell lines, which has been patented [6]. The analgesic activity of Carica papaya leaves (CPL) extract was investigated in mice model using acetic acid induced pain (Siegmund method), Ethanol extract showed the best analgetic activity that was comparable to aspirin [7]. The therapeutic efficacy and nutritional properties of this plant depend on the contents and number of these bioactive compounds, which vary according to the environment. Due to the lack studies in Iraq, the current study aimed to cultivate this plant in southern Iraq and determine bioactive compounds of the papaya leaf extract which will help to explore potential use of this plant in food and pharmaceutical industries.

2. Materials and Methods

2.1. Plant cultivation and Collection of plant specimen

Planting two varieties of papaya (Local, Red lady) in the province of Basra, southern Iraq, During the growing season 2020 as an experiment to cultivate this plant in southern Iraq successfully. The leaves of the cultivated papaya plants of both varieties were collected after five months of planting. After that, the leaves were washed under a continuous stream of tap water for 5 minutes and then dried aerobically, The dried samples were ground into a powder using a grinding machine and then sieved. Samples were stored at 4°C in airtight glass containers until extraction.
2.2. Preparation of the Extracts

Dry papaya leaves 25 grams used and it dissolved in 250 ml of ethanol and left for 24 hours. The suspension was filtered with filter paper through a Whatman No. 41, The filtrate was concentrated in a rotary evaporator at 45°C under reduced pressure.

2.3. GC-MS system

GC-MS analysis was carried out at the Basra oil company laboratory, by using an Agilent Technologies, 7890B GC system coupled to an Agilent Technologies 5977A MSD with EI Signal detector, using HP-5ms 5% phenyl, 95% methyl siloxane (30m*250um*0.25) , the oven temperature was set at 40 C hold for 5 min then raised to 10 C/min to 300 C for 20 min, Helium carrier gas flow rate was 1ml /min and purge flow of 3 ml/min . The injection mode was pulsed Splitless with injection temperature 290 C and the injection sample volume was 1 micro letter. The mass spectrometer used Ion Source Temperature 230 C , With scan speed 1562 (N2) , and the mass range 44-750 m/z . Data was run through the NIST 2014 ,and Wiley 9 Library data base as an additional tool to confirm identity of compounds.

3. Results and Discussion

The phytochemicals present in papaya leaf extract with their corresponding retention time, molecular formula and molecular weight as well as their relative abundance, which was expressed in terms of peak area % are presented in tables 1,2 and depicted in figures 1,2.

Table 1 and figure 1 showed the presence of forty plant components in the ethanolic papaya leaf extract. Through comparative examination, the main components present in the papaya leaf extract of the local variety in terms of their relative abundance were Oleic acid, Tocopherol, Sitosterol, n-Hexadecanoic acid, Dasyacarpidan-1-methanol, acetate (ester ), Campesterol, Neophytadiene, Squalene, Octadecenoic acid, Stigmasterol, Linolenic acid, Phytol and D-Limonene (22.5 , 12.4 , 12.2 , 5.2 , 3.8 , 3.4 , 3.2 , 3.0 , 2.9 , 2.6 , 2.6 , 2.1 , 0.5 )% relative abundance respectively.

Table 2 and figure 2 showed the presence of at least thirty phytochemicals in the papaya leaf extract of the hybrid variety, the main components found in the papaya leaf extract of the hybrid variety in terms of their relative abundance were Octadecatrienoic acid, Tocopherol, Neophytadiene, Sitosterol, Butyl 9,12,15-octadecatrienoate, Phytol, Tetramethyl-2-hexadecen, n-Hexadecanoic acid, Campesterol, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, Octadecenoic acid, Stigmasterol, Octadecadienoate, Squalene, Hexadecene, 6-Hydroxy-4,4,7 a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one which corresponded to (18.6 , 11.5 , 9.6 , 9.6 , 5.6 , 4.8 , 4.2 , 4.2 , 2.8 , 2.5 , 1.7 , 1.6 , 1.4 , 1.2 , 1.0 , 0.53 ) % relative abundance respectively. Findings from previous studies on the papaya leaves contain active compounds like carpaine, sitosterol, choline, carposide, vitamin C and E which have health benefits. leaf of the papaya possess carotenoids, vitamins, anthraquinones, glycosides and hence possess medicinal properties like anti-inflammatory, antioxidant, hepatoprotective, wound healing, recently its antihypertensive and antitumor activities [3, 8].

The results of the current study revealed the presence of many effective and important compounds in papaya leaf extract, these compounds differed in terms of their presence and availability according to the variety. The papaya leaf extract of the local variety was distinguished by its high content of Tocopherol, Sitosterol and Dasyacarpidan-1-methanol, acetate (ester ). Whereas, the papaya leaf extract of the hybrid variety recorded the highest content of Neophytadiene, Butyl 9,12,15-octadecatrienoate and Phytol compared with the local variety. The current study showed that Tocopherol and hexadecanoic acid were major plant components present in papaya leaf extract. Which is known for its antioxidant, antimicrobial and anticancer activity [3,9]. That n-hexadecanoic acid and Phytol which were present in appreciable amounts in papaya leaf extract, were likely antimicrobial and antioxidant, hypcholesterolemic and antihypertensive agents [3]. Results of the study indicate the presence of Phytosterols in papaya leaves extract. Compounds identified were Sitosterol, Stigmasterol and Campesterol. It is an initial principles of hormones, these compounds increase the production of the hormone estrogen. Usually used for heart disease, high cholesterol, immune system modulation, cancer prevention, anti-inflammatory activities and induction of apoptosis in cancer cells [10, 11], found that several biologically active compounds, including Sitosterol, in the Moringa oleifera are responsible for the anti-cancer properties. The presence of high content of Sitosterol (12.2%) in papaya leaf extract is an encouraging result. The results of Table 2, showed that papaya leaf extracts contain neophytadiene (9.6%), which may be responsible for the antibacterial activity. Antimicrobial activity of plant essential oil containing neophytadiene [12]. Neophytadiene is also reported to possess antibacterial activity as well as helping in treatment of headaches, rheumatism and some skin disease [13]. It is noteworthy that some of the plant components present in papaya leaf extract are novel substances in that their therapeutic properties and biological activities have not been previously reported.
### Table 1. Phytochemicals identified in ethanolic leaf extract of C. papaya (Local variety) by GC-MS.

| No. | Name                                                                 | Formula          | RT   | Area%      |
|-----|----------------------------------------------------------------------|------------------|------|------------|
| 1   | 17-Octadecyenoic acid                                               | C18H32O2         | 4.248| 0.12303    |
| 2   | N-Ethyl-2-phenethylamine                                             | C10H15N          | 5.778| 0.08342    |
| 3   | I-Alanine, N-methoxy carbonyl-, heptyl ester                         | C12H23NO4        | 6.95 | 0.07762    |
| 4   | p-Xylene                                                             | C8H10            | 8.641| 0.20978    |
| 5   | 1-(3,3,3-Trifluoro-2-hydroxypropyl)piperidine                         | C8H14F3NO        | 9.915| 0.07818    |
| 6   | 2-Azido-2,4,4,6,6-pentamethylheptane                                 | C12H25N3         | 11.321| 0.07487   |
| 7   | D-Limonene                                                           | C10H16           | 11.819| 0.51814   |
| 8   | .beta.-D-Glucopyranose, 1-thio-,1-[N-hydroxy-5-(methylthio)pentanimidate] | C12H23NO6S2      | 12.09| 0.19512    |
| 9   | 9-Decenoic acid                                                      | C10H18O2         | 13.261| 0.15835   |
| 10  | Benzenepropanoic acid., .alpha.- (hydroxyiminio)-                    | C9H9NO3          | 13.752| 0.64493   |
| 11  | Melezitose                                                           | C18H32O16        | 14.469| 1.29557   |
| 12  | 4-Mercaptophenol                                                     | C6H6OS           | 15.311| 0.28951   |
| 13  | Cyclohexanone, 2-(2-butynyl)-                                       | C10H14O          | 16.402| 0.28996   |
| 14  | 2,4-Difluorobenzene, 1-benzoxyl-                                    | C13H10F2O        | 17.135| 0.41063   |
| 15  | 2,4-Di-tet-butylphenol                                              | C14H22O          | 18.958| 0.39649   |
| 16  | 1-Dodecanol, 3,7,11-trimethyl-                                      | C15H32O          | 19.829| 0.43682   |
| 17  | Tetradecanoic acid                                                  | C14H28O2         | 21.777| 0.89685   |
| 18  | Cyclopentadecane, 2-hydroxy-                                        | C15H28O2         | 22.077| 0.69591   |
| 19  | Neophytopadiene                                                      | C20H38           | 22.589| 3.21657   |
| 20  | Phytol, acetate                                                      | C22H42O2         | 23.029| 1.14473   |
| 21  | n-Hexadecanoic acid                                                 | C16H32O2         | 23.864| 5.24537   |
| 22  | Hexadecanoic acid, ethyl ester                                      | C18H36O2         | 24.12 | 1.26373   |
| 23  | 1-Heptatriacetanotol                                                 | C37H76O          | 24.332| 0.15036   |
| 24  | Phytol                                                                | C20H40O          | 25.299| 2.19468   |
| 25  | Oleic Acid                                                           | C18H34O2         | 25.635| 2.5856    |
| 26  | 1-Heptatriacetanotol                                                 | C37H76O          | 26.785| 0.30511   |
| 27  | 2H-Benzof[loxiren][2,3-E]benzofuran-8(9H)-one, 9-[[2-       | C19H32N2O3       | 28.293| 0.27626   |
| 28  | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester            | C19H38O4         | 28.681| 1.39327   |
| 29  | 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester | C21H38O4       | 30.08 | 1.13393   |
| 30  | Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Z,Z,Z)-      | C21H36O4         | 30.146| 2.65095   |
| 31  | Squalene                                                             | C30H50           | 31.024| 3.04685   |
| 32  | dl.-alpha.-Tocopherol                                                | C29H50O2         | 33.851| 12.4316   |
| 33  | Campesterol                                                          | C28H48O          | 35.073| 3.46363   |
| 34  | Stigmasterol                                                         | C29H48O         | 35.469| 2.6458    |
| 35  | .gamma.-Sitosterol                                                   | C29H50O         | 36.311| 12.2495   |
| 36  | Cholest-5-en-3-ol, 24-propyli dine, (3.beta.)-                       | C30H50O         | 36.501| 1.4471    |
| 37  | 13,27-Cyclourasan-3-one                                             | C30H48O         | 37.197| 2.67255   |
| 38  | 9,19-Cyclolanost-24-en-3-ol, acetate, (3.beta.)-                     | C32H52O2        | 37.438| 2.1492    |
| 39  | 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-              | C57H104O6       | 38.91 | 2.9504    |
| 40  | Dasyrcarpidan-1-methanol, acetate (ester)                            | C20H26N2O2      | 40.418| 3.85696   |
| 41  | 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-              | C57H104O6       | 41.165| 0.98926   |
Figure 1. The GC-MS analysis of C. papaya (Local variety).

Table 2. Phytochemicals identified in ethanolic leaf extract of C. papaya (Red lady) by GC-MS.

| No. | Name                                                                 | Formula            | RT       | Area%       |
|-----|----------------------------------------------------------------------|--------------------|----------|-------------|
| 1   | 1-(1,4-cyclohexadienyl)-2-methylaminopropane                          | C10H17N            | 5.808    | 0.057167    |
| 2   | Formamide, TMS derivative                                             | C4H11NOSi          | 6.979    | 0.071481    |
| 3   | p-Xylene                                                             | C8H10              | 8.663    | 0.350353    |
| 4   | D-Limonene                                                           | C10H16             | 11.841   | 0.190945    |
| 5   | Benzenepropanoic acid, alpha-(hydroxyimino)-                           | C9H9NO3            | 13.766   | 0.328324    |
| 6   | Melezitose                                                           | C18H32O16          | 14.477   | 0.107359    |
| 7   | Formamide, TMS derivative                                             | C4H11NOSi          | 15.26    | 0.736578    |
| 8   | Cyclohexanone, 2-(2-butynyl)-                                        | C10H14O            | 16.417   | 0.295882    |
| 9   | 1-Tetradecyl acetate                                                 | C16H32O2           | 17.325   | 0.209124    |
| 10  | Melezitose                                                           | C18H32O16          | 18.504   | 1.904339    |
| 11  | 2,4-Di-tert-butylphenol                                              | C14H22O            | 18.965   | 0.522125    |
| 12  | 1-Dodecanol, 3,7,11-trimethyl-                                        | C15H32O            | 19.836   | 0.305894    |
| 13  | Tetradecanoic acid                                                   | C14H28O2           | 21.784   | 0.462411    |
| 14  | Acetic acid, 3,7,11,15-tetramethyl-hexadecyl ester                    | C22H44O2           | 22.531   | 0.618028    |
| 15  | Neophytadiene                                                        | C20H38             | 22.611   | 10.10803    |
| 16  | 2-Hexadecene, 3,7,11,15-tetramethyl                                  | C20H40             | 22.663   | 11.05005    |
| 17  | Phytol, acetate                                                      | C22H40O2           | 22.846   | 1.231821    |
| 18  | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol                               | C20H40O3           | 23.043   | 4.420287    |
| 19  | n-Hexadecanoic acid                                                  | C16H32O2           | 23.878   | 4.41944     |
| 20  | Hexadecanoic acid, ethyl ester                                       | C18H36O2           | 24.127   | 0.739618    |
| 21  | Phytol                                                               | C20H40O            | 25.306   | 5.053318    |
| 22  | 9,12,15-Octadecatrienoic acid,                                       | C18H30O2           | 25.665   | 18.64854    |
| 23  | Ethyl 9.cis.,11.trans.-octadecadienoate                               | C20H36O2           | 25.738   | 1.468316    |
| 24  | 3-trns-(1,1-dimethylethyl)-4-trans-methoxycyclohexanol               | C1H22O2            | 28.301   | 0.836893    |
| 25  | Hexadecanoic acid, 2-hydroxy-1-ethyl ester                           | C19H38O4           | 28.696   | 2.683286    |
| 26  | 9,12-Octadecadienoic acid                                           | C21H38O4           | 30.087   | 1.639524    |
| 27  | Butyl 9,12,15-octadecatrienoate                                      | C22H38O2           | 30.168   | 5.870105    |
| 28  | Squalene                                                             | C30H50             | 31.024   | 1.296497    |
| 29  | gamma.-Tocopherol                                                    | C28H48O2           | 32.965   | 2.906746    |
| 30  | Octadecanoic acid, 4-hydroxy-, methyl ester                          | C19H38O3           | 33.17    | 0.814729    |
| 31  | dl.-alpha.-Tocopherol                                                | C29H50O2           | 33.829   | 11.81368    |
| 32  | Campesterol                                                          | C28H48O            | 35.081   | 2.924318    |
| 33  | Stigmasterol                                                         | C29H48O3           | 35.469   | 1.678376    |
| 34  | gamma.-Sitosterol                                                    | C29H50O            | 36.318   | 9.93223     |
| 35  | 9-Octadecenoic acid, (E)-                                           | C18H34O2           | 38.917   | 1.81382     |
| 36  | 9-Octadecenoic acid, 1,2,3-propanetriyl ester,                       | C57H104O6          | 40.411   | 1.46866     |
Figure 2. The GC-MS analysis of C. papaya (Red lady)

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