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Research Article

Identification of Flavonoid Glycosides of Methanol Extract from *Cucumis dipsaceus* Ehrenb. (Fruit) by using HPLC-UV-ESI-MS Methods

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ABSTRACT
The present work is to identify the Chemical Composition of methanolic extract from *Cucumis dipsaceus* Ehrenb. (Fruit) by using HPLC-UV-ESI-MS Methods. It is very proficient method with amalgamation of liquid chromatography attached to electrospray ionization mass spectrometry in tendem means with positive and negative ion recognition. This is a simple and rapid method for characterization of flavonoid glycosides by using Reversed Phase High Performance Liquid Chromatography coupled to Electrospray Ionization Quadropole Time – of – Flight Mass Spectrometry (RP-HPLC-ESI-Q-TOF-MS). The correctness of mass information generated by Q-TOF-MS jointly with the fragmentation blueprint of the complete scan sprint of MS/MS investigation has been a valuable tool to cautiously characterization of 12 flavonoid glycosides (Molecular weight: 497.04 (RT:3.586), 475.06 (RT:6.801), 393.48 (RT:12.594), 336.50 (RT:16.473), 723.00 (RT:17.774), 452.52(RT:18.253), 978.95 (RT: 25.665), 836.20 (RT: 26.889), 893.00 (RT: 27.925), 838.32 (RT: 31.592), 507.44 (RT: 31.592), 415.18, (RT:33.849) in the methanol extract of this fruit. All these flavonoid glycosides are the first time reported from fruit of *Cucumis dipsaceus* Ehrenb., and also highlighting the importance of this fruit as a rich source of likely flavonoid glycosides as antioxidants and hepatoprotective agent. It was concluded that these flavonoid glycosides were Myceritin -3-O- β -D-glucopyranoside (497), Apigenin 7 – O – 6'' – O acetyl – β – D - glucopyranoside (475), 5,6 Dihydroxy 7,8 – dimethoxyapigenin - 6 – O – sulfate (393), 5-p-coumaroylquinic acid (336 or 338), 6'' -O-(3-hydroxy-3- methylglutaroyl)-2'' - O - pentosyl-C - hexosyl-luteolin (723), Catechin-3-O-glucoside (452), 3',4',5',5,7 – Methyl derivative of quercetin 3 – O – (2" - feruloylglucosyl)(1->6) – [apiosyl1->2)] glucoside (978.859), Quercetin 3 – O – β – D -(6 – O – sinnapoyl – 2 – O - – O – β – D glucopyranosyl) glucopyranoside (833), Apigenin 7-*O*-(6"-dihydrogalloyl)-glucosyl-8-*C*-rhamnosyl-6-*C*-glucoside (893), precursor or isomer ion of Quercetin 3 – O – β – D -(6 – O – sinnapoyl – 2 – O - – O – β – D glucopyranosyl) glucopyranoside 838.32 , 3',4',7 – Methyl derivative of quercetin 3 – O – β – D - glucopyranoside (507) and 415 (Daidzein 7 – O – glucoside).

Keywords: *Cucumis dipsaceus* Ehrenb. (Fruit), Methanol extract, Chemical Composition and HPLC-UV-ESI-MS Methods.

INTRODUCTION

The chemical composition of methanol extract obtained from fruits and vegetables of genus *Cucumis*, *Cucurbitaceae* family containing flavonoid glycosides is a very strong antioxidant. The antioxidants are the excellent scavengers of free radicals which decreased the oxidative stress and lowered the risk of developing chronic diseases (Hepatitis, cardiovascular diseases and cancer etc) caused due to free radicals[1]. So, the present scenario of daily intake of fruit and vegetables has become the enlarged attention for getting relieve from these problems. The towering status of in vitro evidence of cellular processes like gene expression, apoptosis, platelet aggregation and intercellular signaling which can be in vitro hepatoprotective activity on HepG2 cell line by MTT assay, anti- atherogenic implications and anti-carcinogenic, hypolipidemic, antiaging and anti-inflammatory activities are first step for the achievement of effective herbal remedy for chronic diseases[2-7]. Polyphenols are divided into various classes depending upon the presence of number of hydroxyl containing rings and substituents that are attached to these rings. The main class of phenolic component is phenolic acids, flavonoids, stilbenes, hydrolysable and condensed tannins and lignans[8].

Cucumis dipsaceus (Ehrenb.) is a sprawling yearly herb with common name teasel / hedgehog guard (family: *Cucurbitaceae*). It is found in countries like Africa, Somalia, Ethiopia, Sudan Tanzania, Kenya, Uganda and Southern Egypt but now also available in Karnataka (Mysore) and Maruthamalai's forest and its foothills (Western Ghats), District Tamil Nadu (Coimbatore) India.

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Traditionally, a variety of parts of the herb are used as anti-emetic, gastrointestinal diseases, diarrhoea, hepatitis, stomach pain, constipation, gallstone, haemorrhoid and meningitis and rabies. The secondary metabolites reported from fruit extracts (*Cucumis dipsaceus* (Ehrenb.) are flavonoids, tannin, alkaloids, resins, saponin, steroids and pharmacological activities of extracts of fruit as analgesic, anti-inflammatory activity, cytotoxic activity, antioxidant activity and antimicrobial activity. It is reported to facilitate fruit of (*Cucumis dipsaceus* Ehrenb.) contains phenolic and flavonoids compounds in considerable quantity. It has strong antioxidant activities which were evaluated on chloroform, ethyl acetate, methanolic and water extracts. Other information which exhibits that flavonoids are accountable for healing of inflammation of liver injure or hepatotoxicity[9 – 11]. The HPLC-UV-ESI-MS methods were intended to identify about individual phenolic content (polyphenolic or flavonoid glycosides), its molecular weight and fragmentation pattern. This present efforts also exercised for the knowledge of how many types of flavonoid glycosides were present in methanol extract of fruit of (*Cucumis dipsaceus* Ehrenb.).

MATERIALS AND METHODS

Plant material matrix

Fruits from herb (*Cucumis dipsaceus* Ehrenb.) were collected in November- December 2014 from Mysore, (Karnataka, India). Dr. Sunita Garg (Head of Raw material, Herbarium & meuseum) at National institute of science communication and information resources (NISCAIR CSIR), New Delhi 110067 has identified these fruits and a voucher specimen no. (Reference No. NISCAIR/RHMD/Consult/2014/2367-147 was issued from the same herbarium.

Sample treatment

Powdered (1.0 Kg) was made after slicing the shade dried of fruits of (*Cucumis dipsaceus* Ehrenb.) and was methanol extract prepared after treatment with n-hexane using soxhlet apparatus by refluxing at temperature 35˚C. Afterward concentrate and dried the methanol extract through rotary vacuum distillation apparatus. The yield of the methanol extract obtained was 120gm and then recrystallized after dissolving in methanol solvent.

Chemicals and reagents[10,11].

HPLC-grade acetonitrile and methanol were used. Analytical grade acetic acid (assay >99.5%) was used. Water was used which prepared by Milli-Q system and other used reagents were also analytical grade.

Separation by HPLC

Phenolic compounds were separated from methanol extract of fruits of (*Cucumis dipsaceus* Ehrenb.) on an Agilent 1200 series Rapid Resolution which composed of a vacuum degasser, binary pump and auto-analyzer. Zorax C18 column (4.6x150mm, 1.8µm) of Agilent Technology was used. Mobile phase made of acetonitrile and acidified water (0.5% acetic acid, v/v) was used. Programmed gradient was set as 0 min, 0%B; 10 min, 20%B; 15 min, 30%B; 20 min, 50%B; 25 min, 75%B; 30 min, 100%B; 32 min, 0%B and finally, start on conditions was seized for 8 min as a re- stability pace. The flow rate of gradient program was set at 0.80mL/min. The T-type splitter was of mass spectrometry was used for the added effluent from HPLC column and 0.2 mL/min was arrived at ESI-Q-TOF-MS detector for this study. The volume of injection injected was 10 µL and 25℃ temperature was maintained for this study.

ESI-Q-TOF-MS analysis

The Quardrpole- time-of – Flight was coupled to HPLC system (Micro –TOP-QTM, Bruker, Germany) and it is Mass spectrometer with orthogonal accelerated Q-TOF and ESI (an electrospray ionization source). Analysis was done by parameter using positive ion mode through spectra found over a mass range from m/z 50 to 1100. The ESI-MS parameters values were optimized; drying gas temperature, 190℃; capillary voltage, +4.0 killo volt; drying gas flow, 9.0L/mm; collision RF, 150Vpp; nubilizing gas pressure, 29psi; Pre- pulse storage, 5µs and transfer time 70µs. Additionally, automated MS/MS experiments were done by varying the collision energy values as follows: m/z 100, 20 eV; m/z 500, 30eV; m/z 1000, 35 eV, and nitrogen gas was used as collision gas. The obtained MS data was processed and analyzed in 4.0 software (Bruker Daltonics, Germany). This software gives a list of potential basic formulas by using Generate Molecular Formula^{TM} editor. The CHNO algorithm was used by editor to to gives standard usefulness which was minimum/maximum elemental range, ring-plus double bond equivalents, electron configuration and sophisticated comparison of the theoretical values along with the measured isotope pattern by m sigma values for molecular formula. The chemical composition was differing only by 5 ppm, this becomes more confidential and also accepted with accuracy. The external instrument for calibration was interfaced with HPLC to develop method. A Cole Plamer Syringe pump was directly connected for passing the sodium formate mixture formed by 0.2% formic acid and 5Mm sodium hydroxide in 1:1water/isopropanol (v:v). The exact calibration curve was obtained which depends upon different groups of masses by differing 68 Da ($NaCHO_2$). By doing temperature drift in the Q-TOP, this external calibration gives a correct mass values for a total run lacking the necessity of a double sprayer system for inner mass calibration[12,13].

RESULTS AND DISCUSSION

Identification process

The summing up of all the characterized flavonoid glycosides in the methanol extract of fruit of (*Cucumis dipsaceus* Ehrenb.) by HPLC-ESI-Q-TOF-MS using the positive mode is the tabulated in table 1 and Percentage of peak area and retention time was shown in Figure 1. These flavonoid glycosides were showed beside among its retention time, m/z fragmentation pattern and also molecular weight which were generated by software for detection deprotonated flavonoid glycosides, classification array in the list of potential. In the present research work 12 flavonoid glycosides were cautiously recognized in this extract through using blend with MS and MS/MS statistics and the data previously reported in the literature.

Flavonoids glycosides

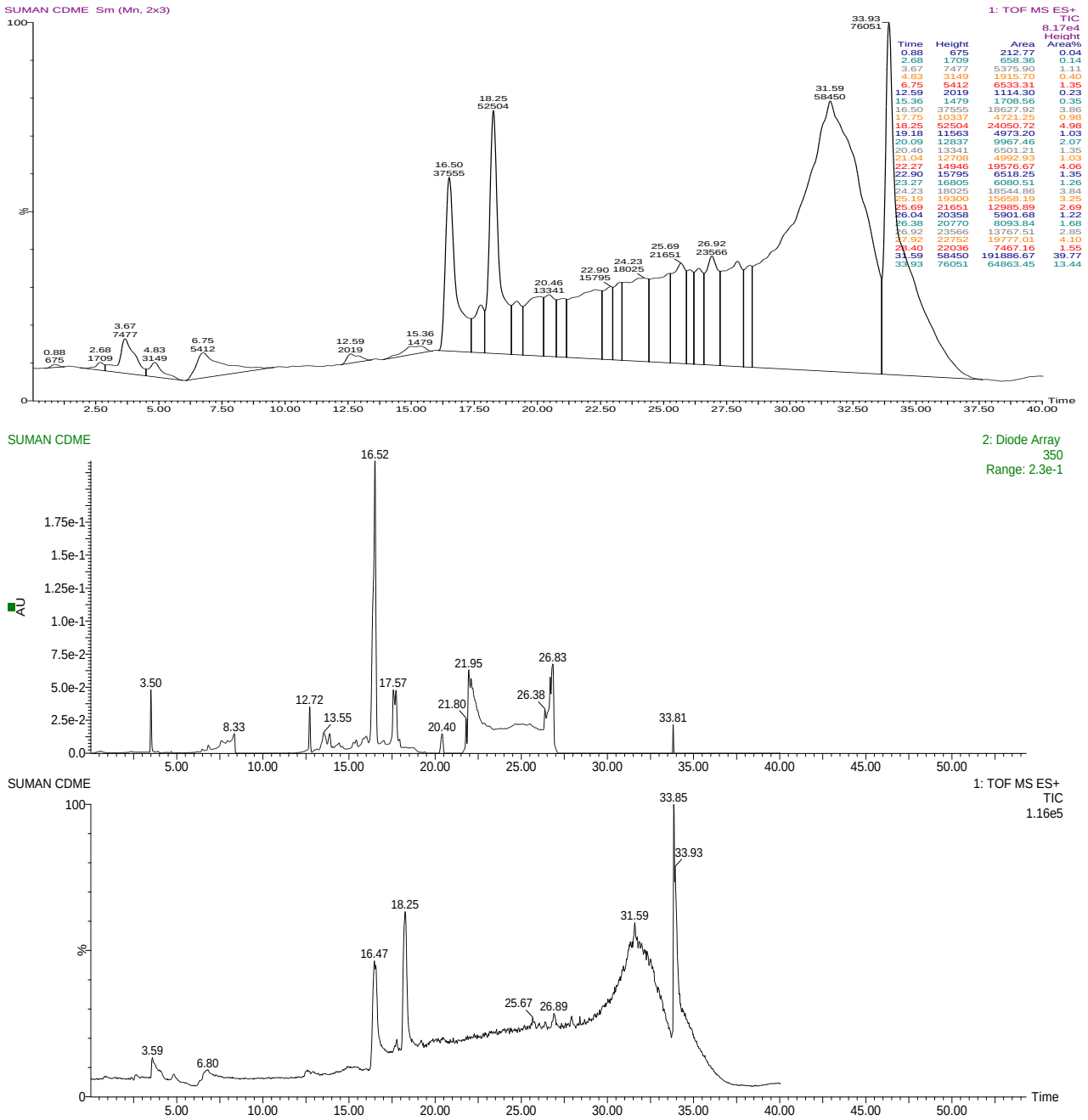


Figure 1: Graph of retention time of elution for different phenolic acids, flavonoids and glycosides by LCMS.

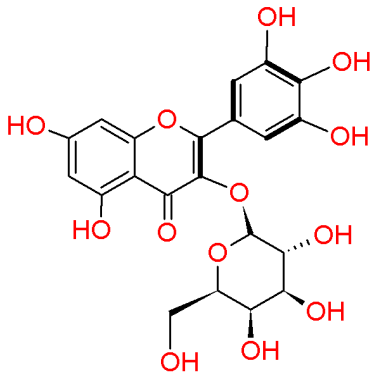
The flavonoid posses a basic structure made up of two aromatic benzene rings divided with an oxygenated heterocyclic ring. Flavonoid glycosides coming to under different types of flavonoid classes which are flavanones, flavones, flavonols and others related were recognized in this methanol extract of fruit of (*Cucumis dipsaceus* Ehrenb.). The methanol extract of fruit of (*Cucumis dipsaceus* Ehrenb.) contained 12 number flavonoid glycosides which are C-glycoside or O- glycoside flavonoids shown in table 1. The C-glycoside flavonoids were reported from many species of *Cucurbitaceae* family and these phytoconstituents gives special blueprint of fragmentation than O-glycoside flavonoids. A sequence of deprotonated ions were bring about as m/z [M-H-18(H_2O)], [M-H-60], [M-H-90], [M-H-120 (pentose sugar)], [M-H-180 (Hexose sugar)] and [M-H-210] due to C-hexosyl and C-pentosyl rings which were the fragmentation pattern of flavonoids (C-glycoside flavonoids)[14].

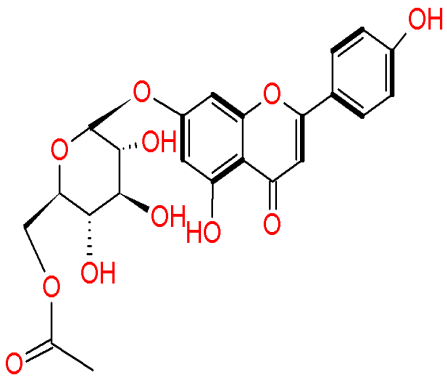
The peak 1 was 497.04 (RT: 3.586) Myricetin – 3 - O – β - D - glucopyranoside (M Wt = 497.04g/mol) and RT 3.58 has the precursor ion at m/z 497.0420 at RT: 3.586 (Figure 1) and its fragmentation pattern of the ESI – MS spectra was 131.38 (base peak), 180.3 (hexose sugar), 317 (Methyl ether of aglycone / flavone) and 497.0420 parent ion peak[14,15]. The peak 2 was 475.06 (RT: 6.801) Apigenin 7 – O – 6'' – O – β – D - glucopyranoside , (M Wt = 452.52g/mol) and its mass fragmentation pattern of ESI – MS spectra was Apigenin 7 – O – 6'' –O -acetyl – β – D – glucopyranoside and its fragmentation ions 117.36 (base

Table 1: RT and Fragmentation pattern of polyphenolic glycosides of CDME using LCMS.

S No.	RT of CDME by LCMS	MW	Fragmentation pattern of polyphenolic glycosides
1	3.586	497.04	71.33, 75.39, 99.32, 99.34, 99.35, 115.39, 131.38, 149.38, 149.42, 172.42, 175.42, 177.41, 179.39, 205.39, 219.36, 219.39, 220.37, 237.40, 265.36, 273.33, 289.30, 289.31, 315.28, 317.26, 317.29, 317.33, 339.24, 349.37, 363.37, 363.41, 364.39, 382.38, 383.21, 421.23, 421.23, 455.08, 481.13, 497.04
2	6.801	475.06	59.38, 59.39, 99.33, 101.38, 117.36, 135.37, 149.40, 159.37, 163.40, 177.38, 195.39, 203.40, 219.37, 219.42, 221. 41, 257.30, 265.38, 279.35, 279.36, 279.36, 279.41, 293.28, 317.27, 317.32, 361.32, 377.21, 382.36 382.41, 399.16, 437.20, 475.06.
3	12.594	393.48	100.48, 249.50, 250.51, 325.44, 349.45, 393.48.
4	16.473	336.50	228.49, 237.49, 246.51, 249.49, 303.37, 304.36, 336.50.
5	17.774	723.00	98.36, 158.42, 199.39, 222.35, 287.38, 317.31, 317.34, 317.37, 317.41, 318.37, 318.39, 339.26, 396.29, 437.25, 479.25, 501.19, 551.34, 585.31, 646.96,647.02, 647.04, 647.09, 647.15, 648.06, 648.09, 649.17, 701.36, 723.00
6	18.253	452.52	100.49, 141.49, 164.03, 172.53, 182.53, 205.04, 209.54, 210.53, 213.54, 227.53, 228.04, 244.59, 277.06, 277.56, 309.56, 326.56, 326.58, 342.51 343.59, 344.59, 359.48, 359.53, 359.99, 360.03, 360.03, 360.08, 360.54 452.49, 452.52
7	25.665	978.95	375.56, 415.50, 416.51, 423.58, 518.86, 631.37, 949, 978.95, 979.95, 981.05
8	26.889	836.20	102.41, 140.39, 181.42, 225.93, 282.38, 298.38, 339.30, 423.56, 441.57, 475.44, 475.47, 475.51, 475.55, 476.48, 476.54, 477.54, 631.39, 679.36, 701.30, 701.33,701.41, 701.45, 702.39, 702.52, 750.35, 751.35, 751.44, 751.51, 830.14, 833.13, 834.15, 835.16, 836.20,
9	27.925	893.00	191.44, 245.41, 261.37, 321.36,353.50, 407.60, 425.59, 426.60, 814.18, , 817.17, 818.18, 818.34, 819.17, 819.30, 893.00
10	31.592	838.32	69.40, 181.52, 251.45, 289.50, 319.55, 331.33, 333.32, 334.34, 393.49, 413.48, 414.48, 461.56, 483.51, 527.25, 595.37, 621.34, 639.38, 679.40, 680.41, 680.49, 723.24, 737.28, 738.24, 780.31, 806.31, 838.32
11	31.592	507.44	69.40, 181.52, 207.48, 223.46, 251.45, 270.59, 289.50, 319.55, 331.33 333.32, 334.34, 356.56, 391.47, 393.49, 394.49, 411.32, 413.48, 414.48, 445.50, 461.56, 483. 53, 507.44.
12	33.849	415.18	94.42, 99.36, 102.41, 111.42, 123.43, 140.40, 165.44, 181.42, 182.42 256.34, 257.32, 263.34, 284.34, 289.32, 299.33, 316.29, 339.29, 359.51, 360.02, 381.54, 415.18.

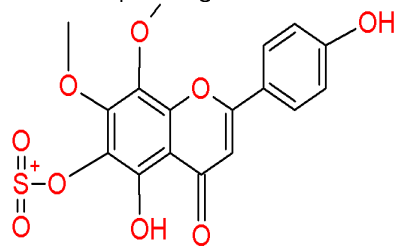
peak), 219.37, 317.27, 361.32, 382.36, 337.20 and 475.06 (parent peak)[16 -19]. Peak 3 was 393 (5, 6 – Dihydroxy 7, 8 – dimethoxyflavone – 6 – sulfate) and its mass pattern 100.48, 249.50, 325.44, 349.45 and 393.48 (M – 3H) 395[20]. Peak 4 336.50 was 5-p-coumaroylquinic acid (7) $C_{16}H_{18}O_8$) or 4-p-coumaroylquinic acid (M Wt = 338 and 336 (M-2H) and its fragmentations were 249.49, 303.37 (base peak and its M+1 ion 304.36) and 336.50 (parent peak)[21]. Peak 5 of 723.006 g/mol was 7- Methyl ether of myricetin – 3- O – 6'' – O - glucosyl - glucoside (M Wt = 723, / 722(M-H) and fragmentation m/z ions were 317.37 (base peak), 437.25, 479.25, 551.34, 647.09 (98%), 701.36 and 723.006 (parent ion peak)[22]. Peak 6 of M Wt = 452.52 g/mol was Catechin-3-O-glucoside and its m/z fragments pattern were 164.03, 182.53, 309.55 326.56, 347.59 (base peak), 360.54, 452.52 (Parent ion peak)[17]. Peak 7 of 836.20g/mol (RT = 26.889) of 3',4',5' Trimethyl ether quercertin – 3- glucosyl – rhamnosyl – glucoside and its m/z ions were 181.42, 339.30, 475.47, 477.53, 631.39, 679.36, 702.52, 750.35 (Base peak), 751.51, 803.14, 833.13 [830.7 M+2H] (834.15, 834.16, 835.16), 836.20(Parent ion peak)[23]. Peak 8 of M Wt = 978.95 g/mol (RT = 25.666) was Myricetin-3-O-(2"-O-galloyl) glucoside and its m/z fragments pattern were 375.56, 415 50, 416.51, 423.58, 518.86, 631.37, 949.00, 978.95(979.94 / 981.05)[24]. Peak 9 of Apigenin 7-O-(6"-dihydrogalloyl)-glucosyl-8-C-rhamnosyl-6-C-glucoside M Wt 893 and its m/z ions were 191.44, 245.41, 261.37, 321.36, 353.54.37, 407.60, 425.59, 817.17(base peak), 893(parent)[25]. Peak 10 of 838 was precursor or isomer ion of Quercetin 3 – O – β – D -(6 – O – sinnapoyl – 2 – O - – O – β – D glucopyranosyl) glucopyranoside and its m/z were 181.52, 251.45, 289.50, 319.55, 331.33 (base peak), 333.32, 334.34, 393.49, 413.48, 414.48, 461.56, 483.51, 527.25, 595.37, 621.34, 639.38, 679.40, 680.49, 723.24, 737.28, 738.24, 780.31, 806.31 and 838.32(parent ion peak)[23]. Peak 11 of 3',4',7 – Methyl derivative of quercetin 3 – O – β – D - glucopyranoside M Wt = 507 and its m/z ions were 181.52, 270.59, 289.50, 319.55, 331.33(base ion peak), 333.32, 334.34, 356.56, 391.47, 393.49, 394.49, 411.32, 413.49, 414.48, 445.50, 481.56, 483.51, 484.53, 507.45(parent ion peak)[14]. Peak 12 of Daidzein 7 – O –

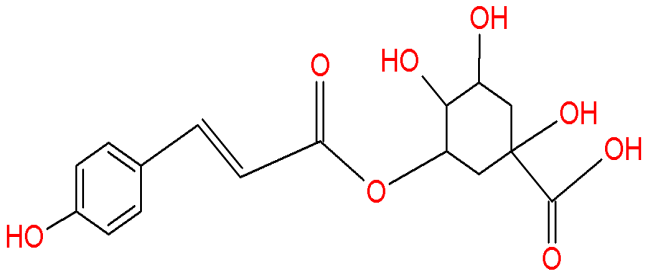




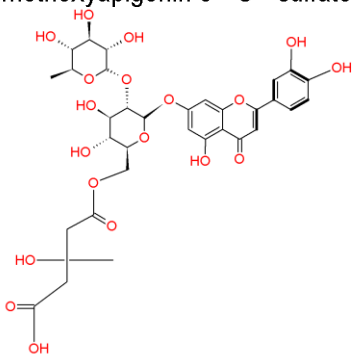
Apigenin - 7 - O - 6" - O - acetyl - β - D - glucopyranosid

Myricetin - 3 -β –D - glucoside M Wt 497

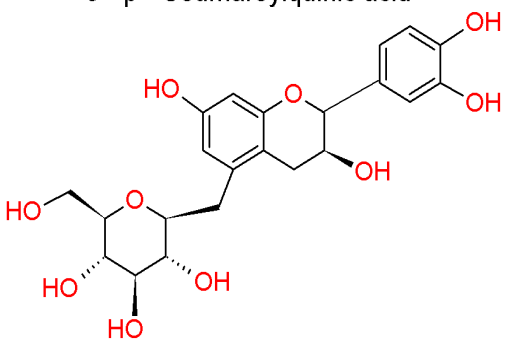




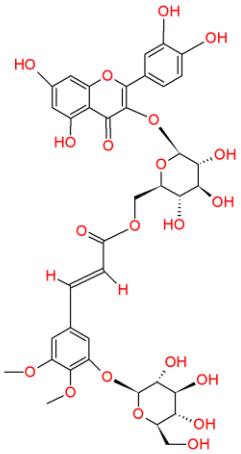
5,6 Dihydroxy 7,8 - dimethoxyapigenin 6 - O - sulfate



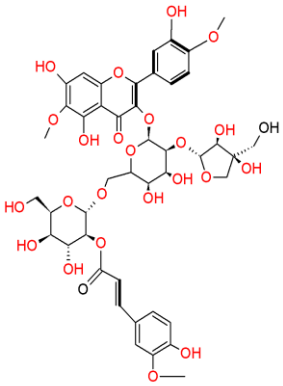
5 - p - Coumaroylquinic acid



6" - O - (3 - hydroxy -3 - methylglutaroyl) - 2" - O -hexosyl - C - hexosyl - luteolin

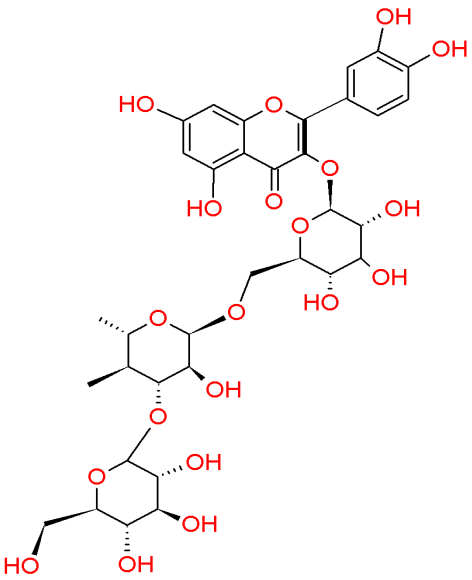


Catechin 3 - O - glucoside or p - Coumaric acid

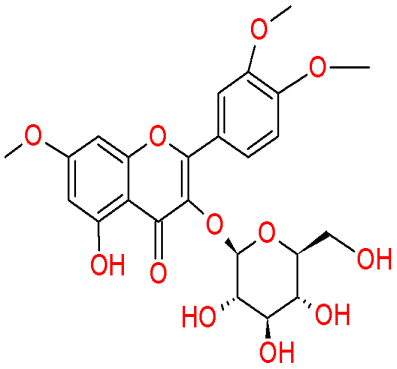


3',4',5',5,7 - Methylated derivative of quercetin 3 -O - (2" - feruloylglucosyl)(1 ->6) - [apiosyl(1 ->2)] glucoside

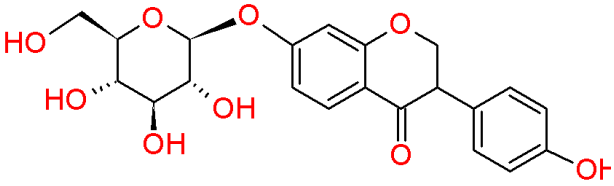
Quercetin 3 - O - β – D - (6 - O - sinnapoyl 2 - O β - D - glucopyranosyl) glucopyranoside



Quercetin 3 - O glucosyl - rhamnosyl - glucoside



3', 4',7 - trimethylether of quercetin - 3 - β - O - glucoside



Daizein - 7 - O - glucoside

glucoside M Wt = 415 and its m/z ions were 181.42, 298.32(base ion peak), 299.33, 316.33, 339.29, 359.51, 360.02, 381.54 and 415.18 (parent ion peak)[14,26].

CONCLUSION

In the present research work HPLC-ESI-Q-TOF-MS methods excellent identifier, a confirmed powerful analytical tool for the segregation and identification with molecular weight of 12 flavonoid glycosides of methanol extract of fruit of (*Cucumis dipsaceus* Ehrenb.). This separation and characterization were performed by MS data and MS/MS patter of fragmentation. To our knowledge this research work by HPLC-ESI-Q-TOF-MS methods was first time done. This report explained that methanol extract was rich source of flavonoid glycosides and these were very strong antioxidant for scavenging the free radicals causing chronic diseases as hepatoprotective activity, cardiac disorders, various types of cancers and other related diseases. So, these identified flavonoid glycosides can be the future herbal remedies for right treatment and also as scavenger too alone or in synergistic pattern.

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REFERENCES

1. Song W (1), Derito CM, Liu MK, He X, Dong M and Liu RH. Cellular antioxidant activity of common vegetables. Journal of Agricultural and Food Chemistry. 2010, 9; 58(11): 6621–6629.
2. Leopoldini M, Russo N, and Toscan M. The molecular basis of working mechanism of natural polyphenolic antioxidants. Food Chemistry, 2011; 125: 288–306.
3. Mates JM (1), Perez-Gomez C and Nunez de Castro I. Antioxidant enzymes and human diseases. Indian Journal of Clinical Biochemistry, 1999; 32(8): 595-603.
4. Aruoma OI. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Nutritional Research**,** 2003, 523-524: 9-20.
5. Rhee MH, Park HJ *and* Cho JY. *Salicornia herbaceae:* Botanical, Chemical and pharmacological review of halophyte marsh plant. Journal of Medicinal Plants Research. 2009; 3*(*8): 548-555.

6. Han LK, Sunmiyoshi M, Zhang J, Liu MX, Zhang XF, Zheng YN, Okuda H and Kimura Y. Anti-obesity effects of chikusetsusaponins isolated from Panax japonicus rhizomes. Phytotherapy Research, 2003; 17(10): 1188- 1194.
7. S Sultana, M Saleem. *Salix caprea* inhibits skin carcinogenesis in murine skin: inhibition of oxidative stress, ornithine decarboxylase activity and DNA synthesis. Journal of Ethanopharmacology, 2004; 91: 267-276.
8. Alam M S, Kaur G, Jabbar Z, Javed K and Athar M. Evaluation of antioxidant activity of *Salix caprea* flowers. Phytotherapy Research, 2006; 20 (6):479-483.
9. Lata S and Mittal SK. Pharmacognosy, Phytochemistry and pharmacology of *Cucumis dipsaceus* Ehrenb. International Journal Pharmacognosy and Phytochemical Research, 2015; 7(3):446 – 449.
10. Lata S and Mittal SK. In vitro and in vivo hepatoprotective activity of flavonoids rich extract on *Cucumis dipsaceus* Ehrenb. (Fruit). International Journal Pharmacology, 2017; 13: 563 – 572.
11. Lata S and Mittal SK. Identification of isolated flavonoid glycoside from methanolic extract of *Cucumis dipsaceus* Ehrenb. (Fruit). International Journal Pharmacognosy and Phytochemical Research, 2017; 9(7): 1051 – 1059.
12. Ibrahim M. Abu R, David AR, Rosa QRP, Salvador FA, Antonio SC and Alberto FG. HPLC – ESI – Q – TOF – MS for a comprehensive characterization of bioactive phenolic compounds in cucumber whole fruit extract. Food Research International, 2012; 46: 108 – 117.
13. Eman A. El – Wakil, El – Sayed S. Abdul H, Mortada M. El – Sayed and Ezzat E. Abdel – L. Identification of the chemical composition of the methanolic extract of Salix tertrasperma Roxb. Using LC – ESI – MS and evaluation its potential as antioxidant agent. 2015; 7: 168 – 177.
14. Wang Y, Yang L, He YQ, Wang CHW, Ed WW, Bligh SWA and Wang ZT. Characterization of fifty – one flavonoids in Chinese herbal prescription Long gen Xiegan decoction by high - performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry and photodiode array detection. Rapid Communication in Mass Spectrometry, 2008; 22: 1767 – 1778.
15. Sun J, Liang F, Bin Y , Li P and Duan C. Screening non – colored phenolic in red wines using liquid chromatography / ultraviolet and mass spectrometry / mass spectrometry libraries.Molecules, 2007; 30: 679 – 693.
16. Gabrieila M C. Mass spectrometry in the structural elucidation of natural products: glcosides. Phytochemisyry: advance in research, 2006; 81: 1 – 22.
17. Gul AK, Nushrat S, Innayat UR, Murad AK, Kamal UA and Viqar UK. Naturally occurring flavonoids. Journal of Chemical Society of Pakistan, 2001; 23: 50 – 60.
18. Long ZL, Pei C, Mustafa O and James MH. Chromatographic profiles and identification of new phenolic components of *Ginkgo biloba* leaves and selected products. Journal of Agricultural and Food Chemistry, 13; 56: 6671 – 6679.
19. Marius BP, Simona D, Floricuta R, Florinela F and Carmen S. Comparative HPLC – DAD – ESI (+) MS fingerprint and quantification of phenolic and flavonoid composition of aqueous leaf extracts of *Cornus mas* and *Crataegus monogyna* in relation to their cardiotonic potential. 2014, 42; 2-18.
20. Wang H, Cao J, Xu S, Gu D, Wang Y and Xiao S. Depletion of high – abundance flavonoids by metal complexation and identification of low – abundance flavonoids in *Scutellaria.* Journal of Chromatography A, 2013; 1315: 107 – 117.
21. Bamawa CM, Ndjele LM and Foma FM. Characterization of leaf phenolic compounds of *Hymenocardia ulmoides* (wild plant tea) by HPLC – MS. International Journal of Pharmaceutical and Biological Research, 2013;4:170 – 174.
22. Giuseppina N, Daniel DS and Ricardo T. Chemical composition of hydroethanolic extract from *Siparuna guianensis*, medicinal plant used as antioxidant in Amazon region. Brazilian Journal of Pharmacognosy, 2012; 22(5): 1024-1034.
23. Meng Z Qiao L, Wang Z, Jia J, Chang Q, and Li W. Analysis of the constituents in semen descurainiae by UPLC/Q – TOF – MS/MS. Journal of Chinese Pharmaceutical Sciences, 2015; 24(5): 303 – 309.
24. Fereidoon S and Marian N. Phenolics in food and nutraceuticals. 1st Edition, CRC press, Boca Raton London New York Washington, D.C. 2006, 196.
25. Zakia B, Carmen GC and Nour EES. Characterization of flavonoid glycosides from Fenugreek (Trigonella foenum - graecum) crude seeds by HPLC – DAD – ESI/MS analysis. International Journal of Molecular Science, 2014; 15:20668 – 20685.
26. Yan Z, Qing X, Xiaozhe Z and Jiping C. High – Performance liquid chromatography – tandem mass spectrometry for identification of isoflavones and description of the biotransformation of kudzu root. Journal of Analytical and Bioanalytical Chemistry, 2005; 383: 787 – 796.