Section A-Research paper
ISSN 2063-5346

Synthesis of 4'O-Methyl Quercetin, 4'O-Sulphate Quercetin and 4'O-Glucose Quercetin Novel Flavonoid Compounds

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Abstract

Synthesis of any organic compounds are depending on each and every atom of the compounds and all substitutes are linked with the rings to exhibit novel properties with the involvement of minimum atoms for the organic synthesis using carbon and hydrogen molecule. To investigate the novel flavonoid compounds of quercetin derivatives were synthesized using ethyl and methyl alkyl substituents of saturated groups. All the synthesized derivatives of quercetin separated by chromatography and characterized by spectroscopy such as infrared (IR) and fourier transmission infrared (FTIR), liquid chromatography and mass spectroscopy (LCMS, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) to elucidate the compounds. The synthesis of quercetin derivatives like 4'O-methyl quercetin, 4'O-sulphate quercetin and 4'O-glucose quercetin obtained as compared to standard compounds. Quercetin compounds were reacted with a multi fold molar excess of ethyl and methyl mixtures, and the obtained products were analyzed and confirmed by HPLC and LCMS. All the three disulfates were identified, and structural inferences were drawn by 1H and ^{13}C NMR spectrometry of HPLC peak isolates.

Keywords: Organic compounds, Chromatography, Spectroscopy, Quercetin compounds

Introduction:

Flavonoid compounds possess significant impact in medicinal chemistry and they are commonly found in huge percentage in biomolecules as an active secondary metabolite such as enzymes, piments, vitamins, natural products and biologically active compounds. Quercetin is one of the plant compounds as flavonol in the flavonoid group of polyphenols [1]. Naturally found in most of the fruits, and vegetables, limited amount also available in leaves, seeds, and grains. It is bitter in nature generally and used as key ingredient as a dietary supplement, nutritional foods and most of the beverages [2]. Its molecular formula is $C_{15}H_{10}O_7$, and the chemical structural formula is illustrated in Figure 1. It act as naturally occurring inhibitor during transport of polar auxin [3]. It has a ketocarbonyl functional group

and their oxygen atom on the first carbon is basic and can generate salts with strong acids. Its molecular structure contains four active groups, namely, a dihydroxy group between the A ring, o-dihydroxy group B, C ring C_2, C_3 double bond, and 4-carbonyl [2]. The presence of a phenolic hydroxyl group and double bonds endows quercetin with a strong antioxidant activity. Its antioxidant and anti-inflammatory properties are closely related to the prevention and treatment of cardiovascular diseases and cancer. All the synthesized compounds exhibit potential antioxidant property as per the literature in particular quercetin derivative containing 4'O-methyl compounds.

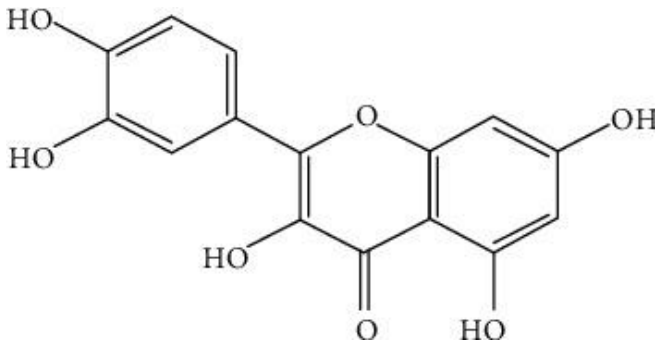


Figure: Structure of Quercetin

In this context current experiment designed to synthesis of flavonoid compound such as 4'O-Methyl Quercetin, 4'O-Sulphate Quercetin and 4'O-Glucose Quercetin using ethyl and methyl alkyl substituents of saturated groups. Two step synthesis process were used to determine the pure compounds of quercetin in the step of intermediate and final to obtain the all the derivatives. The further study planned for preliminary investigation of biological property and also planned for each compound molecular docking to know their structural interaction with DNA and various properties.

Materials and Methods

Chemicals, Methods and Structural Studies

Required chemicals for the synthesis were purchased from commercial sources sigma Aldrich, Chem. Pvt. Ltd. The progress of the reaction was monitored by Thin Layer Chromatography (TLC) using Merk CCM Gel de silica F_{252} plates as stationary phase and ethyl acetate/petroleum ether as mobile phase. Melting points of all the synthesized compounds were recorded in open capillaries using Guna melting point apparatus and are uncorrected. The IR spectra were recorded on a Nicolet iz10 FT IR spectrophotometer in the range 400-4000 cm^{-1}. The ^{1}H and ^{13}C NMR were recorded on a Bruker-400 spectrometer at 400 MHz using DMSO-d6 solvent with TMS as internal standard. The Mass spectra of the prepared molecules were recorded on Agilent technologies 6110 Quadrupole LC/MS. Quercetin is a analytical grade from Sigma Aldrich, St. Louis, MO, USA. Other chemicals were procured from SRL chemicals, India.

Intermediate synthesis

Step 1: 2-(2,2-diphenylbenzo[d][1,3] dioxol-5-yl)- 3,5,7-trihydroxy-4H-chromen-4-one.

Diphenyl ether (43.80g. 2.5 mol) was added to the quercetin (30g 1mol) with stirring. The reaction mixture was refluxed by using Dichloro-diphenyl-methane as a solvent for about 30-40 min at 175 ^{0}C using oil bath. After completion of the reaction mixture were cooled too room temperature the treated with hexane to obtain solid with 49.6% Yield (22.95g).

Step 2: 2-(2,2-diphenylbenzo[d][1,3]-dioxol-5-yl)-5- hydroxy-3,7-bis (methoxy-methoxy) – 4-Hchromen- 4-one

Chloromethyl ether (9.03ml, 2.5 mol) was added to the product obtained in the previous step maintaining basic condition using potassium chloride using acetone as a solvent. Suspension was refluxed for about 8-10 hour. The reaction mixture obtained was cooled to attain room temperature and Chloromethyl ether solvents was removed by vacuum distillation. Further the solid obtained was purified by the Column Chromatographic method. With 70% yield 15.9g

Step 3: 2-(3,4-dihydroxyphenyl)-5-hydroxy-3,7-bis(methoxymethoxy)-4H-chromen-4-one

To a solution of compound obtained from previous (15g, 1 mmol) dissolved in ethanol (30 ml) and THF (30 ml) 10% Pd/C (6 mg) was added with vigorous stirring. Then the reaction vessel was evacuated and the atmosphere replaced with hydrogen. After 8 h, the reaction mixture was filtered through celite and the filtrate concentrated. The crude material was then chromatographed on silica gel (50% ethyl acetate in petroleum ether) to obtain desired product with yield of 85%,9.6 g as a yellow solid.

Step 4: 5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)- 3,7-bis (methoxy methoxy)-4H-chromen-4-one

Iodomethane (2.04 ml, 1.5 mmol) was added to previous compound (9-gram 1mmol) dissolved in dry DMF (20 ml) K_2CO_3 (60 mg, 0.47 mmol) at room temperature. After 8 h, the reaction mixture was partitioned between 100 ml ethyl acetate and 100 ml water. The ethyl acetate layer was washed with brine, dried over $MgSO_4$, filtered and concentrated. The crude material was purified by column chromatography (25% ethyl acetate in petroleum ether) to obtain yellow colour solid with 90% yield 8.49 g.

Final synthesis

Step 1: 4'-O -Methyl Quercetin

Hydrochloric acid (10 ml) was added to a stirred solution intermediate-4 (2.0g, 1 mmol) in Dichloromethane (100 ml) and Diethyl ether (100 ml) by maintaining temperature to less than 25 °C. The dark brown coloured solution obtained was stirred at room temperature for 6 h, completion of the reaction was monitored by the TLC. The reaction mixture was diluted with a large amount of ethyl acetate and washed with water and brine. The ethyl acetate layer

was dried over MgSO4, filtered, then concentrated and the crude material purified by column chromatography (50% ethyl acetate in petroleum ether). Recrystallization from EtOH gave yellow colour solid with 82% yield 1.63 g. [4, 5].

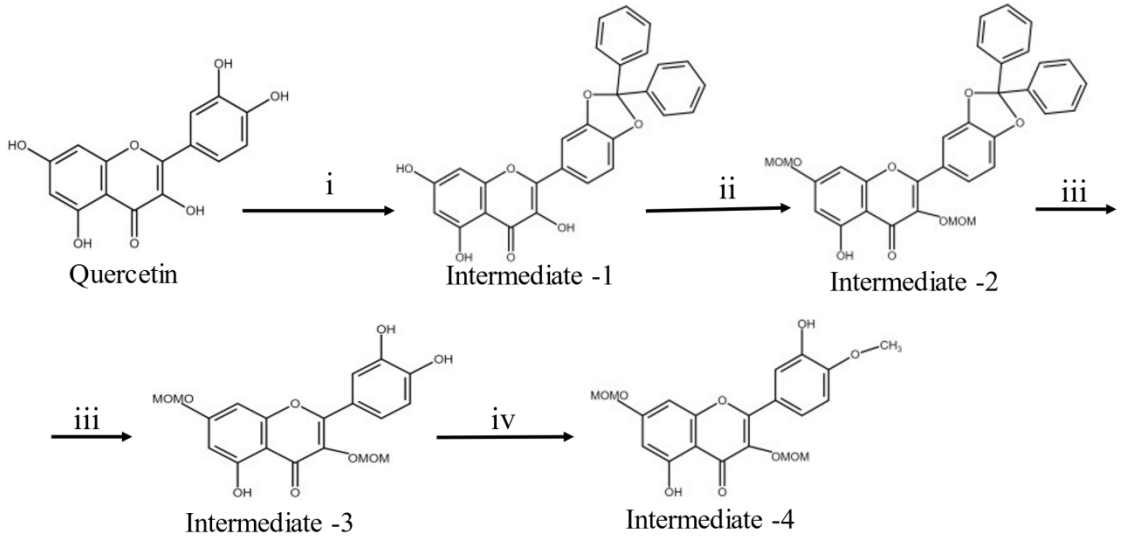
Step 2: 4'-O Sulphate Quercetin

8 cm3 of concentrated sulfuric acid (d = 1.84 g cm−3) was added to 2 g of intermediate-4 in a 100 cm^3 round-bottom flask. The reaction mixture was vigorously stirred for 2 h at 18–20 °C. Then, 20 cm^3 of very cold water was added into the reaction mixture. The yellow precipitate was filtered at a reduced pressure and recrystallized twice from the hot saturated water solution. Next, the yellow sediment was dried in air at room temperature. The synthesis yield was 70 % 1.5g.[6]

Step 3: 4'-O- Quercetin Glucoside

4'-O- quercetin glucoside obtained by dissolving intermediate-4 with tetraacetyl-R-1-bromoglucose in a dimethyl sulfoxide (08 ml, 1.5mmol) and then stirred overnight after the addition of potassium carbonate (1.7g, 2mmol). The resulting mixture was then adjusted to an acidic condition by adding a few drops of formic acid. The precipitate in the acidic solution was separated by centrifugation. The precipitate was then washed and concentrated in vacuum. Sodium methylate solution was added to the residue and kept at room temperature for 20 min. The solution was then neutralized and filtered. The quercetin glucoside obtained were further purified by Column Chromatographic technique with an 75% yield 1.3g [7,8].

SCHEME OF INTERMEDIATES SYNTHESIS



Scheme: i) Ph_2CCl_2, Ph_2O.°C, reflux 30 min. ii)MOMCl, K_2CO_3, acetone, reflux, 6 h. iii) Pd/C (10wt %), H_2 (1 atm), THF/EtOH, 8 h. iv) MeI, K_2CO_3, DMF, 8 h.

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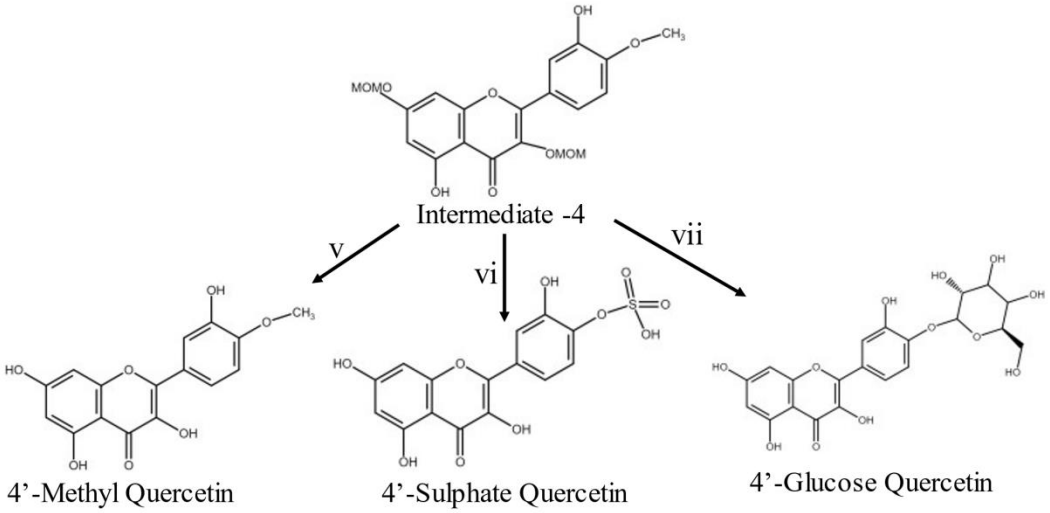
SYNTHESIS OF DERIVATIVES



Scheme: v) HCl (1.0 M) in Et_2O/CH_2Cl_2 (1:1), 25°C, 6 h. vi) Fuming H_2SO_4 (stirred for 2 h at 18–20 °C. vii) etraacetyl-R-1-bromoglucose, DMSO & K_2CO_3.

Results

After the synthesis of all the quercetin derivatives chromatography and spectroscopy study were carried out to know the basic properties of the compounds. It is very much useful to elucidate the compound for structure identification and help it for the characterization.

Synthesis of compound 1: 4'-O -Methyl Quercetin

Yellow solid, M.P. 252-254 °C; IR (cm^{-1}): 3212 (phenolic OH), 2924 (aromatic C-H), 1661 (C=O); ^1H NMR (400 MHz DMSO-d_6): δ 11.46 (s, 1H), 10.39 (s, 1H), 9.40 (s, 1H), 9.29 (s, 1H), 7.89 (d, *J* = 1.9 Hz, 1H), 7.52 (dd, *J* = ,1.9 Hz, 1H), 7.29 (d, *J* = 8.6 Hz, 1H), 6.42 (d, *J* = 2.0 Hz, 1H), 6.13 (d, *J* = 2.0 Hz, 1H), 3.83 (s, 3H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 55.4, 94.8, 105, 114.2, 128.2, 131.5, 149.6, 153.9,156.4, 158.6, 160.4, 162.5, 178.6 ; LC-MS (ESI Positive): *m/z* =$(M_+H)_+$: Anal. Calcd for $C_{16}H_{12}O_7$: C, 60.76; H, 3.82. Found: C, 60.69; H, 3.84.

Synthesis of compound 2: 4'-O Sulphate Quercetin

Yellow solid, M.P. 216-218 °C; IR (cm^{-1}): 3214 (Phenolic OH), 2924 (Aromatic C-H), 1669 (C=O), 1726 (S=O); ^1H NMR (400 MHz DMSO-d_6): δ 11.2 (s, 1H), 7.72 (m, *J* = ,1.9 Hz, 2H), 6.52 (d, *J* = 8.6 Hz, 1H), 6.46 (d, *J* = 2.0 Hz, 1H), 6.26 (d, *J* = 2.0 Hz, 1H), 4.59 (s, 4H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 174.2, 163, 158.6, 149, 144, 136.2, 123, 121, 115, 11.2, 106, 96.7; LC-MS (ESI Positive): *m/z* =$(M_+H)_+$: Anal. Calcd for $C_{15}H_{10}O_{10}S$: C, 47.13; H, 2.64; O, 41.85; S, 8.39. Found: C, 47.13; H, 2.53; O, 41.67; S, 8.82

Synthesis of compound 3: 4'-O- Quercetin Glucoside

Yellow solid, M.P. 182-184 °C; IR (cm^{-1}): 3214 (Phenolic OH), 2924 (Aromatic C-H), 1669 (C=O), 1032 (C-O); ^1H NMR (400 MHz DMSO-d_6): δ 11.2 (s, 1H), 7.72 (m, *J* = ,1.9 Hz,

2H), 6.52 (d, *J* = 8.6 Hz, 1H), 6.46 (d, *J* = 2.0 Hz, 1H), 6.26 (d, *J* = 2.0 Hz, 1H), 4.59 (s, 4H); ^{13}C NMR (500 MHz, DMSO-d_6): δ 174.2, 163, 158.6, 149, 144, 136.2, 123, 121, 115, 11.2, 106, 82.5, 64.8; LC-MS (ESI Positive): *m/z* =$(M_+H)_+$: Anal. Calcd for $C_{21}H_{20}O_{12}$: C, 54.31; H, 4.34; O, 41.34; S, 8.39. Found: C, 54.73; H, 4.26; O, 41.64.

Table 1: Chromatography and spectroscopic test parameters of synthesized quercetin derivatives

SL No.	**Test Parameters**	**Quercetin 4' O-Methyl ($C_{16}H_{12}O_7$)**		**Quercetin 4' O-Sulphate ($C_{15}H_{10}O_{10}S$)**		**Quercetin 4' O-Glucoside ($C_{21}H_{20}O_{12}$)**	
1	**Melting Point**	252–254 °C		216-218°C		182–184 °C	
2	**FTIR Report (using KBr, ν cm^{-1})**	**3212** - phenolic (OH), **2924** - aromatic (C–H), **1661** – Stretching (C=O)		**3214** - phenolic (OH), **2924** - aromatic (C–H), **1669** – Stretching (C=O), **1726** - Stretching (S=O)		**3214** - phenolic (OH), **2924** - aromatic (C–H), **1669** – Stretching (C=O), **1032** – Stretching (C-O)	
3	**LCMS *m/z***	**Calculated**	**Analyzed**	**Calculated**	**Analyzed**	**Calculated**	**Analyzed**
		C - 60.76; **H** - 3.82	**C** - 60.69; **H** - 3.84	**C** - 47.13; **H** - 2.64; **O** - 41.85; **S** - 8.39	**C** - 47.13; **H** - 2.53; **O** - 41.67; **S** - 8.82	**C** - 54.31; **H** - 4.34; **O** - 41.34.	**C** - 54.73; **H** - 4.26; **O** - 41.64

Table 2: NMR spectroscopic test parameters of synthesized quercetin derivatives

SL No.	**Test Parameters**	**Quercetin 4' O-Methyl ($C_{16}H_{12}O_7$)**	**Quercetin 4' O-Sulphate ($C_{15}H_{10}O_{10}S$)**	**Quercetin 4' O-Glucoside ($C_{21}H_{20}O_{12}$)**
1	**^{1}H NMR (DMSO-*d*6, 400 MHz)**	3.83 (s, 3H, -OCH3), 6.13 (d, *J* = 2.0 Hz, 1H, 6-H), 6.42 (d, *J* = 2.0 Hz,	4.59 (s, 4H, -OH), 6.26 (d, *J* = 2.0 Hz, 1H, Ar-H), 6.46 (d, *J* = 2.0 Hz, 1H, Ar-H),	3.42-3.86 (m, 7H, alicyclic CH of glucose), 4.2 (s, 4H, -OH of glucose), 4.8 (s, 4H, Ar-OH),

		1H, 8-H), 7.29 (d, *J* = 8.6 Hz, 1H, 5′-H), 7.52 (dd, *J* = ,1.9 Hz, 1H, 6′-H), 7.89 (d, *J* = 1.9 Hz, 1H, 2′-H), 9.29 (s, 1H, 3-OH), 9.40 (s, 1H, 3′-OH), 10.39 (s, 1H, 7-OH), 11.46 (s, 1H, 5-OH);	6.52 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.72 (m, *J* = ,1.9 Hz, 2H, Ar-H), 11.2 (s, 1H, OH attached to sulphur)	5.4 (d, 2H, Ar-H), 6.52 (d, 2H, Ar-H), 7.42 (d, *I*H, Ar-H). 10.3 (s, 1H, OH attached to glucose)
2	**^{13}C NMR (DMSO, 500 MHz)**	55.4 (O-C-OCH3), 94.8 (2C, Ar-C), 105 (1C, OH-C=C), 114.2(1C, Ar-C, C=C), 128.2 (2C, Ar-C, C=C), 131.5(1C, Ar-C, C=C), 149.6 (1C, Ar-C, C-OH), 153.9(1C, Ar-C, C=C), 156.4 (Ar-C, C=C-O), 158.6 (1C Ar-C, C=C), 160.4 (2C, C-C), 162.5 (1C, C=C-O), 178.6 (1C, Ar C-OH)	96.7 (2C, Ar-C), 106 (1C, OH-C=C), 111.2(1C, Ar-C, C=C), 115.6 (1C, Ar-C, C=C) 121(1C, Ar-C, C=C), 123 (1C, Ar-C, C=C), 136.2 (S, 1C, Ar-C-OH), 144 (1C, Ar-C, O-C=C), 149(1C, Ar-C, C-OH), 158.6 (2C Ar-C, C-O-C), 163 (2C, C-OH), 174.2 (s, 1C, Ar C=O),	64.8-82.5 (6C, Alicyclic-C of Glucose) 106 (3C, -C=C), 111.2 (1C, Ar-C, C=C), 115.6 (1C, Ar-C, C=C), 121 (1C, Ar-C, C=C), 123 (1C, Ar-C, C=C), 144 (1C, Ar-C, O-C=C), 149 (1C, Ar-C, C-OH), 136.2 (1C, Ar-C-OH), 158.6 (2C Ar-C), 163 (2C, C-OH), 174.2 (s, 1C, Ar C=O)

Fig: 1a

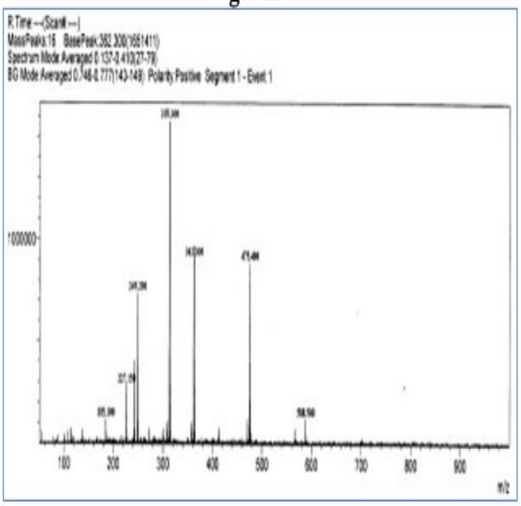


Fig: 1b

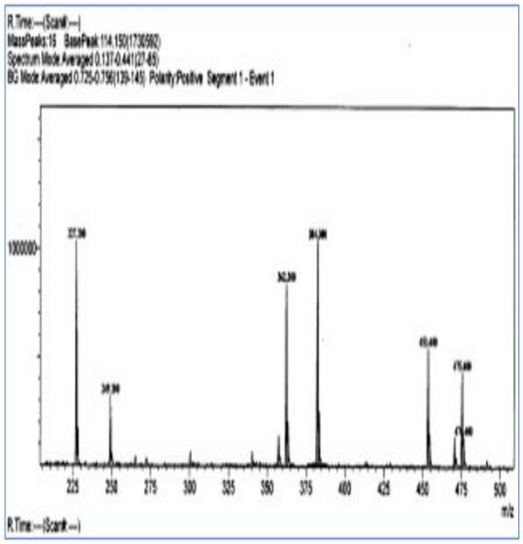


Fig: 1c

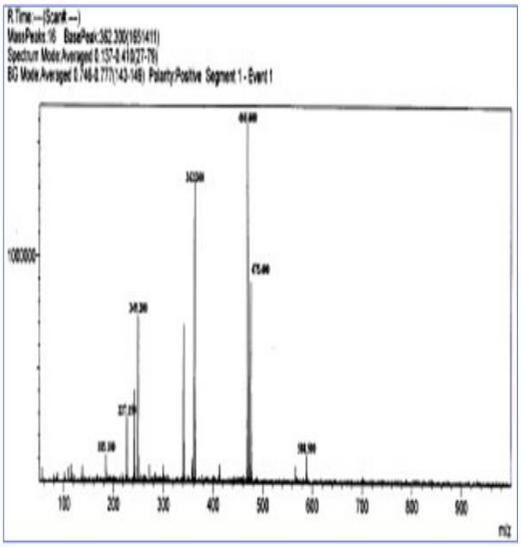


Figure 1: LCMS*m/z*: 466 [M$^+$]: Fig-1a: 4´Methyl Quercetin,Fig-1b: 4´Sulfhate Quercetin Fig-1c: 4´Methyl Quercetin

Fig:2a

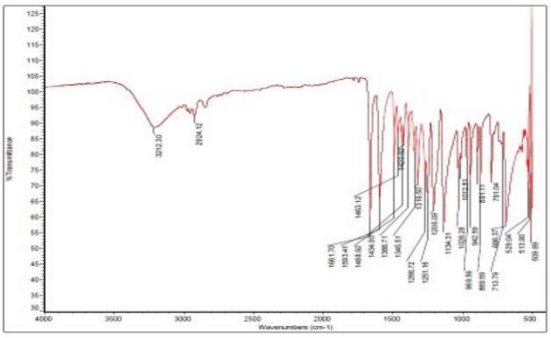


Fig:2b

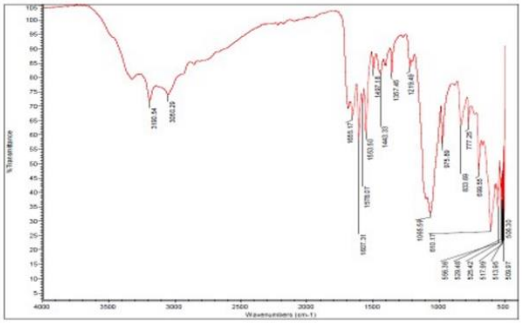


Fig: 2c

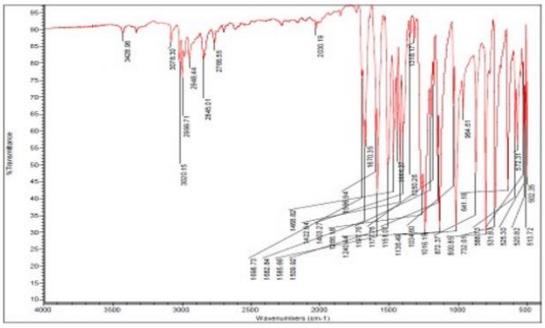


Figure 2: FTIR (KBr, ν cm−1):Fig-1a: 4´Methyl Quercetin,Fig-1b: 4´Sulfhate Quercetin Fig-1c: 4´Glucose Quercetin

Fig: 3a

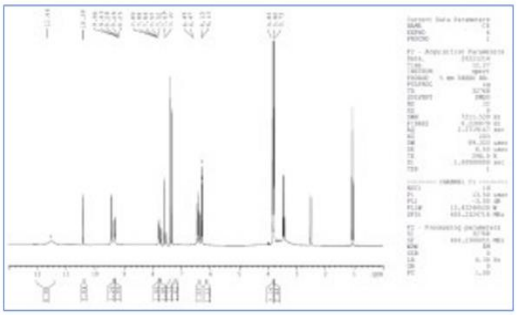


Fig: 3b

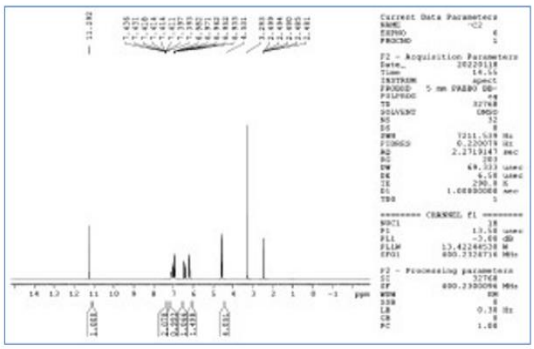


Fig: 3c

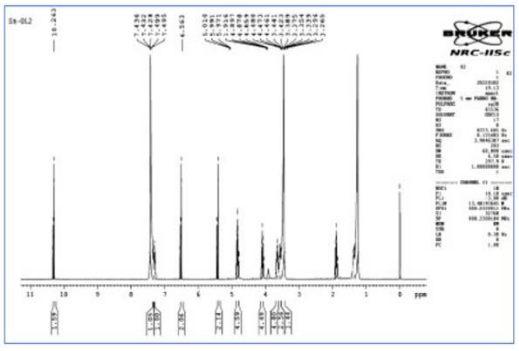


Figure 3: ^{1}H NMR (DMSO -*d*6, 400 MHz): Fig -3a: 4´Methyl Quercetin, Fig -3b: 4´Sulfhate Quercetin Fig -3c: 4´Glucose Quercetin

Fig: 4a

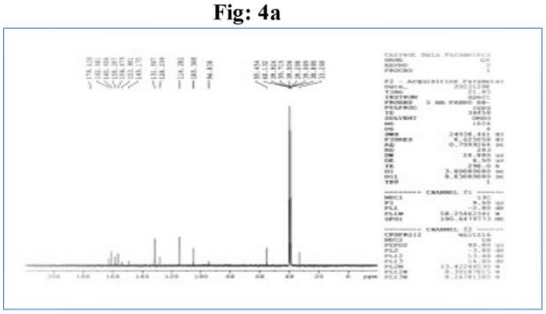


Fig: 4b

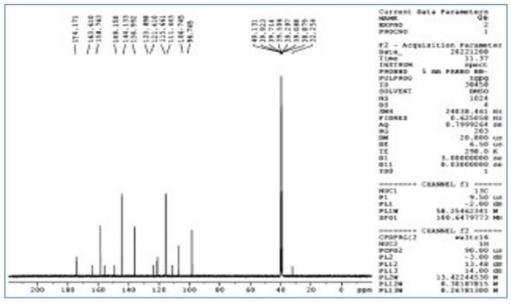


Fig: 4c

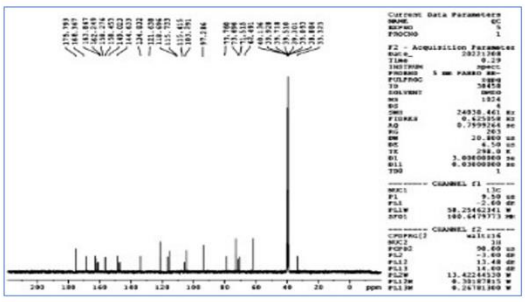


Figure 4: ^{13}C NMR (DMSO, 500 MHz): Fig-4a: 4´Methyl Quercetin, Fig-4b: 4´Sulfhate Quercetin Fig-4c: 4´Methyl Quercetin

Discussion

Synthesis of flavonoid compounds are very useful in pharmacological industry, in current investigation we synthesized quercetin derivatives such as 4'O-Methyl Quercetin, 4'O-Sulphate Quercetin and 4'O-Glucose Quercetin using ethyl and methyl alkyl substituents of saturated groups. It is evidence with chromatographic and spectroscopic studies to confirm the compound as novel new moieties of flavonoid. Similar investigation of Mrkus et al., (9) peptidyl-quercetin derivatives is 2-[3-$(aa)_n$-4-hydroxyphenyl]-3,5,7-tri-hydroxy-4H-1-benzopyran-4-on, and described formula of the main peptidyl-resveratrol derivatives is (E)-5-[4-$(aa)_n$)styryl]benzene-1,3-diol and reported for their antioxidant and anticancer potential. In other studies of Massi et al., (10) reported modified compound of flavonoid quercetin (3,3',4',5,7-pentahydroxyflavone) is widely distributed in plants, foods, and beverages. This polyphenol compound exhibits varied biological actions such as antioxidant, radical-scavenging, anti-inflammatory, antibacterial, antiviral, gastroprotective, immune-modulator, and finds also application in the treatment of obesity, cardiovascular diseases and diabetes (11-1)6. In this context our synthesized compound were equally valuable and novel when compared naturally derived Quercetin and their biological properties.

Conclusion

It is one of the major challenges to researchers to find alternative compound in higher scale to combat the health issues of animal and mankind. Quercetin is well known drug using it in pharmaceutical industry to treat the many diseases. To find purer and cost-effective production, we synthesized 3 derivatives in very low-cost method and almost similar to commercial Quercetin compound which is highly applied in medical sector for various health issues and may it will be useful compound for future perspective to meet the global market to supply it in very cheaper and available for all.

Reference

1. Mrkus, Ljiljana et al., Synthesis and biological evaluation of quercetin and resveratrol peptidyl derivatives as potential anticancer and antioxidant agents. Amino Acids., 2019, 51(2): 319-329. doi:10.1007/s00726-018-2668-6
2. Yang D., Wang T., Long M. and Li P. Quercetin: Its main pharmacological activity and potential application in clinical medicine. Oxid Med Cell Longev. 2020, 30: 2020:8825387. doi:10.1155/2020/8825387.
3. Fischer C., Speth V., Fleig-Eberenz S., Neuhaus G. Induction of zygotic polyembryos in wheat: influence of auxin polar transport. Plant Cell., 1997, 9(10):1767–1780. doi: 10.1105/tpc.9.10.1767
4. Danihelováa M., Viskupicovaa J., Ernest Sturdíka Acta Chimica Slovaca, 2012, 5(1): 59-69. Doi: 10.2478/v10188-012-0010-6.
5. Li N., Shi Z., Tang Y., Yang J. and Jin-Ao Duan. An efficient partial synthesis of 4′-O-methylquercetin via regioselective protection and alkylation of quercetin. 2009, 5(60). doi:10.3762/bjoc.5.60
6. Woz´nicka E., Pienia-zek E., Zapała Ł., Byczyn´ ski L., Trojnar I. and Kopacz m. New sulfonic derivatives of quercetin as complexing reagents: synthesis, spectral, and thermal characterization. J Therm Anal Calorim. 2015, 120:351–361.
7. Massi A., Bortolini O., Ragno D., Bernardi T., Sacchetti G., Tacchini M. and De Risi C. Research progress in the modification of quercetin leading to anticancer agents. Molecule, 2017, 22(8):1270, 2017.
8. Donald J. L., et al. A synthetic approach to the generation of quercetin sulfates and the detection of quercetin 3'-O-sulfate as a urinary metabolite in the rat. Bioorg Medicin Chem. 2005, 13(24): 6727-6731. doi:10.1016/j.bmc.2005.07.021
9. Suganthy N., Devi K. P., Nabavi S. F., Braidy N.and Nabavi S. M. Bioactive effects of quercetin in the central nervous system: focusing on the mechanisms of actions. Biomed Pharmacother. 2016, 84:892–908. doi: 10.1016/j.biopha.2016.10.011
10. Ganesh D., Fuehrer H. P., Starzengrüber P., et al. Antiplasmodial activity of flavonol quercetin and its analogues in *Plasmodium falciparum*: evidence from clinical isolates in Bangladesh and standardized parasite clones. *Parasitology Research*. 2012, 110(6):2289–2295. doi: 10.1007/s00436-011-2763-z.
11. Sun L., Lu B., Liu Y., Wang A., Li G., Zhao L. and Zhao C. Synthesis, characterization and antioxidant activity of quercetin derivatives, Synthetic Communications, 2021, 51(19): 2944-2953, doi: 10.1080/00397911.2021.1942059
12. Ketan H.V., et al. Quercetin and its derivatives: synthesis, pharmacological uses with special emphasis on anti-tumor properties and prodrug with enhanced bio-availability. Anti-cancer Agents in Medicinal Chem. vol. 2009, 9(2): 138-161. doi:10.2174/187152009787313855
13. Shi Z.H., Li N.G., Tang Y.P., et al., Synthesis, biological evaluation and SAR analysis of O-alkylated analogs of quercetin for anticancer. Bioorg Med Chem Lett. 2014, 15, 24(18): 4424-4427. doi: 10.1016/j.bmcl.2014.08.006.

14. Haleshappa R., Keshamma E., Girija C.R., Thanmayi M., Nagesh C.G., Lubna Fahmeen G.H., Lavanya M. and Patil SJ., Phytochemical Study and Antioxidant Properties of Ethanolic Extracts of *Euphorbia milii*. Asian J Biol Sci. 2020, 13(1): 77-82.
15. Kolgi R.R., Haleshappa R., Sajeeda N., Keshamma E., Karigar C.S., Patil S.J. Antioxidant studies, *in vitro* cytotoxic and cell viability assay of flavonoids and alkaloids of *Leucas aspera* (Wild.) Linn leaves. Asian J Biol Life Sci. 2021, 10(1): 165-171.
16. Haleshappa R., Patil S.J. and Siddalinga Murthy K.R. Phytochemical analysis, in vitro evaluation of antioxidant and free radical scavenging activity of *Simarouba glauca* seeds. Adv Pharmacol Pharm. 2021, 9(1-8): doi.org/10.13189/app.2021.090101.