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CHARACTERIZATION OF RUTIN ISOLATED BY LEAVES *ANNONA SQUAMOSA* BY MODERN ANALYTICAL TECHNIQUES

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ABSTRACT
Flavonoids are principal active constituents have been used to treatment of various human diseases. Rutin (quercetin-3-rhamnosyl glucoside) as the flavonoids display anticancer, antiviral, antiinflammatory and heart disease protective activities. Rutin by acting as antioxidants exhibited several beneficial effects, such as antiinflammatory, antiallergic, antiviral as well as an anticancer activity. *Annona squamosa* Linn is used in the treatment of diabetes, hepatotoxicity and in cancer cell line, It has been recognized that flavonoids display anticancer, antiviral, anti-inflammatory, and heart disease protective activities. The present study was carried out to isolation, characterization of rutin by NMR and Mass spectroscopic method.

KEYWORDS: Rutin, Annona *Annona squamosa*, NMR & Mass.

INTRODUCTION
Medicinal plants serve as important source of phytochemicals (secondary metabolites) which have protective or disease preventive properties including - antibacterial, anticancer, antifungal, and antioxidant. The naturally occurring antioxidants in them possess the ability to reduce the oxidative damage associated with many diseases, including cancer, cardiovascular disease, cataracts, atherosclerosis, diabetes, arthritis, immune deficiency diseases and aging[1]. Annona squamosa L. (Annonaceae), commonly known as custard apple is a native of west Indies, it is widely grown throughout the tropics in India and popularly cultivated in the north eastern parts of Thailand, mainly for its edible fruit[2]. Rutin is a flavonoid present in the plant kingdom as *allelopathic* substances. Rutin (Fig. 1) is the rhamnoglucoside of the flavonoid quercetin and found in many plants and used for treatment of various diseases related to the vascular5. It is quercetin-3-rutinoside or 3,3',4', 5,7-pentahydroxy flavones-3-rutinoside and has a chemical formula $C_{27}H_{30}O_{16}$[3].

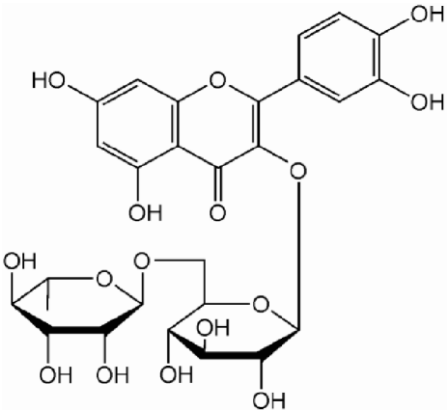
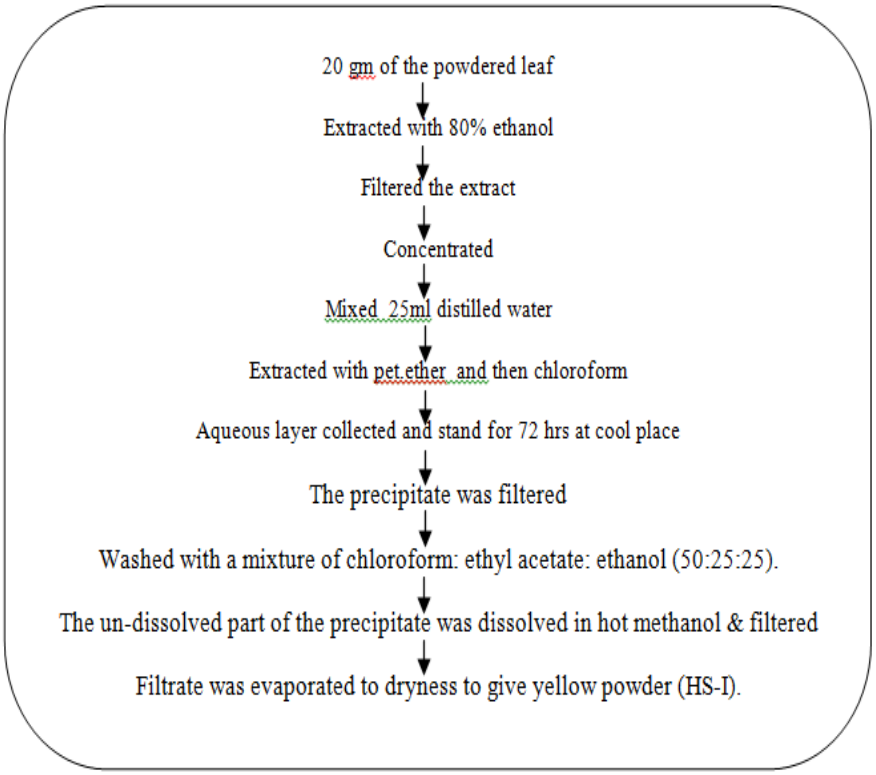


Fig. 1: Structure of rutin.

MATERIAL AND METHOD
Plant material collection
The leaves were collected from botanical garden L.N.C.P. Bhopal (M.P.) and authenticated (Voucher No. 004/bot/LNCP/10 & 004/bot/LNCP/11). They were dried in shade for several days at room temperature and then grinded as powder. The standard rutin was obtained as a gift sample from Jamia hamdard, Delhi.

Extraction and isolation



Characterization of Rutin

NMR analysis

NMR spectra of sample and standard were recorded using a Bruker Avance [SAIF (Sophisticated Analytical Instrumentation Facility) Punjab university, Chandigarh] operating at a frequency of 300.00 MHz (1H). The temperature of the measurements was 295.7 K. NMR samples were prepared by dissolving the compound DMSO d6. The 1H NMR chemical shifts were referenced to the signals of TMS (0 ppm).

Mass analysis

The isolated compound (HS-I) was analyzed by mass spectroscopy in SAIF (Sophisticated Analytical Instrumentation Facility) Punjab university, Chandigarh. The electro spray mass spectra were recorded on WATERS-Q-TOF Micromass (LC-MS). The sample was (dissolved in methanol) introduced into the ESI source through a syringe pump at the rate of 5 ml per min. The ESI capillary was set at 3.5 KV and cone voltage was 40v and the spectra were collected.

Table 1: Interpretation of ^{1}HNMR spectra of isolated compound (HS-I).

Molecular Formula	$C_{27}H_{30}O_{16}$
Mol.wt.	610.51
Chemical name	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]-4*H*-chromen-4-one
^{1}HNMR(DMSO) (δ ppm)	1.00(3H-CH_3) 5.35(H) 6.21(1H C-6-H) 6.40(1H C-8-H) 7.55(1H C-2-H) 7.56(1H C-6H) 9.21(1H C-3-OH) 9.71(1H C-4-OH) 10.86(1H C-7-OH) 12.49(1H C-5-OH)

Table 2: Interpretation of Mass spectra of isolated compound (HS-I).

m/z (%)	610[Base peak] 301[M-H- glu-rha]

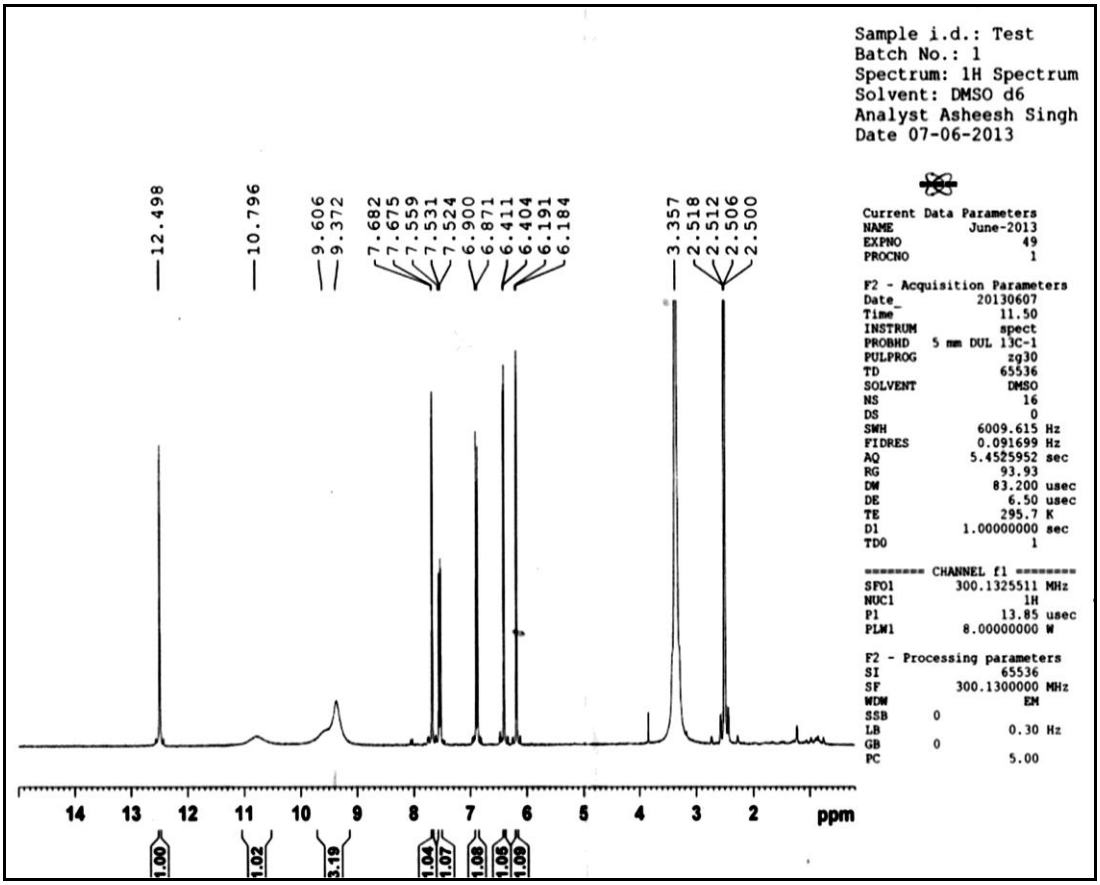


Fig 1: NMR spectra of Test sample(HS-I).

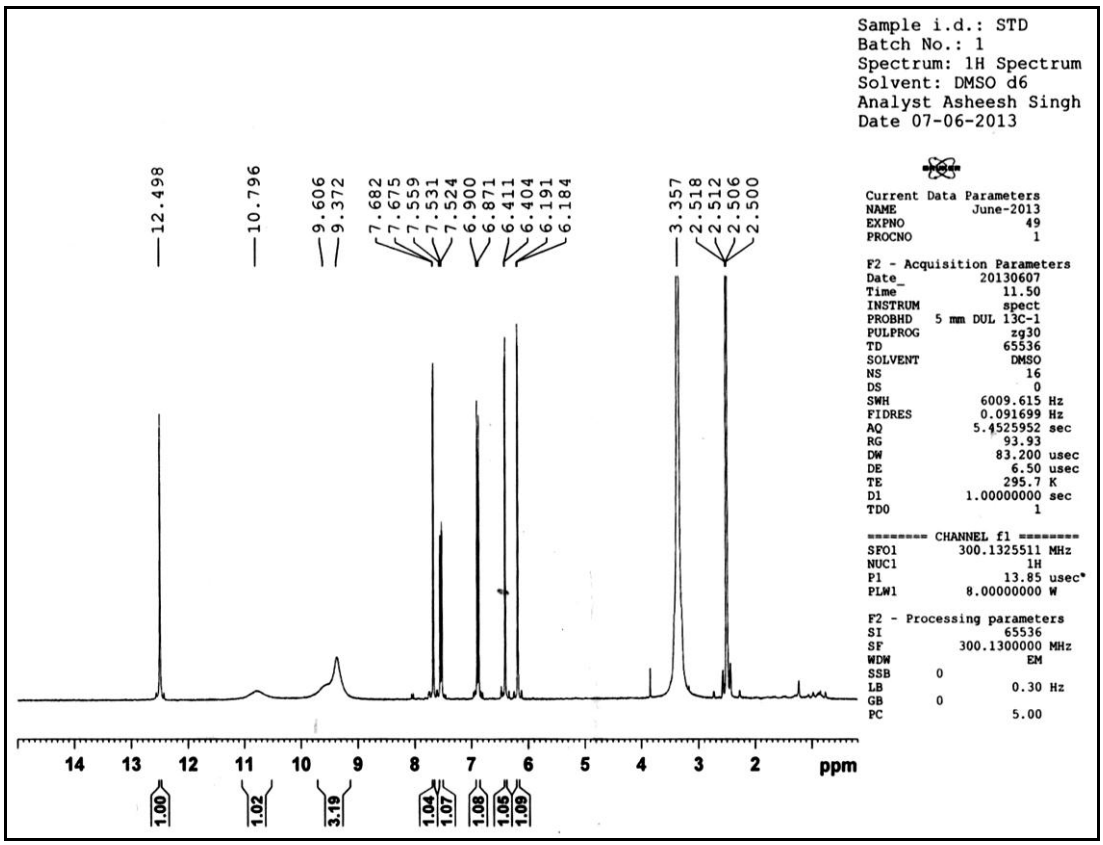
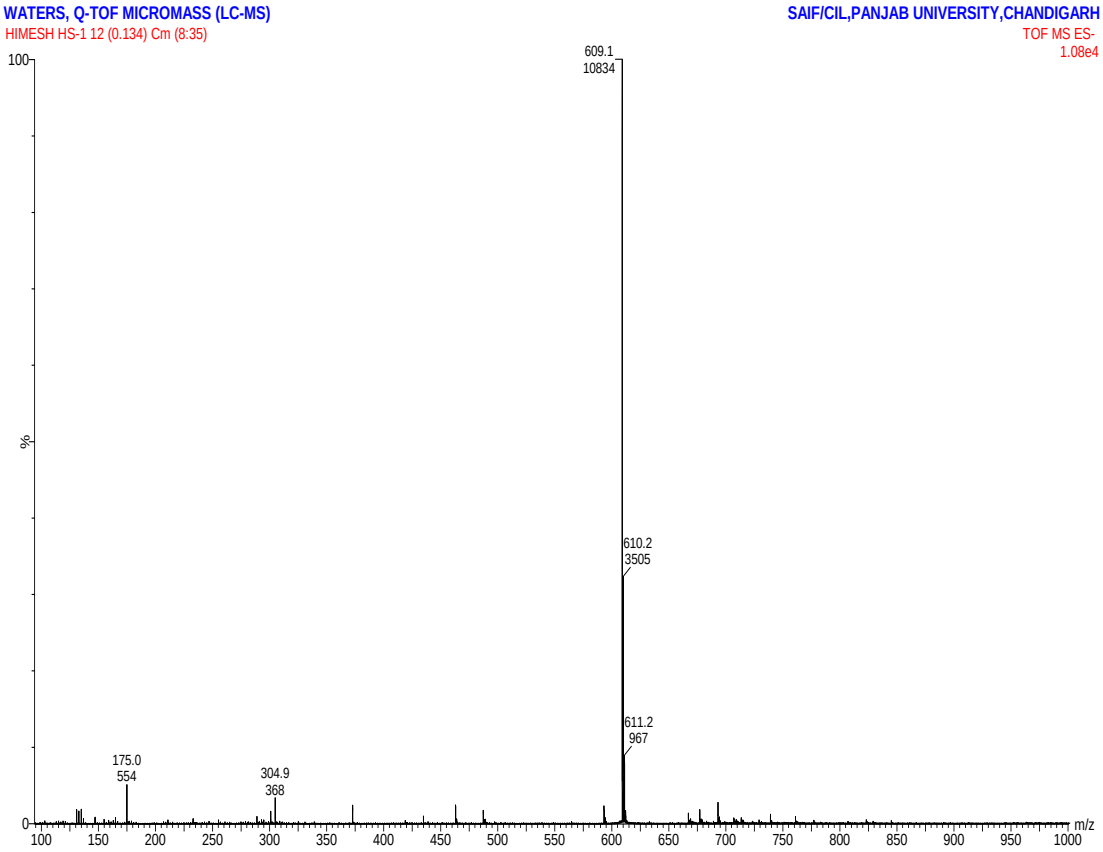
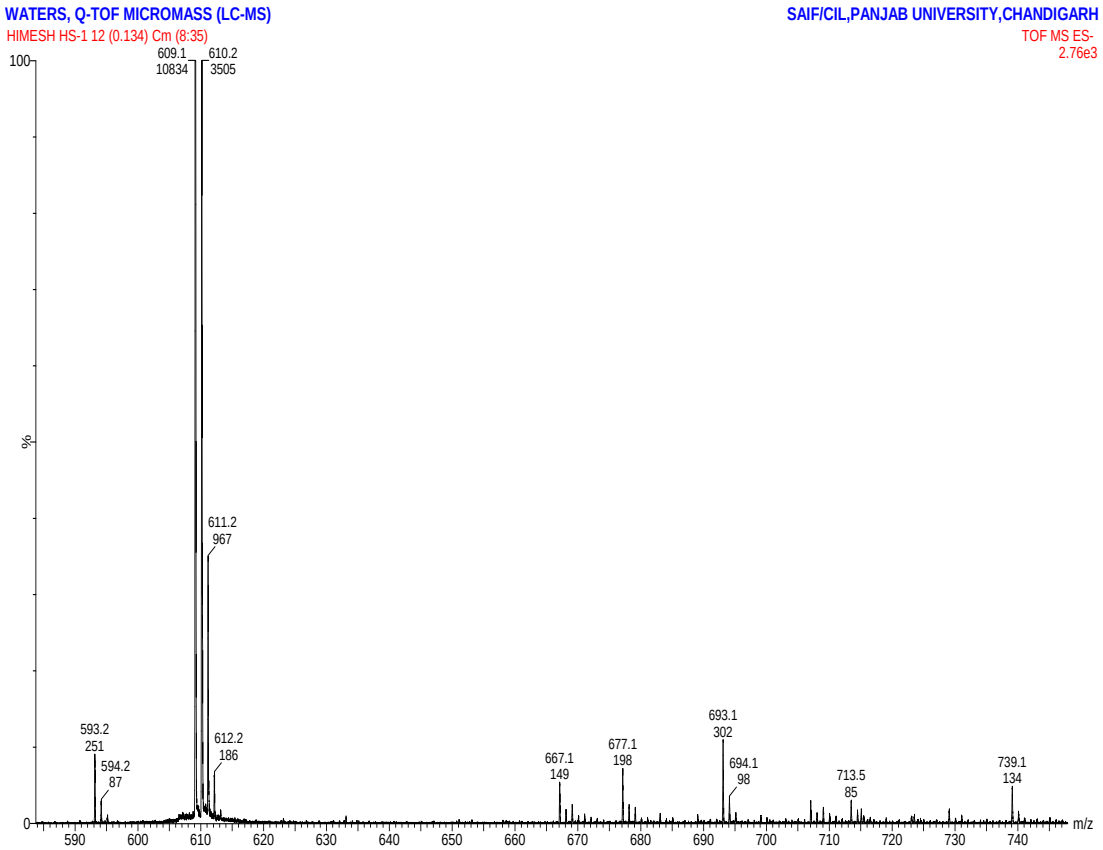


Fig 2: NMR spectra of standard sample.


WATERS, Q-TOF MICROMASS (LC-MS)
HIMESH HS-1 12 (0.134) Cm (8:35)
SAIF/CIL,PANJAB UNIVERSITY,CHANDIGARH
TOF MS ES-
1.08e4
609.1
10834
610.2
3505
611.2
967
175.0
554
304.9
368
%
m/z


WATERS, Q-TOF MICROMASS (LC-MS)
HIMESH HS-1 12 (0.134) Cm (8:35)
SAIF/CIL,PANJAB UNIVERSITY,CHANDIGARH
TOF MS ES-
2.76e3
609.1
10834
610.2
3505
611.2
967
593.2
251
594.2
87
612.2
186
667.1
149
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198
693.1
302
694.1
98
713.5
85
739.1
134
%
m/z

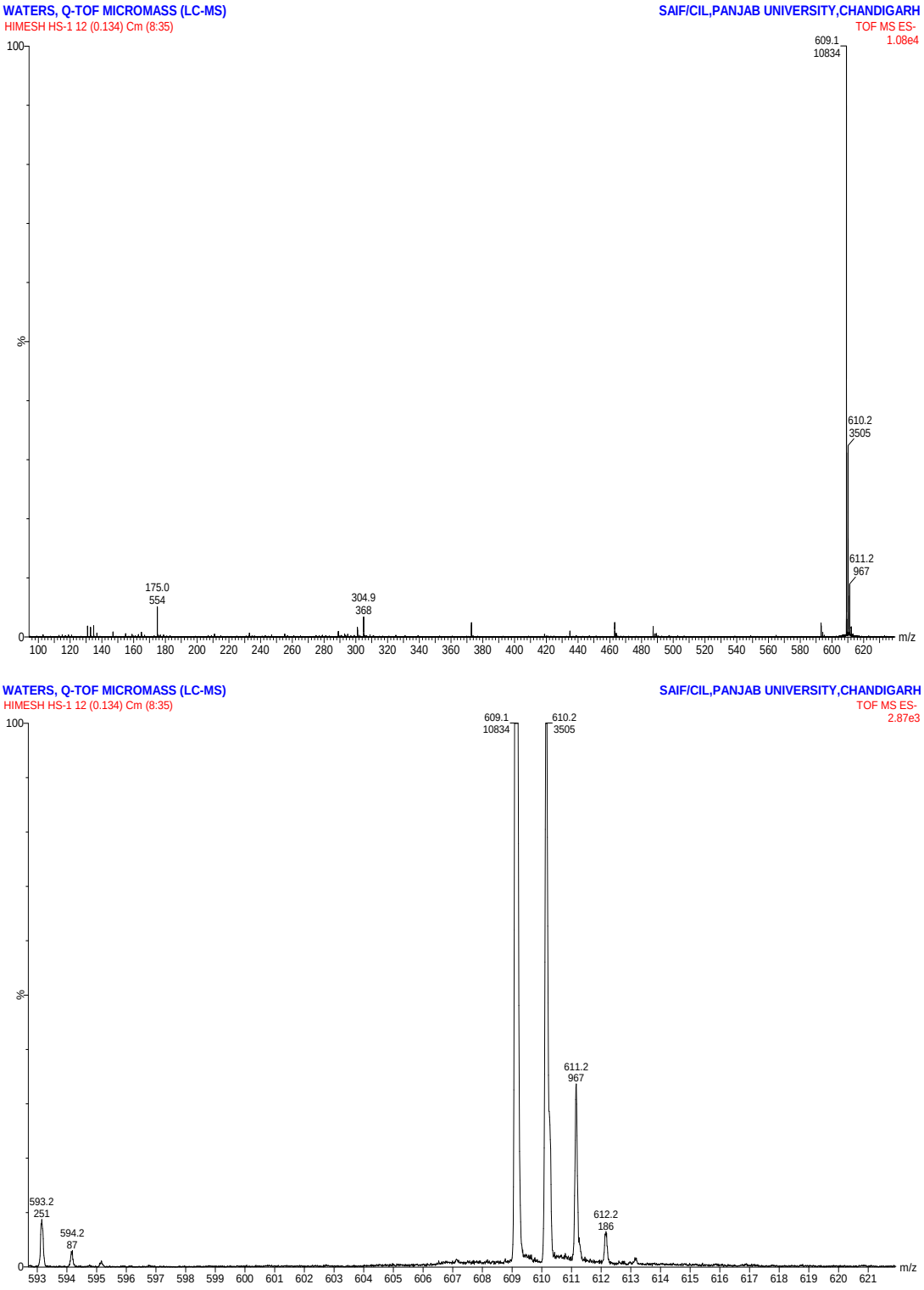

Fig 3: Mass spectra of isolated compound (HS-I).

RESULT AND DISCUSSION

Isolated compounds (HS-I) from leaves of *A.squamosa* was characterized by NMR and Mass spectroscopic techniques. HNMR spectra of isolated compound resembles to the spectra of standard rutin(table 1 & Fig 1-2). In Mass spectra, isolated compound M-H showed peak at 609 (m/z) whereas most abundant peak in spectra is 610(m/z). The calculated molecular mass is 610.59(table 2 & Fig 3).Thus it is confirmed that isolated compound is rutin.

CONCLUSION

Flavonoid is major phenolic compounds are becoming the major subject of medical research. They have been reported to possess many useful properties, including oestrogenic activity, antiinûammatory activity, enzyme inhibition, antimicrobial activity. For centuries,

preparations that contain flavonoids as the principal physiologically active constituents have been used by physicians and lay healers in attempts to treat human diseases.[4-5] Various medicinal importance of rutin are anti-hyperglycaemic and antioxidant activity, Heptoprotective, Improves spatial memory in Alzheimer's disease because of its anti-oxidant and anti-inflammatory activity, in treatment of Type 2 Diabetes by inducing the insulin signaling pathway causing increased GLUT4 translocation and increased glucose uptake, prevents stroke, heart attack, cardiovascular disease,Anti-inflammatory effect on arthritis ,anti-fungal effect & anti-cancerous effect.[6] The results of this study, it is clearly indicate that isolated compound (HS-I) from leaves of *Annona squamosa* is rutin.

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