CHAPTER-3

CHROMATOGRAPHIC STUDY OF THE PLANT *CUDRANIA JAVANENSIS*

3.1 *Cudrania javanensis*, Trecul, Moraceae

See part-I, section 1.1 and page 13

3.2 SAMPLE COLLECTION

The plant materials of the fruits have been collected from different parts of Assam during October-November, 2004. The identification of the plant species is made by comparing with the Herbarium maintained at Botany Department, Gauhati University, Assam. All the samples are well classified and documented with proper labeling after drying in the shed. A voucher specimen has been deposited in the Division of Life Sciences of Institute of Advanced Study in Science & Technology, Guwahati, Assam, India.

Figure 3.1: Fruits of Saru moin (*Cudrania javanensis*)

3.3 CHEMICAL CONSTITUENTS

The plant, *Cudrania javanensis* has been reported to contain isoflavonoids . an important class of compounds having broad medicinal properties. We have isolated two isoflavonoids from the fruits of this plant.

The present investigation deals with the isolation, purification, and structure elucidation of two major compounds, viz. { 5-hydroxy-3-(3-hydroxyphenyl)-8,8 dimethyl-6-(3-methylbut-2-enyl)-4H.8H-pyrano[2,3-h]chromen-4-one]} (MN-01) and {5,7,4'-trihydroxy-6,3'-diprenylisoflavone}[3] (MN-02) from the fruits of *Cudrania javanensis.* One of the compounds isolated (MN-02) has been undertaken for detailed of crystal structure study as the crystal structure of MN-01 has already been reported [3].

3.4 FLAVONOIDS

In this study, it is found that the two compounds isolated from the fruits of this plant *(Cudrania javanensis)* are isoflavonoids { 5-hydroxy-3-(3-hydroxyphenyl)-8.8-dimethy(3-methylbut-2-enyl)-4H,8H-pyrano[2,3-h]chromen-4-one]}and{5,7,4'-trihydroxy- 6, 3'-diprenylisoflavone}[4].

Isoflavones show tremendous potential to fight disease on several fronts. They have been shown to help prevent the buildup of arterial plaque, which reduces the risk of coronary heart disease and stroke[5] Isoflavones may help reduce breast cancer by blocking the cancer-causing effects of human estrogen [6]. They may also prevent prostate cancer by hindering cell growth Isoflavones can fight osteoporosis by stimulating bone formation and inhibiting bone resorption. They may even relieve some menopausal symptoms as well.

Being a weak form of estrogen, isoflavones can compete at estrogen receptor sites, blocking the stronger version naturally produced by the body from exerting its full effect. Since high blood levels of estrogen are an established risk factor for breast cancer; weaker forms of estrogen may provide protection against this disease. Genistein has been found to hinder breast cancer[7] as well as prostate cancer. Results from a new University of California study show that genistein slowed prostate cancer growth and caused prostate cancer cells to die. It acts against cancer cells in a way similar to many common cancer-treating drugs.

The reduction of isoflavones has been actively studied during the last twenty years, owing to the range of interesting biological effects,[8-10] estrogenic activity, promising anti-cancer activity[11], osteoporosis, and coronary heart disease prevention activity shown by the isoflavones themselves and their reduced metabolites. Another strategy involves the hydrogenation of isoflavones using a palladium or platinum catalyst but mixtures of reduction products are often formed [12-13].

The antioxidant behavior of isflavonoids and the related activity-structure relationships have already been investigated[14-15]. The antioxidant activity of a flavonoid depends upon the number of hydroxyl substitutions in its backbone structure, which has no antioxidant action. In general, the more hydroxyl substitutions, the stronger the antioxidant activity. The flavonoids that contain multiple hydroxyl substitutions showed antiperoxyl radical activities several times stronger than Trolox [15], an alpha-tocopherol analogue. The single hydroxyl substitution at position 5 provides no activity, whereas the di-OH substitution at 3' and 4' is particularly important to the peroxyl radical absorbing activity of a flavonoid.

These above mentioned properties of the compounds present in this plant had prompted us to include this particular plant for our investigation to study in details the crystal structures of the phyto constituents of this plant.

3.5 Extraction of fruits of *Cudrania javanensis*

3.5.1 Extraction and isolation of *Cudrania javanensis*

Collected fruits of *Cudrania javanensis* (1 Kg) are first shed dried in an airy room and the finely powdered fruits are extracted with Methanol in a soxhlet apparatus. The extract on concentration under reduced pressure gave a yellow coloured material (45g) (CJ-00). These materials are washed several times by petroleum ether (60-80°C) followed by ethanol. This amount is then column chromatographed using solvents of the following composition in this order as eluent-

i. Pet Eth (60-80^{0}C)

ii. EtOAc

iii. MeOH

After careful column chromatography a light yellow solid is isolated and on crystallization (benzene: MeOH: 9:1) gave crystalline light yellow solid (0.09g). These solid gave two purple spots on T.L.C. plate (Petroleum ether: Ethyl acetate 8:2), when sprayed with vanillin/H_2SO_4 followed by heating at 100°C. The solid yielded two light yellow compounds, MN-01 and MN-02 on further chromatography. After doing different chemical tests, it is confirmed that these two compounds are unsaturated isoflavonoids[16]. These two pure compounds are identified as (MN-01){ 5-hydroxy-3-(3-hydroxyphenyl)-8,8-dimethyl-6-(3-methylbut-2-enyl)-4H.8H-pyrano[2,3-h]chromen-4-

one]} and (MN-02) {5,7,4'-trihydroxy- 6,3'-diprenylisoflavone} by comparison of their m. p., IR, ^{1}H NMR and Mass spectral data[17-19] with the reported values as well as by comparing with authentic samples. The melting points of the samples (MN-01) and (MN-02) are 175^{0}C and 144^{0}C respectively and the IR, NMR and Mass spectra of the samples are shown in the figures 4,5,6,7,8,9,10 and their data are given tables 3.2 & 3.3 respectively.

Table 3.1: Physical and Spectral data of the compound MN-01

Physical and Spectral Data	MN-01
Molecular formula	$C_{25}H_{24}O_4$
Molecular weight (gm)	404.3
Melting point (°C)	175
^{1}H NMR (δppm)	(δ_H 13.086,s,H-2),(δ_H 7.377,s,H-2′),(δ_H 7.353,s,H-4′),(δ_H 7.252 ,d, H-6′), (δ_H 3.359 ,dd,H-5),(δ_H 1.610,d,CH_3)
IR υ_{max} (KBr)cm^{-1}	3405(br-OH), 2971, 2921(-CH), 1645(Conjugated C=O), 1610 (Conjugated C=C), 1245, 1215(C-O-C).
Mass Spectra	m/Z 404.3 $[M]^+$

Table 3.2: Physical and Spectral data of the compound MN-02

Physical and Spectral Data	MN-02
Molecular formula	$C_{25}H_{26}O_5$
Molecular weight (gm)	406.46
Melting point (°C)	144
^{1}H NMR (δppm)	(δ_H 13.217,s,H-5),(δ_H 7.807,s,H-2),(δ_H 6.366,s,H-8),(δ_H 7.236,d,H-2′), (δ_H 7.230 ,dd,H-6′),(δ_H 6.858,d,H-5′)
IR υ_{max} (KBr)cm^{-1}	3246(br-.OH),2925(-CH) 1648(Conjugated C=O),1624(Conjugated C=C) 1207(C-O-C),1506(aromatic ring)1419,1358,1265,1171, 1094, 825,789,600,446
Mass Spectra	m/Z 406.46 $[M]^+$

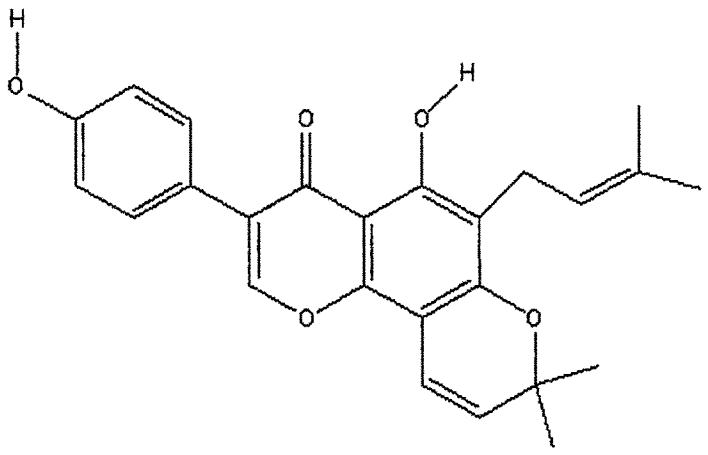

Figure 3.2: Structure of compound MN-01

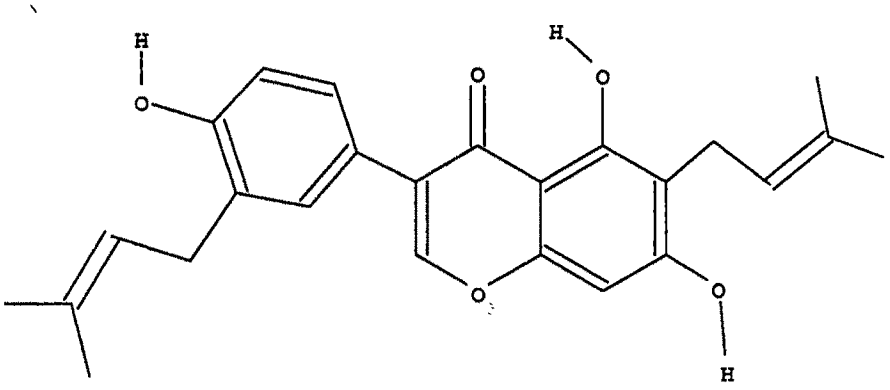

Figure 3.3: Structure of compound MN-02

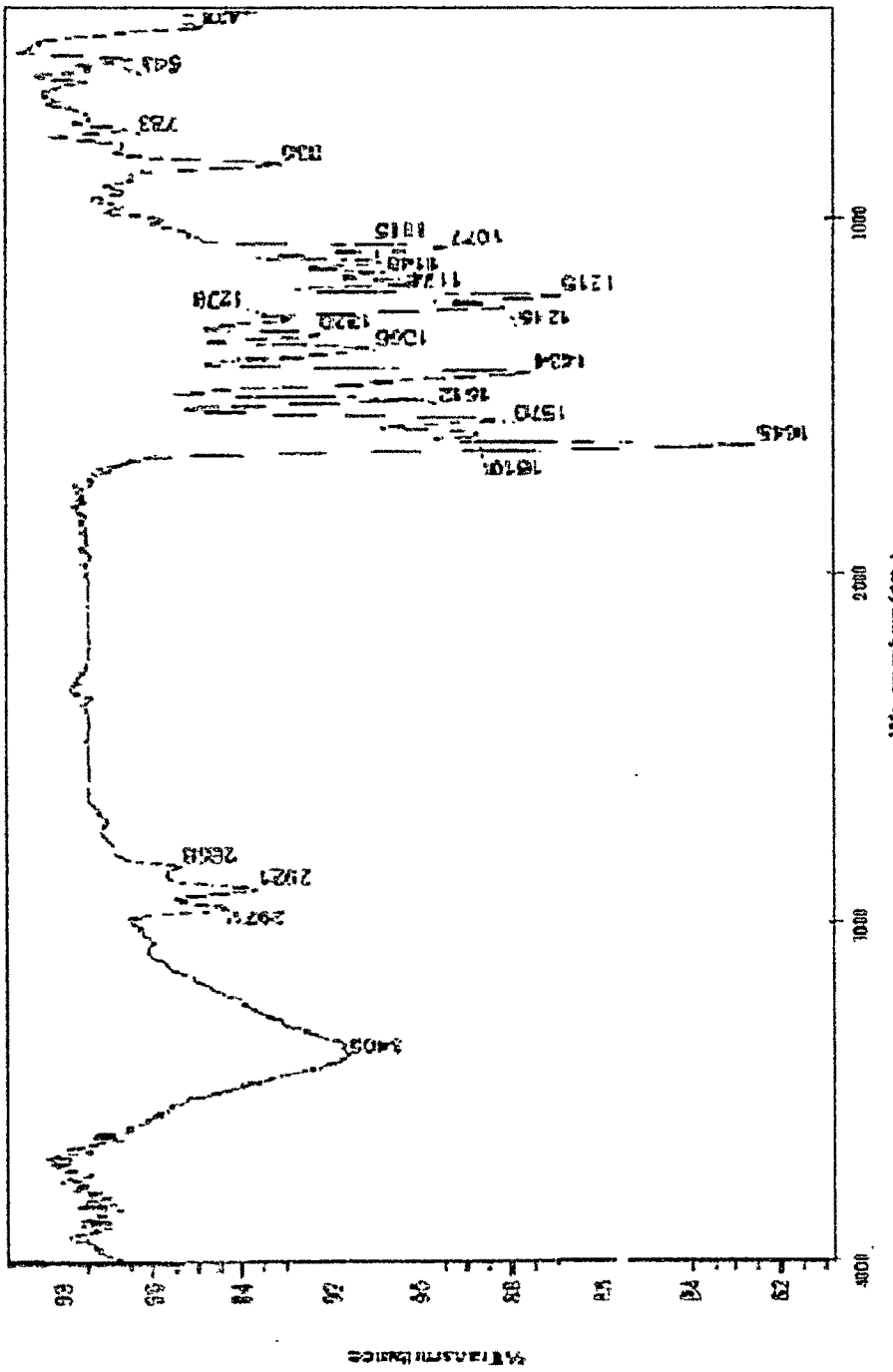

Figure 3.4 IR Spectra of MN-01

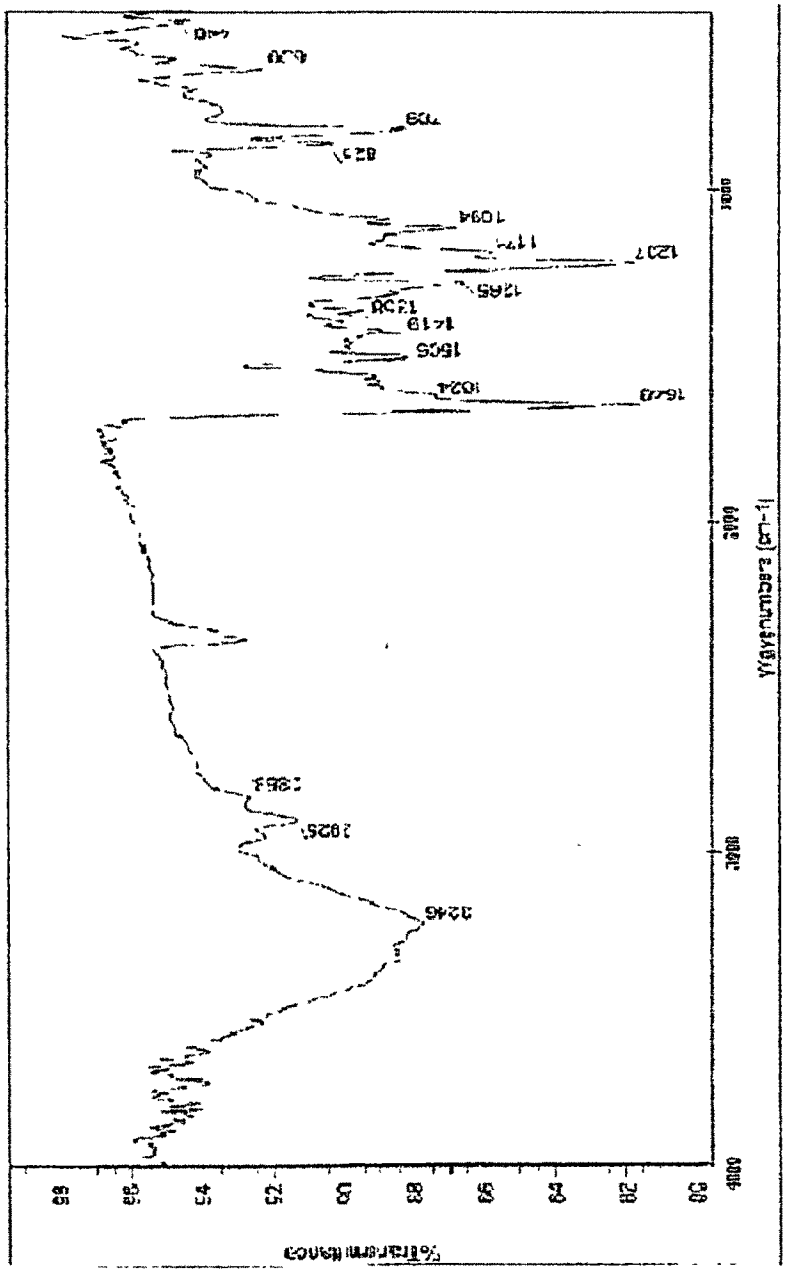

Figure 3.5:IR Spectra of MN-02

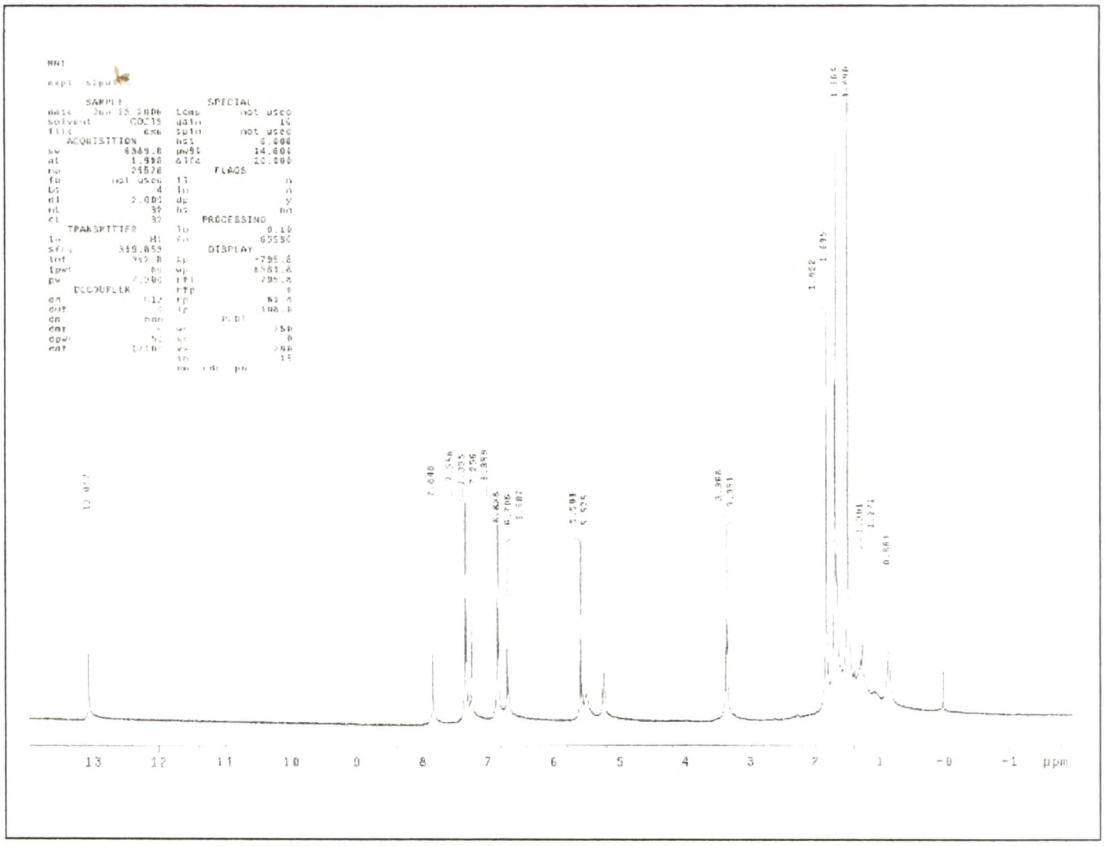

Figure 3.6: ^{1}H NMR Spectrogram of the compound MN-01

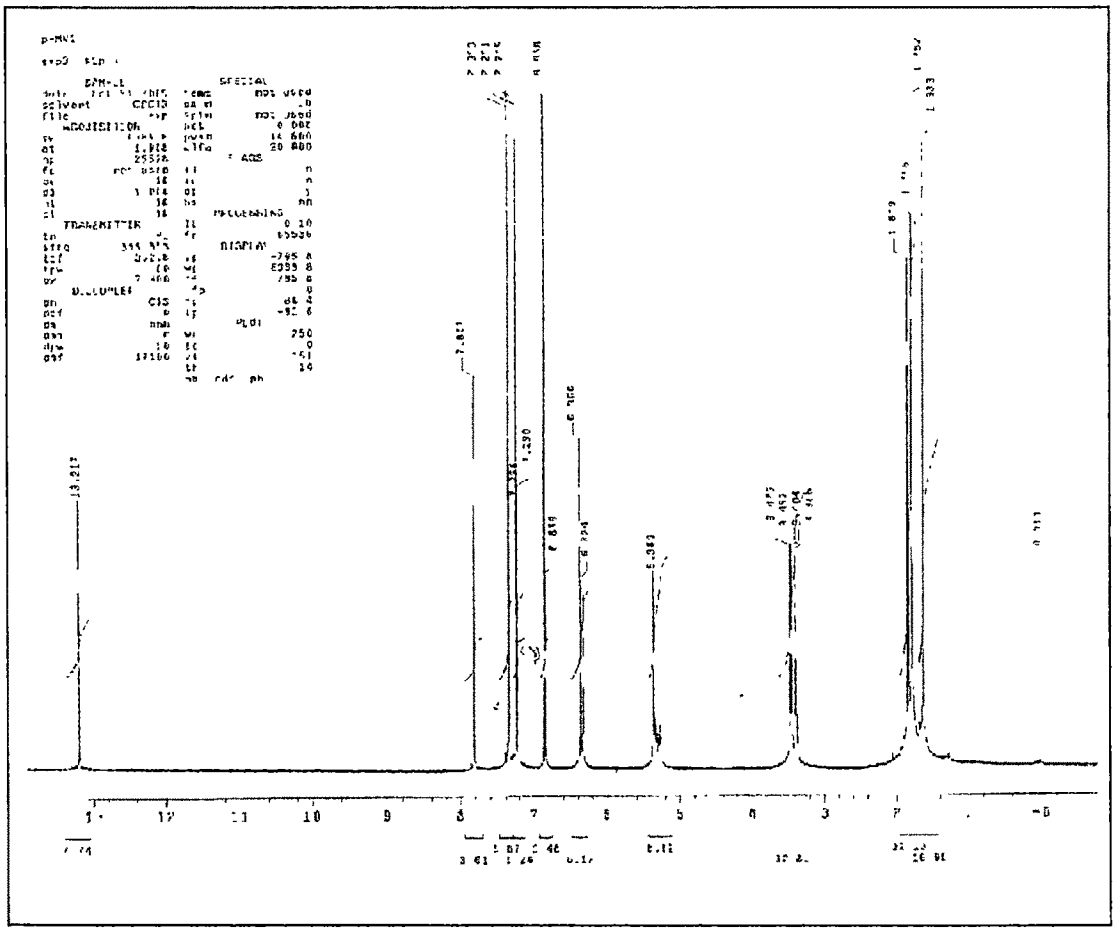

Figure 3.7: ^{1}H NMR Spectrogram of the compound MN-02

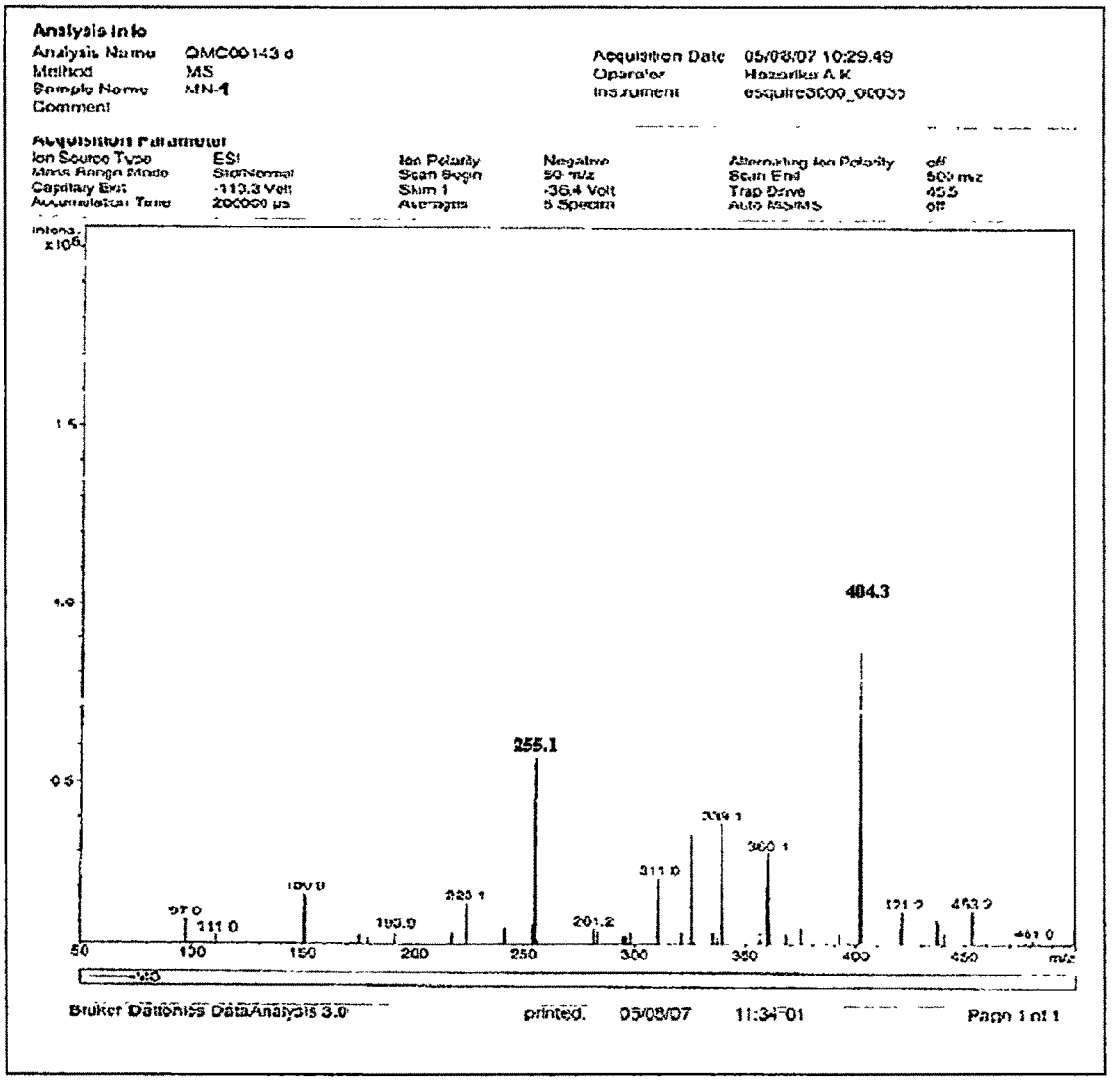

Figure 3.8: Mass Spectrogram of the compound MN-01

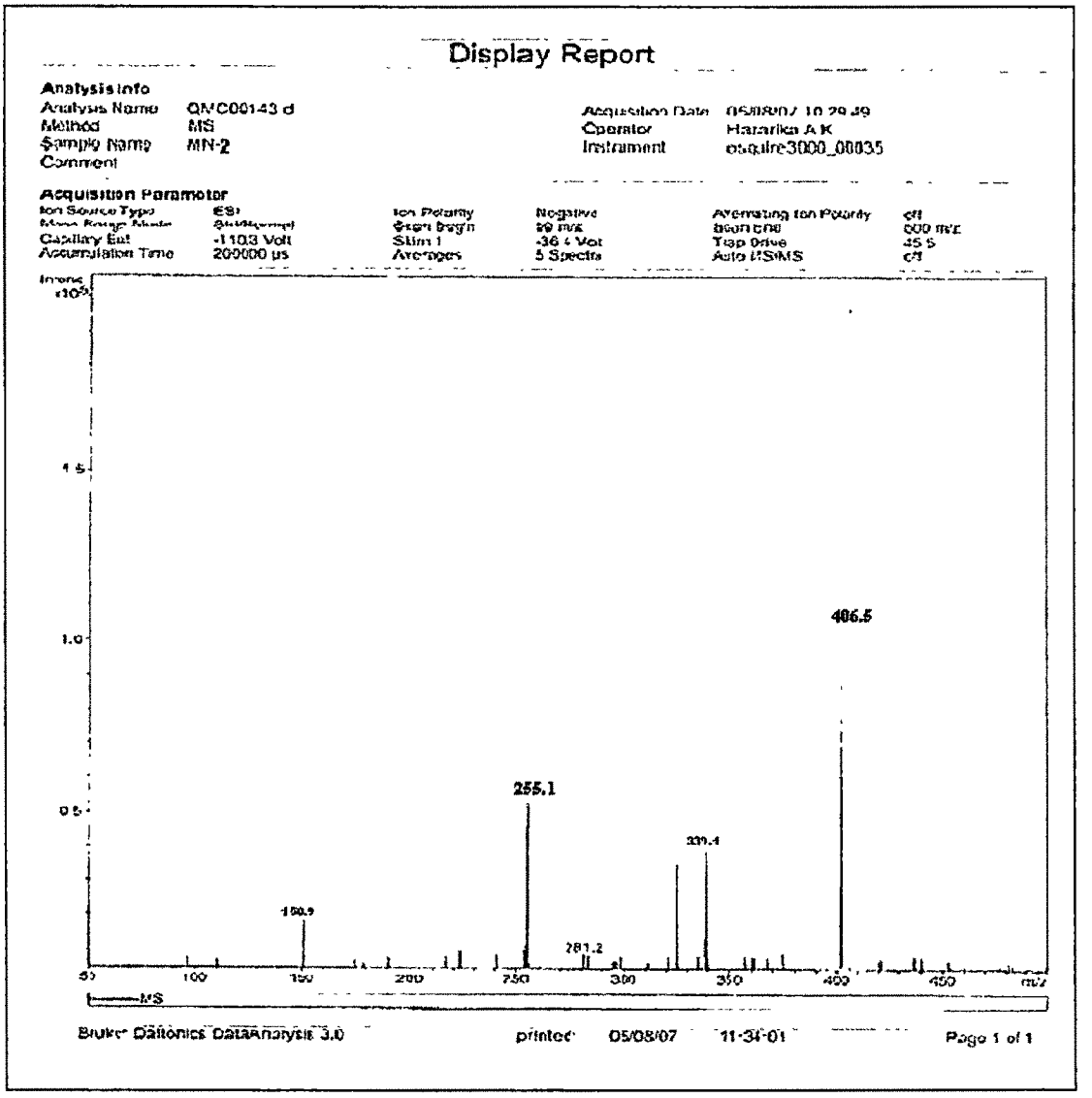

Figure 3.9: Mass Spectrogram of the compound MN-02

2.6. RESULT & DISCUSSION

From the chromatographic separation method of the fruits *Cudrania javanensis* two compounds are isolated from the Petroleum ether fraction and purified by TLC. On repeated crystallization (benzene: MeOH:: 9:1) gave crystalline light yellow solid (0.09g). These two pure compounds are identified as (MN-01){ 5-hydroxy-3-(3-hydroxyphenyl)-8,8-dimethyl-6-(3-methylbut-2-enyl)-4H.8H-pyrano[2,3-h]chromen-4-one]} and (MN-02) {5,7,4'-trihydroxy- 6,3'-diprenylisoflavone} by comparison of their m. p., IR,^{1}H NMR and Mass spectral data[15-17] with the reported values as well as by comparing with authentic samples. The melting points of the samples (MN-01) and (MN-02) are 175^{0}C and 144^{0}C respectively and the IR, NMR and Mass spectra of the samples are shown in the Figure 4,5,6,7,8,9,10 and their data are given table 2 ,table 3 respectively.

The cardio protective effects of the isoflavonoid, MN-01 against ischemia-reperfusion induced injury are studied[5]. This is the first report of the isolation of these two isoflavonoids from this species.

The crystal and molecular structure of MN-01 & MN-02 have been discussed in this thesis.

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