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Phytochemical Evaluation of *Chrozophora rottleri* (Geiseler) A. Juss. *ex* Spreng.

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A B S T R A C T

Chrozophora rottleri belongs to Euphorbiaceae family commonly known as Suryavarti. The plant occurs naturally throughout India, Myanmar, Thailand, Andaman Islands, and Central Java: Malesia. *C. rottleri*, an erect hairy annual common waste lands, blossoms profusely from January to April. It is an erect herb with silvery hairs; lower part of stem is naked, upper part hairy and has slender tap-root. The three-lobe leaves are alternative, thick and rugose. The plants are monoecious, the flowers borne in sessile axillary racemes with staminate flowers in upper and pistillate flowers in the lower part of raceme. The major phytochemicals of *C. rottleri* include Alkaloids, carbohydrate, glycosides, tannins, steroids, flavonoids and saponins, quercetin 3-o-rutinoside (1, rutin), acacetin 7-orutinoside (2), and apigenin 7-o-b-d-[6-(3,4- dihydroxybenzoyl)] -glucopyranoside (named, chrozo phorin, 5). In the present investigation important phytochemicals of aerial parts *Chrozophora rottleri* have been studied in the ethanol extracts using Paper Chromatography, Mass spectroscopy, Thin Layer Chromatography, HPLC, NMR and Mass spectroscopy techniques since there is no systematic phytochemicals carried out in this species. The investigation revealed that the aerial parts of this plant contain flavone, methylated flavones, glycosides and acylated glycosides. The seeds were found to contain a blue dye. *C. rottleri* was found to contain apigenin, apigenin 7-O-methyl ether, apigenin 7-O-β-D glucopyranoside, apigenin 7-O- (6''-E-p-coumaroyl)- β -D- glucopyranoside (a rare flavonoid) and apigenin 7-O-(3''-E-p-coumaroyl)-β -D- glucopyranoside (a new acylated flavonoid). The occurrence of flavanones is the first report from the species *Chrozophora rottleri*.

Introduction

Chrozophora belongs to the the family Euphorbiaceae, the spurge family (Webster, 1967; Webster, 2007; Hyam and Pankhurst, 1995) that encompasses 7,500 species; 422 species are described from India. Most spurges are herbs, but some, especially in the tropics, are shrubs or trees. The family is distinguished by the presence of milky sap, unisexual flowers, superior and usually trilocular ovary, axile placentation and the collateral, pendulous ovules with carunculate micropyle. The species of spurge family

widely occur in warmer climate, also they extend into the temperature regions of Northern and Southern hemisphere but are not found in the arctic region (Lawrence, 1951). This family occurs mainly in the tropics, with the majority of the species in the Indo-Malayan region and tropical America. A large variety occurs in tropical Africa, but they are not as abundant or varied as in these two other tropical regions (Gibbs, 1974). However, Euphorbia also has many species in non-tropical areas such as the Mediterranean Basin, the Middle East, South Africa, and Southern USA. The leaves are alternate, seldom opposite, with stipules. They are mainly simple, but where compound, are always palmate, never pinnate. Stipules may be reduced to hairs, glands, or spines, or in succulent species (Paul *et al.,* 2014; Betancur-Galvis *et al.,* 2002) are sometimes absent.

Chrozophora is the sole genus in the subtribe *chrozophorinae of* Euphorbiaceae. It comprises 11 species, which are mostly monoecious herbs and under shrubs. This genus is distributed in Pakistan, India, West Africa and Mediterranean regions (Tene Vicente *et al.,* 2007; Caius, 1938). Five species of *Chrozophora* are known to occur in India. The plant occurs naturally in tropical African, Asia and India (Rev. Fr. Jean Ferdinand Caius, 1938).

Botanical description

Annual herbs, prostrate or ascending; main stem up to 50 cm long, stellate-pubescent or at times scabrid. Leaves alternate, 2-5 x 1-4 cm, rounded or obtuse at apex, rounded or subtruncate at base, entire or shallowly crenate-sinuate, 3-5-veined from base, somewhat bullate above when young, becoming less so with age, pubescent above, densely so beneath; petiole 1-4 cm long, densely stellate-pubescent; stipules 2 mm long, linear. Inflorescence 1-5 cm long, leaf-opposed. Male flowers: pedicels 1 mm long; sepals c. 3 mm long, lanceolate, stellate-pubescent; petals pink, 3 mm long, elliptic-oblong, lepidote without; stamens 15, united into 4 mm tall column; anthers 1 mm long. Female flowers: pedicels c. 5 mm long, extending up to 1.5 cm or more in fruit; sepals 1.5-2 mm long, linear-lanceolate, stellate-pubescent; petals minute or absent. Ovary 2 mm diameter, densely stellate-pubescent; styles 1-1.5 mm long, bifid almost from base, stellate-pubescent without, densely papillose within. Fruit 4 x 7 mm, rounded, 3-lobed, stellate-pubescent; seeds 3-3.5 x 2-2.5 mm, globose-ovoid, grey.

Scientific classification

Kingdom: Plantae; Clade: Angiosperms; Clade: Eudicots; Clade: Rosids; Order: Malpighilales; Family: Euphorbiaceae; Subfamily: Acalyphoideae; Tribe: Chrozophoreae: Subtribe: Chrozophorinae: Genus: *Chrozophora* Neck. Ex A. Juss. (1824), Pax and K. Hoffm. (1919); Species: *Chrozophora tintoria, Chrozophora rottleri*

The leaves of *C. rottleri* are very much beneficial in treatment of skin diseases (Khari, 2007) and are also used as depurative agent. From this plant, aqueous extract of this leaves has a significant anti-helmintic property against Pheritima posthuma (Priyanka *et al.,* 2010) (Indian Earth worm) and possess phytotoxic activity on rice, wheat and mustard. Suparna and Tapaswi (1999) reported that, the leaf extracts of *C. rottleri* exhibited higher inhibition of shoot, root and radial elongation than the stem and root Juice of the fruit is given in cases of cough and colds, (Khare, 2007) in countries like Nepal and leaf is used as purifying agent and seed is used as laxative (Singh *et al.,* 2010), having bioactive components (Mander, 1998). The seeds are used as cathartic (Sasinath, 2007) and have with purgative properties (Srivastava

and Agarwal, 1953). Chrozophora genus has several interesting medicinal uses, the plant ash of *Chrozophora brocchiana*, is applied to sore and the crushed leaves were rubbed on the affected sites to treat stitch in the side. The aerial parts are taken in decoction to strengthen lactating mothers and their children, and to treat fever and dysentery. While powdered dried leaves in water are taken to treat diarrhea. Root sap in water is used as ear drops to treat otitis. (Yushau, 2011). Analysis of the chemical content shows no particular reason for a beneficial action as a wound-dressing; however, there is an unusually high silica content. While *Chrozophora senegalensis* plant has been reported is an astringent for treatment diarrhea mainly caused by Salmonella specie, and in Senegal a root decoction is given to suckling babies to treat diarrhea (Etkin, 1997).

It is boiled with cereal foods and the pregnant women used a decoction of it as a body wash, also used as a remedy for syphilis; and treatment of intestinal pain, typhoid and boils (Usman *et al.,* 2007; Benoit- Vical *et al.,* 2008). The fruit juice is used as eye drops to treat more severe cases, a maceration of leaves and roots is drunk to treat loss of hair and diabetes, and a water extract of aerial parts caused an in-vivo hypoglycemic response in rats (Delazar *et al.,* 2005). It has been reported that leaves and stems extracts of *Chrozophora senegalensis* showed a high anti-plasmodial activity against two chloroquine-resistant Plasmodium falciparum strains, without toxicity in vitro and no toxicity in vivo by oral way in mice. While the leaf extracts alone showed antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*; with highly active on Salmonella typhi. In Sudan, *C. oblongifolia* stem and leaf extracts are used to treat gonorrhea and the chloroform and methanol extracts showed considerable antidiabetic activities. Ugulu *et al.,* (2009) reported that *Chrozophora tinctoria*, has a high solubility in water, and produced dark red color, but it did not show reaction with wool fiber. The plant is used traditionally to treat warts, also has been used as an emetic, cathartic, and for the treatment of fever elsewhere (Gamble, 1967).

Chrozophora plicata has an emetic, drastic and corrosive property. Its seeds are used as cathartic (Manandhar *et al.,* 2000). The leaf extracts exhibited strong fungi toxicity against *P. aphanidermatum*, the plant poisoning causes salivation, dyspnea, bloat, dullness, diarrhea, paresis of the hind limbs, recumbence and lateral deviation of the head and neck. While *Chrozophora rottleri* is traditionally used for the treatment of various diseases. In Sudan people use stems or whole plant as powdered and applied it to wounds to improve healing. The plant also used in Saudi Arabia and India to treat Jaundice and purifying blood. An infusion of seeds and leaves is taken as a laxative in Ethiopia and in Senegal, the plant is not browsed by most stock, except occasionally by sheep and goats, as it causes vomiting and diarrhea, whereas in Kenya, camels graze it. The fruits yield a purplish blue dye, which is used to dye mats in East Africa. The fruit juice is given in cases of cough and cold in Nepal (Khare, 2007).

The leaves of *Chrozophora rottleri* are used as a depurative agent and they are very much beneficial in treatment of skin diseases (Priyanka *et al.,* 2010). The seeds are used as cathartic like Ghodtapde and credited with purgative properties. Priyanka *et al.,* (2010) reported that, the aqueous extract of the leaves of this plant has a significant anti-helmintic property against *Pheritima posthuma* (Indian Earth worm). The aqueous extract of *Chrozophora rottleri* possessed phytotoxic activity on rice, wheat and mustard. In an experimental study Suparna and Tapaswi (1999) reported that, the leaf extracts of

Chrozophora rottleri exhibited higher inhibition of shoot, root and radial elongation than the stem and root.

The major phytochemicals of *C. rottleri* include Alkaloids, carbohydrate, glycosides, tannins, steroids, flavonoids and saponins, quercetin 3-o-rutinoside (1, rutin), acacetin 7-orutinoside (2), and apigenin 7-o-b-d-[6-(3,4-dihydroxybenzoyl)] -glucopyranoside (named, chrozo phorin, 5).

The oil from the seed of *Chrozophora rottleri* was reported to be rich in linoleate, while the leaves and root contain xanthone glycosides and chromone glycoside. The tannin was found in the whole plant (Madane *et al.,* 2013). Another study revealed the presence of alkaloids, carbohydrate, glycosides, tannins, steroids, flavonoids and saponins in the chloroform extract of C. rottleri (Maharaj *et al.,* 2013). Maharaj and Prabhakaran (2013) and Mothana *et al.,* (2011) reported that the weed *C.rottleri* had adverse allelopathic effects on the germination and growth of rice seedlings.

In the present investigation important phytochemicals of aerial parts *Chrozophora rottleri* have been studied in the ethanol extracts using Paper Chromatography, Mass spectroscopy, Thin Layer Chromatography, HPLC, NMR and Mass spectroscopy techniques since there is no systematic phytochemicals carried out in this species.

Materials and Methods

Instruments and Chemicals

EI-MS was measured on JEOL JMS600 Hz (Japan) and Shimadzu Qp-2010 plus (Japan). NMR analysis (1H-NMR, 13C-NMR and DEPT) were measured on Bruker Mercury-VX-400 MHz spectrometer (Germany), Varian Mercury VX-300 MHz spectrometer (USA) and JEOL TNM-LA-400 MHz spectrometer (Japan) using TMS as internal standard. Column chromatography was carried on silica gel (70-230, mesh, E-Merck, Germany), Sephadex LH-20 (Fluka, 25-100µm, Sigma-Aldrich chemicals, Switzarland), TLC was carried on precoated silica gel plates G60 F254 (E-Merck, Germany). The plates were examined under UV light at (365 and 254 nm). The spots are sprayed with 10% *v/v* H_2SO_4 in MeOH and heated at 110-140 0C till maximum spot intensity. Authentic reference materials were purchased from Merck, Germany.

The following solvent systems were used for TLC:

Methylene chloride-methanol (95: 5 v/v)
n-hexane - ethyl acetate (80:20 v/v)
Methylene chloride-methanol (93:07 v/v)
Methylene chloride-methanol (90:10 v/v)

All solvent used are of analytical grade.

Plant materials

The aerial parts of *Chrozophora rottleri* were collected in April 2018 from a local garden near the G. D. College, Begusarai. The air dried aerial parts (1 kg) were extracted for three times with boiling 95% ethyl alcohol (3X3L) and concentrated in vacuum. The aqueous alcoholic concentrate was fractionated using benzene, ether, ethyl acetate and ethyl methyl ketone.

Air dried aerial parts (1kg) of *Chrozophora rottleri* were extracted for three times with boiling 95% EtOH (3X3L) and concentrated in vacuum. The aqueous alcoholic concentrate was fractionated using benzene, ether, methyl acetate, ethyl acetate and ethyl methyl ketone. The benzene fraction gave no characteristic spot for flavonoids on paper chromatogram. The ether fraction gave two purple – purple

spots on paper chromatogram (15% AcOH) under UV and UV/ NH_3. This fraction was subjected to column chromatography over sephadex LH-20 using methanol. 25 fractions were collected, each of 10 ml. Fractions 5- 16 yielded a light yellow coloured solid (40mg) indicated as compound I.

Fractions 18- 25 yielded another yellow solid (20mg) indicated as compound II. On Paper chromatography (15% AcOH) were found to contain three compounds using the EAC and MEK fractions. Hence these fractions were mixed then concentrated and subjected to column chromatography using stationary phase as sephadex LH- 20 and mobile phase as methanol. 60 fractions each of 20ml were collected, Fractions 5-28 yielded a homogenous yellow solid (70mg) indicated as compound III. Fractions 34-47 yielded a greyish yellow solid indicated as compound IV and fractions 50-60 deposited an another greyish yellow solid designated as compound V.

Characterization of compound I (5, 7, 4'-trihydroxy flavone: apigenin)

Compound I of molecular formula is $C_{15}H_{10}O_5$ and its melting point is 348- 350□ C, yellow colour is obtained with alkalis, olive green when subjected to with ferric chloride and deep red with Mg-HCl. Under UV and UV/NH3 it gave purple and had Rf (Table 1) which was by the UV spectrum (λmax., MeOH. 267, 296sh, 336nm) further supported for the characteristic of a flavone. The bathochromic shift of 48 nm in band I of AlCl3spectrum indicated presence of a free 5-OH in the compound I when compared to band I of MeOH spectrum and presence of a free 7-OHindicated by the bathochromic shift of 6 nm in band II on addition of NaOAc. presence of a free 4'-OH indicated by the bathochromic shift of 56 nm in band I (without decrease in intensity) of NaOMe spectrum and the absence of any characteristic bathochromic shift in band I of $NaOAc/H_3BO_3$ spectrum gave testimony for the absence of ortho dihydroxy system in B-ring. Thus compound A was characterized as 5, 7, 4'-trihydroxy flavone (apigenin). The 1H NMR spectrum showed signals at δ13.39for 5-OH and at δ10.98 for7-OH beyond that the expected characteristic chemical shift and splitting pattern for the aromatic protons. The typical doublet pattern for 3', 5' and 2', 6'-H was obtained at δ8.34 (J = 7.8 Hz) and δ7.34 (J=8 Hz) respectively that were in exactly agreement with values already reported. Further a singlet at δ7.2for 3-H, two doublets one at 6.89 for 8-H (J=2Hz) and another at δ 6.60for 6-H respectively were observed. In 13 C NMR spectrum, signals at δ164.28(s) for C-7, at δ161.47(s) for C-4' and at 161.23(s) for C-5 confirmed the above characterization (Figure 1) along with other expected signals.

Characterization of compound II (apigenin 7-O- methyl ether: genkwanin)

Compound II of molecular formula is C16H12O5, mp. 324-327^{0}C which gave yellow colour with alkalis, gave red with Mg-HCl and Olive green when reacted with ferric chloride. Under UV and UV/NH3It was purple and had □ max, (MeOH) 268, 295,326nm and Rf (Table 1) characteristic of a flavone. Absence of any shift in band II of NaOAc spectrum and a bathochromic shift of 58nm in band I of NaOMe spectrum compared to MeOH spectrum showed the absence of free 7-OH and the presence of free 4'-OH. The presence of free –5-OH indicated by a bathochromic shift of 56nm in band I of AlCl3 spectrum in comparison with MeOH spectrum showed. Compound II was7- methyl ether of apigenin was identifiedby the formation of 5, 7, 4'- trihydroxy flavone (apigenin) on demethylation with HI. On acylation it gave a diacetate whose mp.198- 201^{0}C and on methylation yielded apigenin trimethyl ether.

Thus compound II was characterized as 5, 4'-dihydroxy-7-methoxy flavones (Figure 2).

Characterization of compound III (Apigenin 7-O-λ-D- glucopyranoside)

Compound CIII is pale yellow needles (MeOH), its mp.251-253^{0}C, the molecular formula is C21H20O10, gave yellow colour when treated with alkali, gave olive green when reacted with Fe^{3+} and red with mixture of Mg and HCl. It answered Molisch's test and Under UV was purple changing to yellow under UV/NH_3. It had Rf (Table 1) for glycoside and (Table 2) for sugar and max. (MeOH) 268, 333nm typical of a flavones glycoside. The bathochromic shift of 49 nm in band I of AlCl3 /HCl spectrum was indicated presence of free 5-OHwhen compared to MeOH spectrum. The presence of 4'- OH group showed by bathochromic shift of 54 nm in band I of NaOAc and NaOMe spectrum when compared to MeOH spectrum. When careful comparison of band II of NaOAc spectrum of glycoside and its methanol spectrum, proposed that 7-OH was involved in glycosylation (Markham, 1983; Mabry *et al.*, 1970). On acid hydrolysis compound III (2N, HCl, 100^{0}C, 2hrs.) gave an aglycone recognized as apigenin and the sugar was identified as D-glucose by the co-chromatography. On enzyme hydrolysis λ-glucosidase also gave the homogenous products as in acid hydrolysis identified the compound λ-D-glucoside of apigenin. In addition to the1H NMR spectrum exhibited signals showa characteristic of a flavone glucoside. A signle at 6.8 was due to 3-H of aglycone and the doublet at 5.05 with J=7.25 Hz was due anomeric proton of the sugar (glucose). The doublets at 7.93 with J=8.7 Hz, 6.98 with J=8.6 Hz 6.84 with J=2.5 Hz and 6.44 with J=2.5 Hz were due to C-2'& 6', 3'& 5', C-8 and C-6 protons of aglycone part and the multiplets between 3.2 to 3.69 were due the other protons of the sugar. The mass spectrum (MS electrospray) showed peaks at m/z, 455 (M+Na+, 100) expected that of molecular formula is $C_{21}H_{20}O_{10}$. Thus compound III was recognized as apigenin 7-O-λ-D-glucopyranoside (Figure 3). Its identity was again confirmed by direct comparison with the reliable sample and co-chromatography.

Characterization of compound IV (Apigenin 7-O-(6''-E-p-coumaroyl)-λ-D-glucopyranoside)

Molecular formula of compound IV is C30H26O12 and which is pale yellow crystal, its mp.337-339^{0}C, gave characteristic colour reactions, chromatographic behavior (Table 1 for glucoside and Table 2 for sugar) and UV spectral analysis with the usual shift Reagents (Voirin, 1983) showing the flavonoid nature of compound IV. Flavones glycoside further indicated by chromatographic mobility, positive Molisch's test and characteristic max. (MeOH) 268, 317 Acid hydrolysis of compound IV (2N HCl, 2hrs.) gave D-glucose, p- coumaric acid and apigenin in approximately equal proportions. Co-paper chromatography glucose, p-coumaric acid and apigenin were identified by authentic samples indicated by a bathochromic shift of 63 nm in band I of AlCl3/HCl compared to band I of MeOH spectrum. A bathochromic shift of 63 nm in band I of AlCl3/HCl compared to band I of MeOH spectrum indicated the presence of a free 5-OH in the compound IV. The presence of a free 4'-OH indicated by a bathochromic shift of 63 nm in band I of NaOMe spectrum compared to band I of MeOH spectrum. The absence of any bathochromic shift of 6-10 nm in band II of NaOAc spectrum compared to MeOH spectrum clearly indicated 7-OH was involved in glycosylation. On cold alkali treatment, compound IV gave apigenin 7-O-β-D-glucopyranoside and an organic acid (p-coumaric acid).

The 1H NMR spectrum of compound IV gave evidences for apigenin and a β-D-glucopyranosyl moiety esterified with trans-p-coumaric acid. The signal appeared at δ5.14, d with J= 7.3 Hz shows the anomeric proton of glucose indicated the β configuration. The olefininc proton exhibiting a coupling constant of 15.9 Hz. The trans sterochemistry of p-coumaric acid was concluded from concluding the trans stereochemistry of p-coumaric acid. The 13 C NMR spectrum with SEPT was confirmed the aglycone as apigenin, the sugar moiety identified as β-D- glucopyranose, the acyl group as trans p- coumaric acid and the site of glycosylation as C-7 which can be compared with the δ values of C-5'' and C-6'' of glucose with those of β-D-glucopyranose of apigenin 7-O-β-D- glucopyranoside (Gabrieli and Kokkalou, 1990). The site of take place at the site at C-6'' was decided by esterification of glucose. This was again supported by the ESIMS which showed peaks at m/z 579 (M+H) +, (C30H26O12 required 578), 433 (glucoside +H) + 271 (aglycone+ H)+ and 155 (P-coumaric acid +H)+. Thus compound IV was recognized as apigenin 7-O-(6''-E-P-comaroyl) and β-D-glucopyranoside (Figure 4) a rare compound which is reported for the first time from this family.

Characterization of compound V (apigenin 7-O-(3''-E-p-coumaroyl)-λ-D glucopyranoside)

Molecular formula of compound V is $C_{30}H_{26}O_{12}$ which was pale yellow crystals and its mp.338-339^{0}C. Its colour reaction, chromatographic behaviour, positive Molisch's test, and UV spectral analysis with usual shift reagents showed the flavonoid glycosidic nature of compound V. It had λmax almost identical with compound IV. On hydrolysis with acid it gave apigenin, D-glucose and p-coumaric acid in the ratio 1:1:1, these were identified by CO-PC with reliable samples. Its Rf values on TLC (cellulose) developed with BAW (4:1:5 upper) indicating slight difference (Rf 88) compared to compound IV (Rf 84) (Table 1) indicating that it could be an isomer of Compound IV. The comparison of UV λmax value of MeOH spectrum with the shift reagent NaOAc was confirmed by the site of glycosylation at C-7. Thus the absence of any shift in the band II of NaOAc spectrum revealed the site of glycosylation at C-7 of apigenin.

The ^{1}H NMR spectrum of compound V was almost same as that of compound IV. The signal appeared at δ5.16, d, with J= 7.32 Hz for the anomeric proton of glucose indicated a β configuration. The olefinic proton exhibiting a coupling constant 16.2 Hz was concluded.

The trans stereochemistry of p-coumaric acid (Gabrielli and Kokkalou (1990) was concluded the site of esterification of glucose at C-3'' by comparison of δ values of glucose protons with the data of apigenin 7-O-(4''-E-p-coumaroyl)-β -D- glucoside given by and of chrysoeriol 7- O-(3''-E-p-coumaroyl)-β -D-glucopyranoside by Tomas *et al.,* (1986). The positions of protons H-β and H-α (CH=CH) of p-coumaroyl moiety were in agreement with a linkage at C-3'' (sugar- coumaroyl). For compound V Tomas *et al.,* (1986) observed the values at δ7.56 and 6.38were very close to the data given by in DMSO-d6 for 3''-E-paracoumaroyl -β-D glucopyranoside (7.58 for H-β and 6.42 for H-α) and were different from the data indicated for 6'' substituted sugar in compound IV. The δ value reported (Tomas *et al.,* 1986) for C-3'' proton at the site of esterification at C-3'' of glucose was confirmed by comparing the δvalue at 5.05 ppm of compound V.

ESIMS showing peaks at m/z 579 [M+H]+ and 271 [aglycone +H+] more supported for the structure was identified as apigenin 7-O-(3''-E-p-coumaroyl) β-D- glucopyranoside. This was again confirmed by co-

chromatography with reliable sample of apigenin 7-O-(4''-E-p-coumaroyl) β-D-glucopyranoside. Mobility from in TLC and PC showed clearly that it is different than that of compound IV. Thus the compound V was recognized as apiginin 7-O-(3''-E-p coumaroyl)-β -D-glucopyranoside, which is a new natural product (Figure 5).

Twenty fractions, each of 10 ml were collected. Of these the fractions 1- 8 yielded as yellow solid (100mg) and fractions 11- 19 obtained as another yellow solid (20mg). These two compounds were recognized as compound VI and VII.

Characterization of compound VI (5, 7, 4' – trihydroxy flavanone: naringenin)

Molecular formula of compound VI is $C_{15}H_{12}O_5$ which is pale yellow needles and its mp. 245-248^{0}C, with Mg-HCl gave magenta red colour. Under UV it was purple and under UV/NH3 yellow. It developed a pink colour when a paper containing a spot of the compound was smeared with $NaBH_4$ and fumed with HCl indicating the nature of the compound as a dihydro flavonoid. It had λmax. (MeOH) 289, 326sh and Rf (Table 3) serving as a type of a flavanone. Bathochromic shift of 14nm in band II of AlCl3/HCl spectrum compared to band II of MeOH spectrum indicates the presence of free 5- OH. Bathochromic shift of 34 nm in band II of NaOAc and NaOMe spectrums compared to band II of MeOH spectrum indicates the presence of free 7-OH and this effect was further confirmed by an increase in the intensity of band II in both cases.

The appearance of signals in 1H NMR at δ5.46 (dd, J = 2.2 & 12Hz) for H-2, 3.41 (dd, J=12 & 15 Hz) for Hax –3 and 2.72(dd, J=2.8 & 15Hz) for Heq-3 were in perfect agreement with reported values for dihydro flavones. This was further supported by appearance of signals in ^{13}C NMR at δ78.69 ppm for (C-2) and δ42.23 ppm for (C-3). Further the presence of 5, 7, 4' free OH were confirmed by the appearance of signals in 13C NMR at δ166.87 ppm (C-7), 163.77 ppm (C-3) and 157.96 ppm respectively. The appearance of peak at m/z 272 (M+, 63.71) in EIMS was in agreement with the molecular formula $C_{15}H_{12}O_5$ of compound VI. Further the flavanone was converted to chalconaringenin by alkali treatment and compared with an authentic sample (Jayprakasam, 1993). Based on these observations the flavanone was identified as 5, 7, 4'- trihydroxy flavanone (Buckingham, 1995) (naringenin) (Figure 6) and the identity confirmed by CO-PC with authentic sample (Zhang *et al.,* 2014) yellow under UV/NH3. It had λmax (MeOH) 287, 325sh and Rf (Table 3) characteristic of a typical flavanone. The presence of free 5-OH was indicated by a bathochromic shift of 15nm in band II of AlCl3 / HCl spectrum compared to band II of MeOH spectrum. The presence of free 7-OH indicated the bathochromic shift of 33nm in band II of NaOAc spectrum and and bathochromic shift of 33nm in band II of NaOMe spectrum when compared to band II of MeOH spectrum. The bathochromic shifts in both cases are accompanied by an increase in the intensity of band II. Demethylation of the compound VII with HI gave a solid, which was found to be identical in all respect with compound VI. These observations suggested that the compound VII must be naringenin 4'-methyl ether (Zhang *et al.,* 2014; Grayer, 1989). This was further supported by the appearance of peak at m/z, 286 (M+, 2) in EIMS, in agreement with the molecular formula C16 H14 O5 of the compoundVII. Thus the compound was identified as 5, 7 dihydroxy 4'-methoxy flavonone: narigenin 4'- methyl ether (Figure 7) and the identity was further confirmed by direct comparison 65 and CO-PC with an authentic sample (Jiang- Hong *et al.,* 2015)

Characterization of compound VII (5, 7-dihydroxy, 4'-methoxy flavonone: narigenin 4'-methyl ether)

Yellow needles (MeOH), C16 H14 O5, mp. 248-250^{0}C, gave magenta red colour with Mg-HCl, pink with alcoholic NaBH4 and HCl. It was dull violet under UV and yellow under UV/NH3. It had □ max (MeOH) 287, 325sh and Rf (Table 1) characteristic of a typical flavanone. The presence of free 5-OH was indicated by a bathochromic shift of 15nm in band II of $AlCl_3$ / HCl spectrum compared to band II of MeOH spectrum. The presence of free 7-OH indicated the bathochromic shift of 33nm in band II of NaOAc spectrum and and bathochromic shift of 33nm in band II of NaOMe spectrum when compared to band II of MeOH spectrum. The bathochromic shifts in both cases are accompanied by an increase in the intensity of band II. Demethylation of the compound VII with HI gave a solid, which was found to be identical in all respect with compound VI. These observations suggested that the compound VII must be naringenin 4'-methyl ether (Grayer, 1989).

This was further supported by the appearance of peak at m/z, 286 (M+, 2) in EIMS, in agreement with the molecular formula C_{16} H_{14} O_5 of the compound VII. Thus the compound was identified as 5, 7 dihydroxy 4'-methoxy flavonone: narigenin 4'- methyl ether (Figure 7) and the identity was further confirmed by direct comparison (Grayer, 1989) and CO- PC with an authentic sample (Jiang- Hong *et al.*, 2015).

Statistical Analysis

Experimental results are expressed as mean ± standard error. Results were statistically analyzed using analysis of variance (one-way ANOVA) followed by student's t test for comparison between different groups. SPSS 20 version was used for the statistical analysis.

Results and Discussion

The species *Chrozopora rottleri* collected G. D College Campus, Begusarai has been systematically analyzed for their phytochemicals especially flavonoids. The investigation revealed that the aerial parts of this plant contain flavone, methylated flavones, glycosides and acylated glycosides. The seeds were found to contain a blue dye. *C. rottleri* was found to contain apigenin, apigenin 7-O-methyl ether, apigenin 7-O-β-D glucopyranoside, apigenin 7-O- (6''-E-p-coumaroyl)- β -D- glucopyranoside (a rare flavonoid) and apigenin 7-O-(3''-E-p-coumaroyl)-β -D- glucopyranoside (a new acylated flavonoid). The occurrence of flavanones is the first report from the species *Chrozophora rottleri*. The flavones apigenin is found to be very common in the species of *Chrozophora*, especially in *C.senegalensis, C. tinctoria, C. brorcchiana, C. rottleri* and *C. plicata*. If the remaining species are subjected to systematic chemical analysis and will proved to show the presence of apigenin then apigenin and their derivatives in the species of *Chrozophora* can be the earmark phytochemical flavone to be used as the chemotaxaonomic marker of the genus *Chrozophora* of Euphorbiaceae family.

The structures of all the seven flavonoids were identified by UV, NMR and MS studies. On hydrolysis compound IV gave the aglycone, (apigenin), sugar (D-glucose) and P-coumaric acid in the ratio of 1:1:1. By observing a characteristic peak at 579 (M+H+, 20) in the Electrospray MS the glycosides as glucoside with paracoumaric acid of apigenin were identified. The glycosylation was at C-7 which was confirmed by UV spectrum in NaOAc. The ^{1}H NMR spectrum of Compound IV confirmed the 5, 7, 4'- tri oxygenated flavone structure of aglycone. Fixing the stereochemistry of the glycosidic linkage as β-linked, is in agreement with the anomeric

configuration of glucopyranoside of flavonoids reported by the anomeric proton of glucose appeared at δ5.14 d with J=7.3Hz, ^{13}C NMR was confirmed by the site of esterification of glucose at C-6''. The appearance of C-6'' at 64.12ppm (down field shift of +3.4) comparing to unsubstituted C-6'' at 60.7 and the appearance of C-5'' (neighbouring carbon) at 74.3 in compound IV (an up field shift of –2.1) comparing to compounds of unsubstituted sugars of C-5'' had confirmed the site of esterification at C-6'' of glucose. The appearance of peaks in ^{1}H NMR at δ6.29 d, with J=15.9Hz was confirmed the Trans stereochemistry of the olefininc protons in p-coumaric acid.

The natural product was identified as apigenin 7-O-(3''-E-p-coumaroyl)-λ-D-glucopyranoside an isomer of Compound IV. On acid hydrolysis of this glycoside gave the same results as compound IV. Its UV spectrum and Mass spectrum in electrospray were closely identical. In all the substituted sugars the H-1'' proton shifts shown in down field. For this compound anomeric proton appeared at □ □ 5.17compared with □ □ at 5.05 of H- 1''when compared with of unsubstituted sugar of compound III revealed that the presence of substitution of paracoumaric acid with one of the OH of the sugar glucose. The careful comparison of ^{1}H NMR values of this compound with the value of chrysoeriol 7-O-(3''- E-P-coumaroyl λ-D-glucosidewas confirmed the site of esterification at C-3'' of glucose. The H-3'' proton signal (usually appear at □ □ 3.4-3.5 as multiplet in unsubstituted sugars) of the compound V appeared at □ □ 5.05 was in closeagreement with the □ □ values at 5.06 for chrysoeriol 7-O-(3''-E-p-coumaroyl) λ□-D-glucoside are reported. Based on all above facts, the structure of compound V was confirmed as apigenin 7-O-(3''-E-p-coumaroyl)-λ-D-glucopyranoside a new natural productis obtained in low concentration when comparing to other compounds have been isolated. The flavanone naringenin, the major component of *C.rottleri* was characterized using fully ^{1}H, ^{13}C NMR and Mass spectral studies. The compound naringenin 4' – methyl ether was identified by comparing the Mass spectrum of this compound (m/z, 286) and that of the demethylated product which exhibited the molecular ion peak at (m/z, 272), identical to that of naringenin. Thus the compound was identified as 4' – methyl naringenin.

The EAC and MEK fractions were concentrated and column chromatographed over sephadex LH-20 using methanol as eluting agent. From these observations50 fractions each of 20ml were collected. Fractions 1- 12 yielded as yellow solid, fractions 13-16 yielded as yellow solid, fractions 19-25 yielded as yellow needles. Similarly, fractions 26-34 and 36-45 obtained two greyish yellow solids. All these compounds were found to be the same as compounds I, II, III, IV, V, VI and VII isolated and identified by chemical and spectral methods from *C.rottleri.*

Structures of Compounds Isolated from *Chrozophora rottleri*

Compound I (Apigenin)

It gave pale yellow needles when reacted with methanol. Its mp 348-350^{0}C, and its (50 mg), molecular formula is C15H10O5. It gave yellow colour with basic solutions (NH_3, Na_2CO_3 and NaOH) and pink colour with Mg-HCl and olive green when reacted with ferric chloride. Under UV it was purple and under UV/NH_3 light yellow.

UV (□ □ max., nm)

MeOH: 267, 296sh, 336
NaOAc: 274, 301, 376
NaOAc/H3BO3: 268, 302sh, 338

AlCl3: 276, 301, 348, 384
AlCl3/HCl: 276, 299, 340, 381
NaOMe: 275, 324, 392

Rf Table 1

^{1}H NMR (200.13 MHz, DMSO-d6,□ □ , ppm) (Spectrum - 1)

13.39 (s, 1H, OH-5), 10.98 (s, 1H, OH-7), 8.34(d, J=7.8 Hz, 1H, H-3', 5'), 7.34 (d, J=7.9Hz, 1H, H-2', 6'), 7.20(s, 1H-3), 6.89 (s-1H-8), 6.60 (s, 1H-6).

^{13}C NMR (50.32 MHz, DMSO-d6, □ , ppm) (Spectrum- 2)

181.78 (C-4), 164.28 (C-7) 163.75 (C-2), 161.47(C-4'), 161.23(C-5), 157.34(C-9), 128.52(2', 6'), 121.18(1'), 116.0 (3',5'), 103.68(C-10), 102.85 (C-3), 98.91(C-6), 94.02 (C- 8).

Acetylation of compound I (apigenin triacetate)

Compound I (5mg) was dissolved in few drops of C5H5N and treated with 2ml of Ac2O.It was poured into broken ice, kept for 3hrs at room temperature for 24 hrs and then filtered. When subjected to recrystallation

The solid when recrystallized by ester and petrol yield colourless needles, mp. 185-187□ C.

Methylation of compound I (apigenin trimethyl ether)

Compound I (5mg.) was dissolved in 10 ml of dry Me_2CO added to the mixture 1ml Me_2SO_4 and 1g of anhydrous K_2CO_3 and refluxed for 36 hrs at 70□ C. The reaction product was cooled, filtered, washed with Me_2CO. The residue from Me_2CO was added to cold water. The white solid formed was filtered, washed with cold water, dried and re-crystallized from MeOH to yield colourless needles, mp. 156-157□ C.

Compound II (5, 4' dihydroxy-7 methoxy flavone: apigenin 7-methyl ether)

It is yellow needles (EtOAc- petrol) and its mp. is 325-327□ C, (20mg), and molecular formula is $C_{16}H_{12}O_5$. It gave permanent yellow colour with basic solutions like NH3, Na2CO3 and NaOH, gave red colour with Mg-HCl and gave Olive green with Fe3+. Purple colour pruduced under UV and yellow colour produced under UV/NH3.

UV (□ □ max., nm)

MeOH: 268, 293, 326
NaOAc: 260, 301, 370
NaOAc/H3BO3: 268, 296, 326
AlCl3: 276, 301, 348, 382
AlCl3/HCl: 276, 299, 340, 381
NaOMe: 275, 324, 384

Rf Table 2

IR (□ □ max., cm-1, KBr)

3320br, 1610, 1490, 1270, 1215, 1190, 1120, 1065, 1000, 860, 840, 820.

Acetylation of compound II (genkwanin diacetate)

Genkwanin diacetate was added to the mixture acetic anhydride and pyridine yielded colourless needles of mp. 199-201□ C and it was recrystallised from the mixture of EtOAc and petrol.

Demethylation of compound II (apigenin)

Compound II (5mg) was dissolved in dimethyl ketone and 2ml of HI then refluxed about 2hrs at 170-180□ C. When treated with saturated

sodium bisulphate solution the excess of iodine and HI were destroyed and the product was extracted from ether.

The extract of dried ether on subjected to crystallisation from methanol gave yellow needles with melting point 348- 350□ C and was similar as apigenin.

Compound III (apigenin 7- O-λ-D-glucopyranoside)

This was pale yellow needles with molecular formula C21H20O10 (55mg) and mp. 251-253□ C.

It gave pink colour with Mg-HCl, produced olive green with Fe^{3+}, with alkali, and gave yellow colour with Molisch's reagent. Under UV showed purple and under UV/NH3 yellow.

UV (□ max. nm)

MeOH: 268, 333
NaOAc: 256sh, 267,355, 386
$NaOAc/H_3BO_3$: 267, 340,
$AlCl_3$: 276, 300, 348, 386
$AlCl_3$/HCl: 277, 299, 341, 382
NaOMe: 245sh, 269, 301sh, 386

Rf Table 1 for glycoside and Table 3 for sugar

1H NMR (400 MHz, DMSO-d6,□ □ , ppm) (Spectrum- 3)

7.93 (d, J= 8.7 Hz, 2H, H-2', 6'), 6.98 (d, J= 8.6 Hz, 2H, H-3',5'), 6.84 (d, J=2.5Hz, 1H, H-8), 6.8 (s, ^{1}H, H-3), 6.44 (d, J=2.5Hz, 1H, H-6), 5.05 (d, J=7.25 Hz, 1H, H-1'').

MS (ESIMS, m/z, rel intensity as % (Spectrum- 4)

455 (M+Na+, 100).

Acid hydrolysis of compound III (apigenin, D-glucose)

Compound III (5mg) dissolved in 2ml of methanol and then refluxed with 2N HCl (2ml) at 100□ C for 1hr. The reaction mixture and an equal volume of water were added and kept in the freeze throughout the night. The solid obtained was filtered and washed with water and then dried. The solid was recrystallized by methanol to obtain the aglycone.

Aglycone of compound III (apigenin)

It gave yellow needles with methanol and its mp. was 348-350□ C and identified as apigenin as described under compound I.

Identification of sugar (D- glucose)

The aqueous hydrolysate was neutralized with PbCO3after removal of the aglycone and the filtrate diffused through DOWEX 50W-X8(H+) and the eluted fraction was concentrated. The sugar was subjected to PC and TLC and was found out as D- glucose by co- Rf with reference sample.

Enzyme hydrolysis of compound III (apigenin, D- glucose)

It was hydrolyzed by enzyme λ- glucosidase to give the same products as in the case acid hydrolysis of compound C. (Rf of glycoside Table 1 and sugar Table 2).

Compound IV (apigenin 7-O- (6''- E- p-coumaroyl- λ- D- glucopyranoside)

It was pale yellow needles with methanol and had mp. of 337- 339□ C with molecular formula, $C_{30}H_{26}O_{12}$ (60mg) and gave yellow colour with alkali, produced olive green in Fe^{3+} and gave pink colour with Mg-HCl. It reacted with Molisch's reagent and under UV

was purple but changed to yellow under UV/NH_3.

UV (□ max. nm)

MeOH: 268, 317
NaOAc: 268, 317, 380
NaOAc/H3BO3: 268, 318
$AlCl_3$: 277, 284, 309, 325,381
$AlCl_3$/HCl: 278, 284, 309, 326,380
NaOMe: 287, 318, 380

Rf Table 1 for glycoside, Table- 2 for sugar and Table 3 for organic acid

1H NMR (400 MHz, DMSO- d6, □ , ppm) (Spectrum- 5)

7.86(d, J= 8.9 Hz, 2H H- 2', 6'), 7.48 (d, J= 15.6 Hz 1H H- trans λ), 7.34 (d, J= 8.6Hz 2H, H-2''', 6'''), 6.79 (d, J= 9.4Hz, 2H, H- 3', 5'), 6.78 (d, J= 2.44Hz, 1H, H-8), 6.73 (s, 1H, H-3), 6.66 (d, J=8.54 Hz, 2H, H-3''', 5'''), 6.44 (d, J=2.1Hz, 1H, H-6), 6.29 (d, J=15.9 Hz, 1H, H-trans □), 5.14 (d, J= 7.3 Hz, 1H, H-1''), 4.45 (d, J= 11.1 Hz, 1H, H-A 6''), 4.45(d,J=11.9 Hz, 1H, H-B 6''), 3.82 (m, 1H, H-5''), 3.35 (m, 3H, H-2'', 3'',4'').

13C NMR (100 MHz, DMSO, d6, □ , ppm) (Spectrum- 6)

182.1(C-4), 66.4 (C=O), 165.2(C-2), 163.0 (C-7), 161.6 (C-5), 61.6(C-4'), 160.9 (C- 4''') 157.3 (C-9), 145.5(C-λ), 130.6(C-2'''), 129.1 (C-2', 6'), 124.9 (C-1'''), 121.0 (C- 1'), 117.3 (C-3''', 5'''), 116.3(C-3', 5'), 113.8 (C-□), 105.7(C-10), 103.0 (C-3), 99.9 (C -1''), 99.8 (C-6), 94.9 (C-8), 76.7 (C-3''), 74.3 (C-5''), 73.4 (C-2''), 70.4 (C-4''), 64.1 (C-6'').

MS (ESIMS, rel intensity as %) (Spectrum- 7)

601(M+Na+, 100), 579(M+H+), 433 (glucoside+H+), 271 (aglycone +H+), 155 (p-coumaric acid +H+).

Acid hydrolysis of compound IV (apigenin, D-glucose and E- paracoumaric acid)

Compound IV (15mg) was dissolved in warm methanol and same volume of 4N HCl was added. The reaction mixture under refluxed condition was heated at 100□ C to about 3hrs. It was subjected to distillation in vacuum to remove excess of methanol and then was diluted with water and left in the ice chest for 5hr. The solid separated out was filtered, washed by cold water and then dried and shaken by Et2O and the residue obtained from ether extract was combined with solid on the filter and total aglycone weighed (5mg).

It was recrystallized from MeOH to obtain an aglycone and phenolic acid. It was neutralized with $PbCO_3$ and then filtered through Whatman No 42 filter paper and passed through column of Amberlite (120H+) resin to remove plumbate ions and then subjected to concentration. It was subjected to PC and CO-PC with authentic sample of D-glucose; both showed identical Rf value (Table 2).

Identification of the phenolic acid (E- 4 – hydroxy cinnamic acid)

The acid crystallized from methanol appear as colourless needles with mp. 219- 220^{0}C. It gave pale yellow colour when reacted with alkalis, greenish brown with ferric ion and decolourised bromine water. It gave brisk effervescence with bicarbonate solution. It was colourless under UV but changed to blue under UV/NH3.

UV (□ max, nm)

MeOH: 255, 305.

IR (□ □ max., cm-1,KBr)

3390, 2750, 1625, 1595, 1510, 1235, 1205, 830.

Rf – Table 3

HPLC

Retention time (Rt min) was determined on Zorbax C8 and Zorbax ODS (C18) column (4.6nm i.d.X 25 cm) using a flow rate of 1ml/min under a pressure of $1.0X10^2$ kg F cm-2 for Zorbax C8 and 1.8X 102 kg F cm-2 for Zorbax ODS. The acid as well as authentic p-coumaric acid had Rt=5.1 min in both the experiments (MeOH: 10% HOAc 6:4).

Identification of aglycone from acid hydrolysis (Apigenin)

It was yellow needles with MeOH and its mp. 348-350□ C and identified as apigenin as described under compound I.

Compound V (apigenin 7- O- (3''- E- p-coumaroyl) - λ- D- glucopyranoside.)

It was pale yellow needles with methanol and its formula was C30H26O12 and mp. 338-340□ C (20mg), and appeared yellow colour with alkali, gave olive green with ferric ion and pink colour with Mg and HCl. It reacted with Molisch's reagent and gave purple colour under UV but changed to yellow under UV/NH3.

UV (□ □ max. nm)

MeOH: 269, 317
NaOAc: 268, 317, 380sh
$NaOAC/H_3BO_3$: 268, 318
$AlCl_3$: 278, 299, 327, 381
$AlCl_3$/HCl: 278, 298, 326, 379
NaOMe: 267, 319, 368

Rf Table 1 for glycoside, Table- 2 for sugar and Table 3 for organic acid

^{1}H NMR (400 MHz, DMSO-d6, δ, ppm) (Spectrum- 8)

7.90(d, J=8.9Hz, 2H, H-2', 6'), 7.56 (d, J= 15.9 Hz, 1H, H-β trans), 7.54 (d, J= 8.9H, 2H H-2''', 6'''), 6.84 (d, J=8.24 Hz, 2H, H-3', 5'), 6.81 (d, J= 2.14Hz, 1H, H- 8), 6.79 (s, 1H, H-3), 6.78 (d, J=8.6Hz, 2H, H-3''', 5'''), 6.42 (d, J= 2.14Hz, 1H, H-6), 6.38 (d, J= 16.17Hz, 1H, H-α trans), 5.16 (d, J= 7.3Hz 1H, H-1''), 5.05 (d, J= 7.31Hz, 1H, H- 3''), 4.45 (d, J=11.1Hz, 1H, H-HA 6''), 4.15 (d, J= 11.9Hz, 1H, H-HB 6''), 3.82 (m, 1H, H-5''), 3.7 (m, 2H, H-2'', 4'').

MS (ESIMS, rel intensity as %) (Spectrum- 9) 601(M+Na+), 579 (M+H+), 271 (aglycone + H+) and 155 (p-coumaric acid+H+).

Acid hydrolysis of compound V (apigenin, D-glucose and E- p-coumouric acid)

Compound V (10mg) was hydrolyzed using 2N HCl as mentioned in compound IV. The aglycone, sugar and the phenolic acid obtained were identified as apigenin, D- glucose, and p-coumaric acid respectively by following same procedure described in compound IV.

Compound VI (5, 7, 4'- trihydroxy flavanone: naringenin)

This compound was yellow coloured needles (MeOH) with molecular formula C15H12O5 and mp. 246-248^{0}C produced magenta-red colour with Mg-HCl, Pink colour with alcoholic solution of NaBH4 and HCl. It appeared purple under UV and yellowish green under UV/NH3. (Found C 66.01, H 4.48, cald C66.15, H 4.45).

UV (□ max. nm)

MeOH: 289,326sh
NaOAc: 284sh, 323
$NaOAc/H_3BO_3$: 293, 330sh
$AlCl_3$: 305,373
$AlCl_3$ /HCl: 305, 371
NaOMe: 245, 273sh, 323

Botanical Description

Fig.1 Apigenin

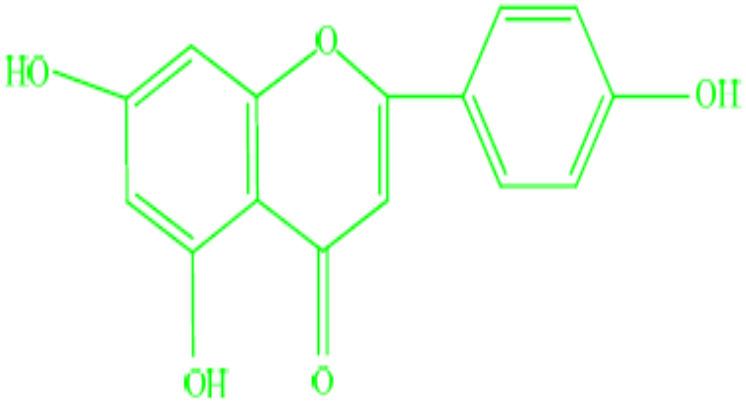


Fig.2 Apigenin- 7- O- methyl ether

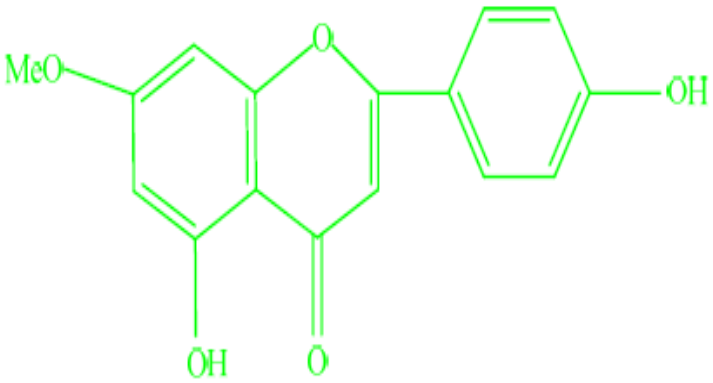


Fig.3 Apigenin- 7- O- β- D- glucopyranoside

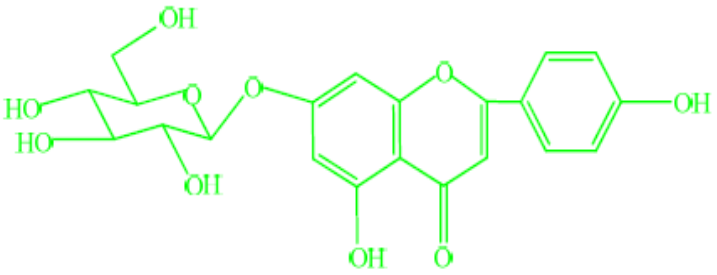


Fig.4 Apigenin- 7- O- β- D- (6”- E- p- coumaroyl) glucopyranoside

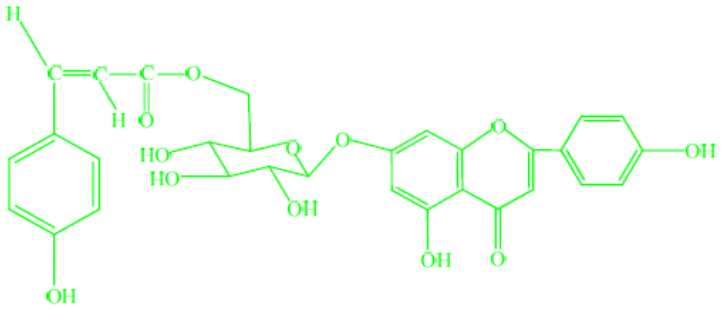


Fig.5 Apigenin- 7- O- β- D- (3” – E- p- coumaroyl) glucopyranoside

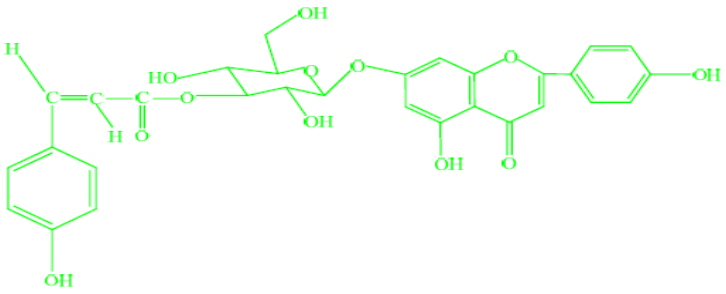


Fig.6 Narigenin

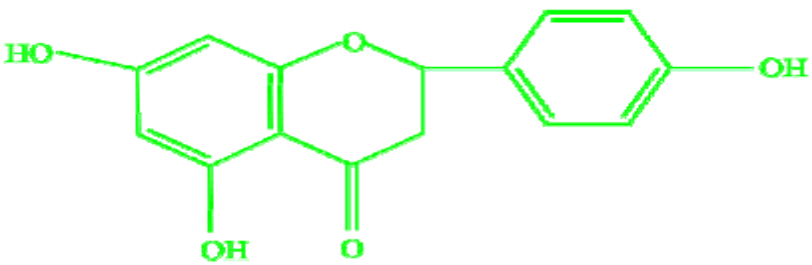
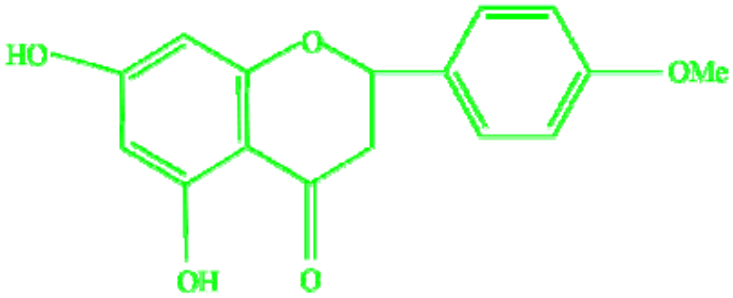
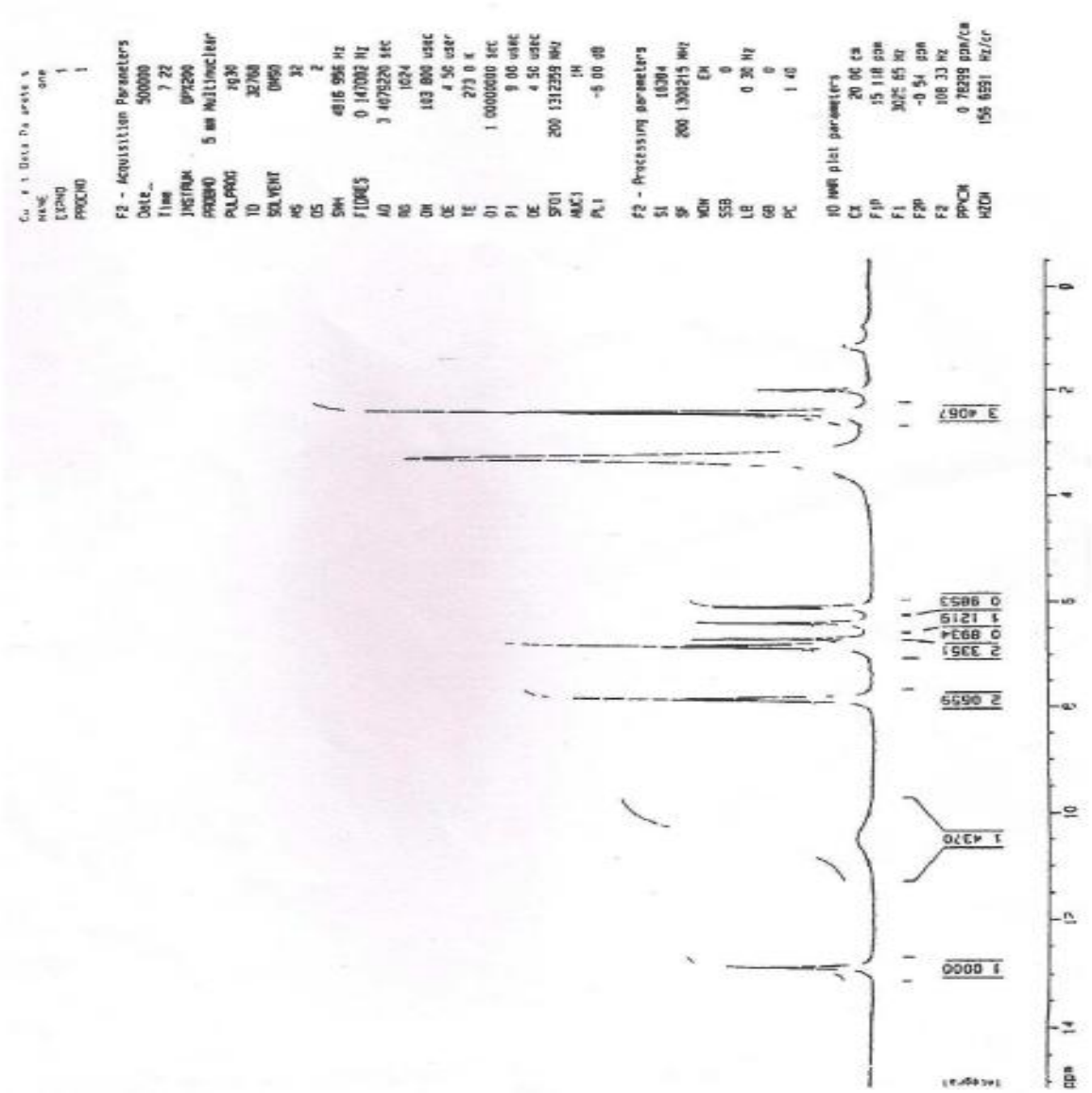


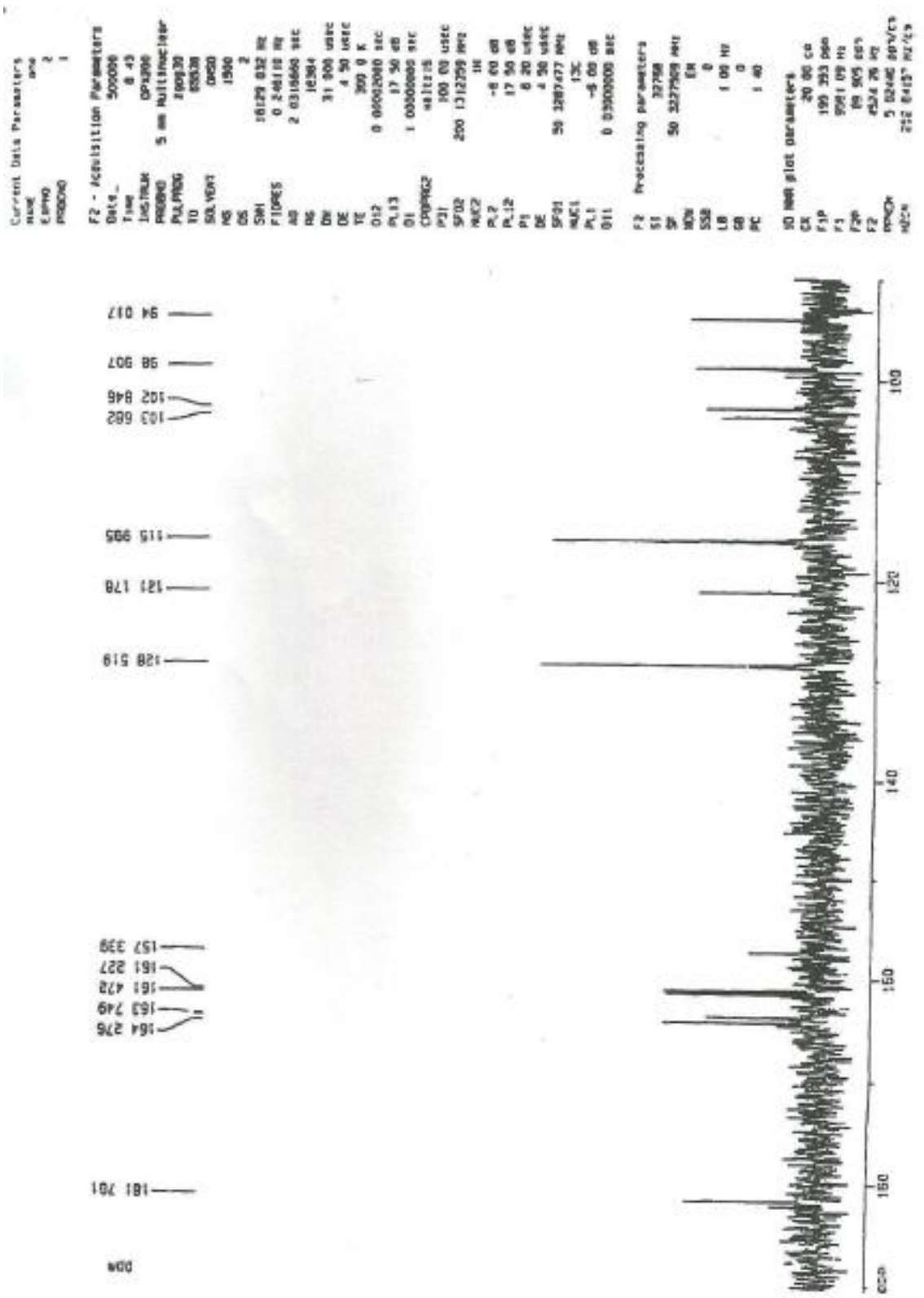
Fig.7 Narigenin- 4' – methyl ether



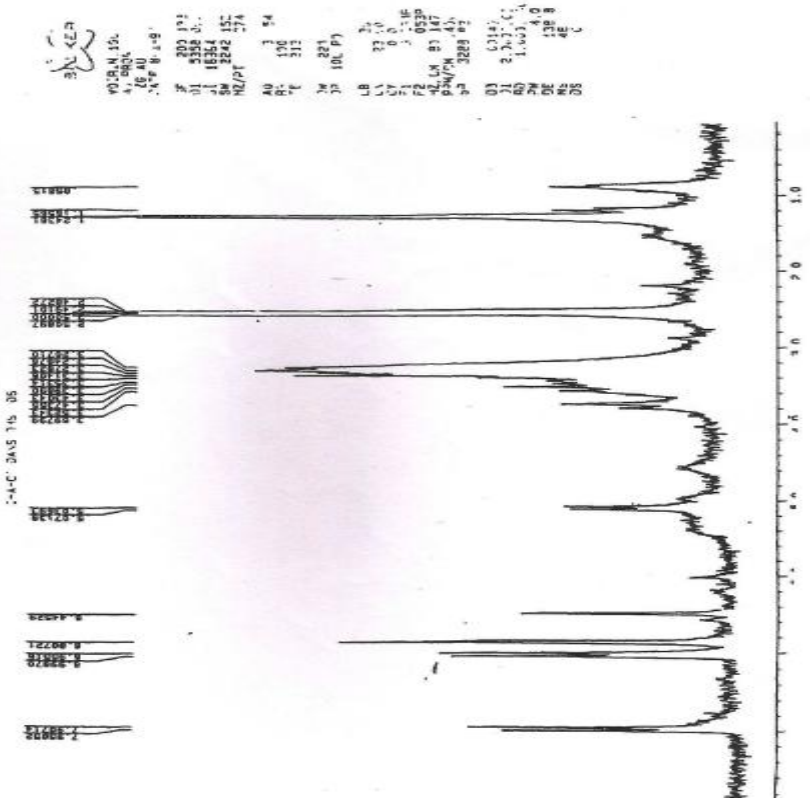
Spectrum.1 ^{1}H NMR spectrum of Apigenin



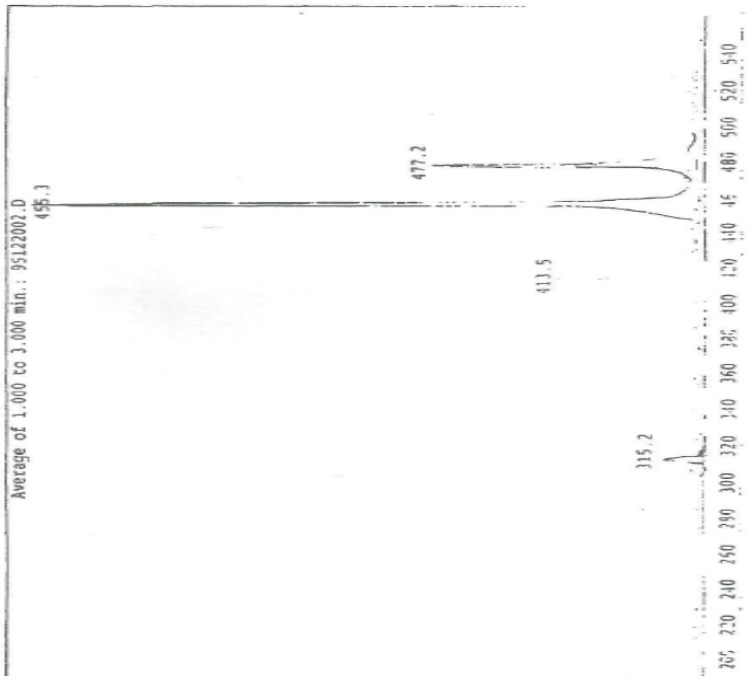
Spectrum.2 ^{13}C NMR spectrum of Apigenin



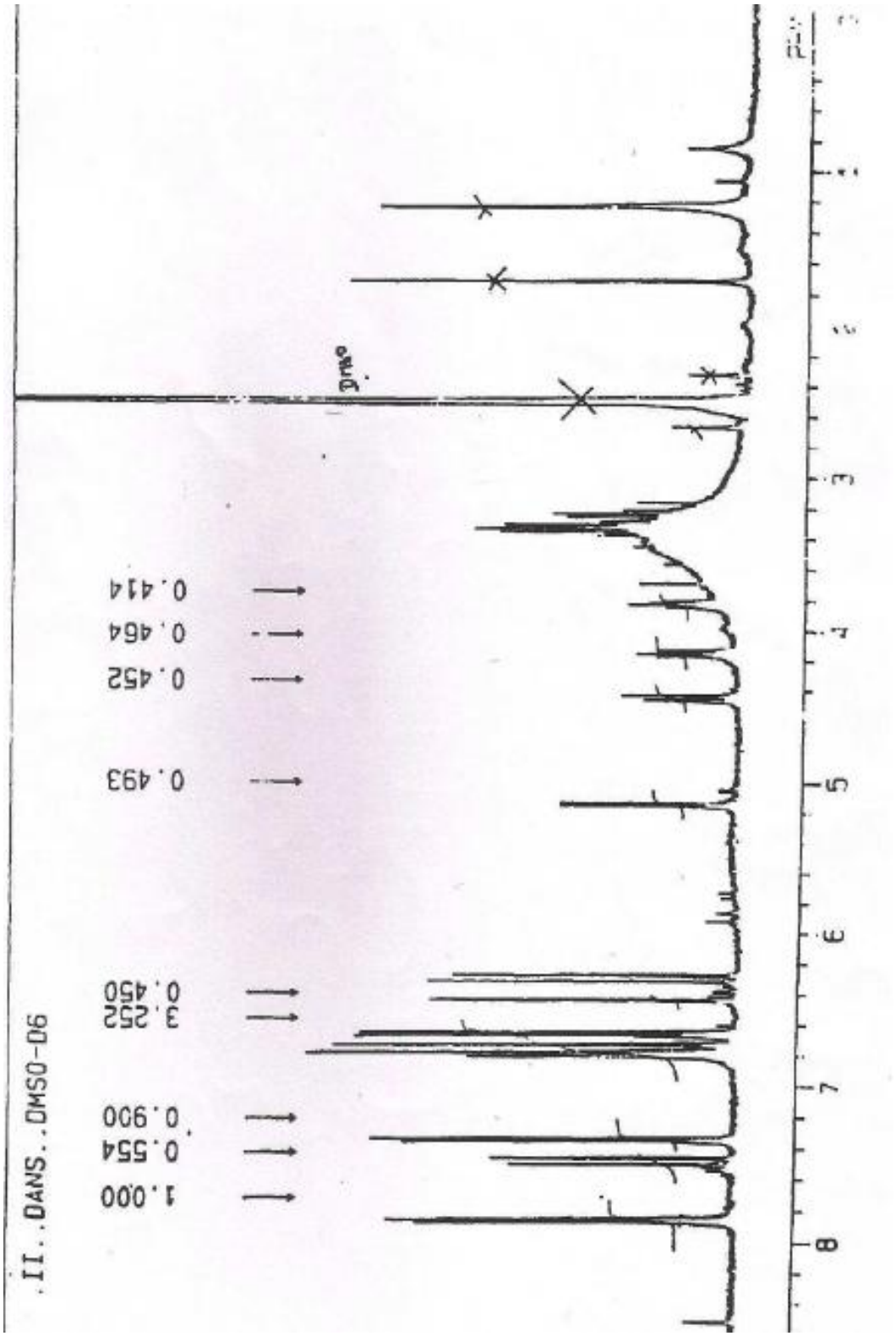
Spectrum.3 ^{1}H NMR spectrum of Apigenin 7- O- β- D- glucopyranoside



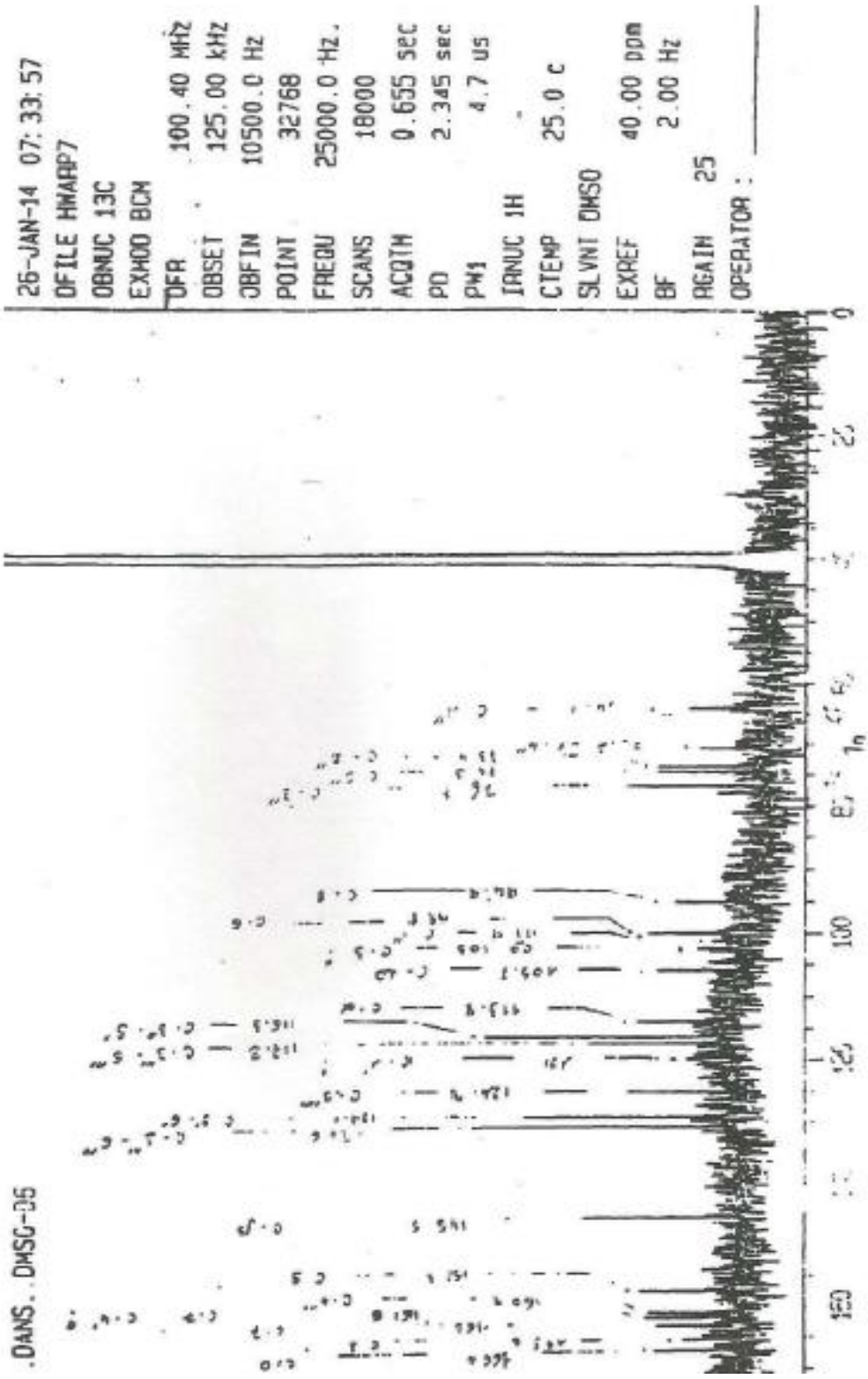
Spectrum.4 Mass spectrum pf Apigenin 7- O- β- D- glucopyranoside



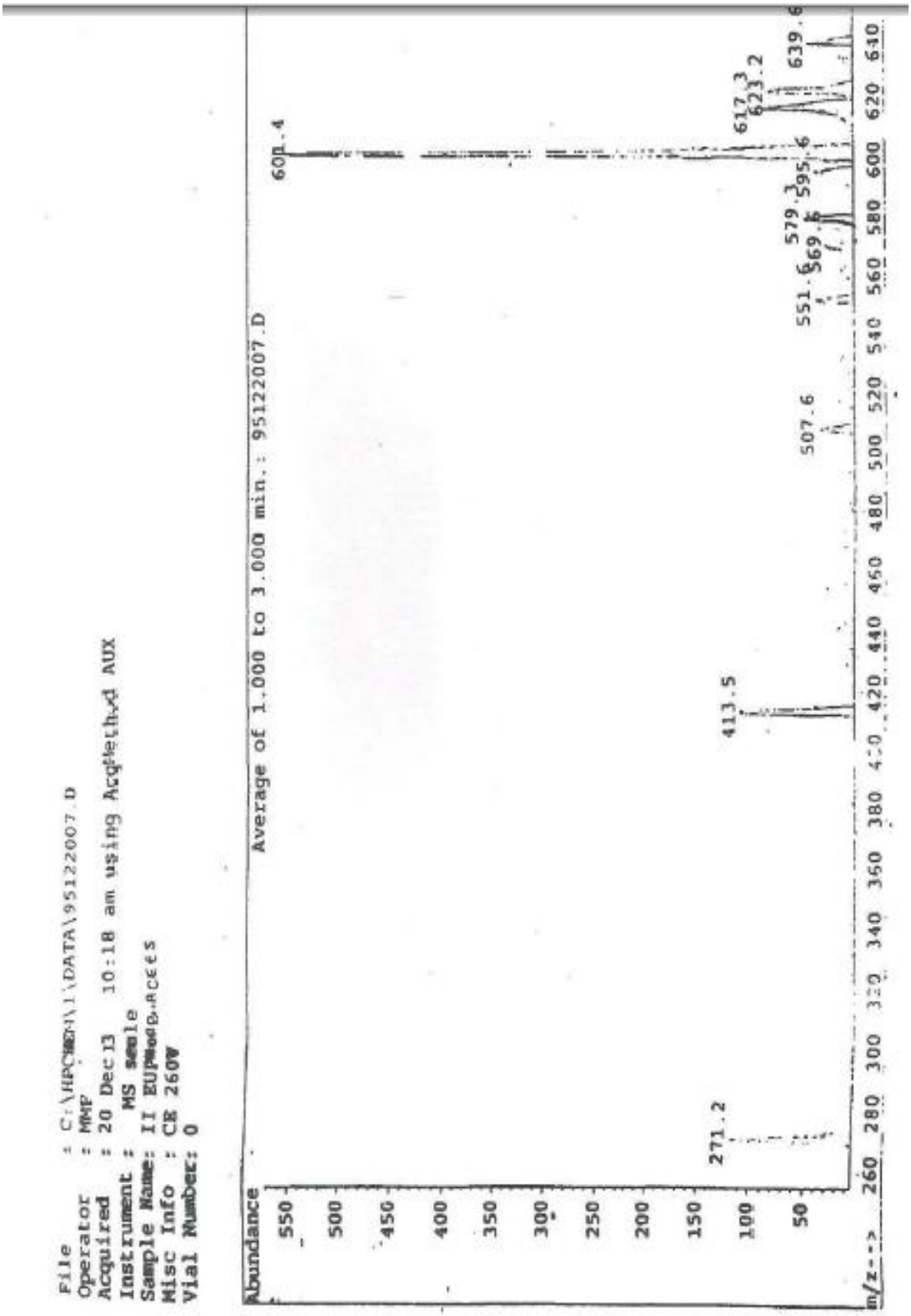
Spectrum.5 [1]H NMR spectrum of Apigenin 7- O- (6" – E- p- coumaroyl)- β- D-glucopyranoside



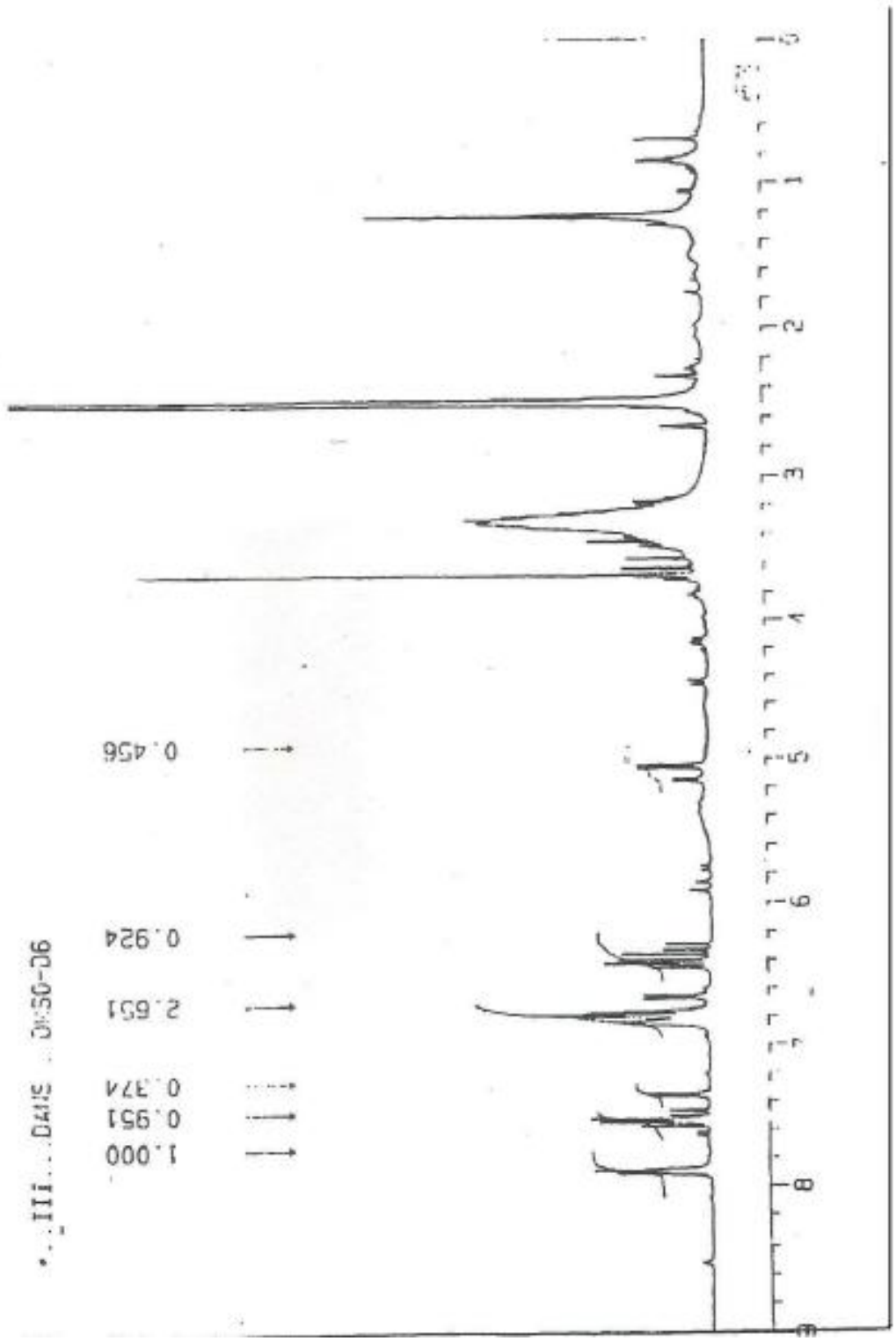
Spectrum.6 ^{13}C NMR spectrum of Apigenin 7- O- (6" – E- p- coumaroyl)- β- D-glucopyranoside



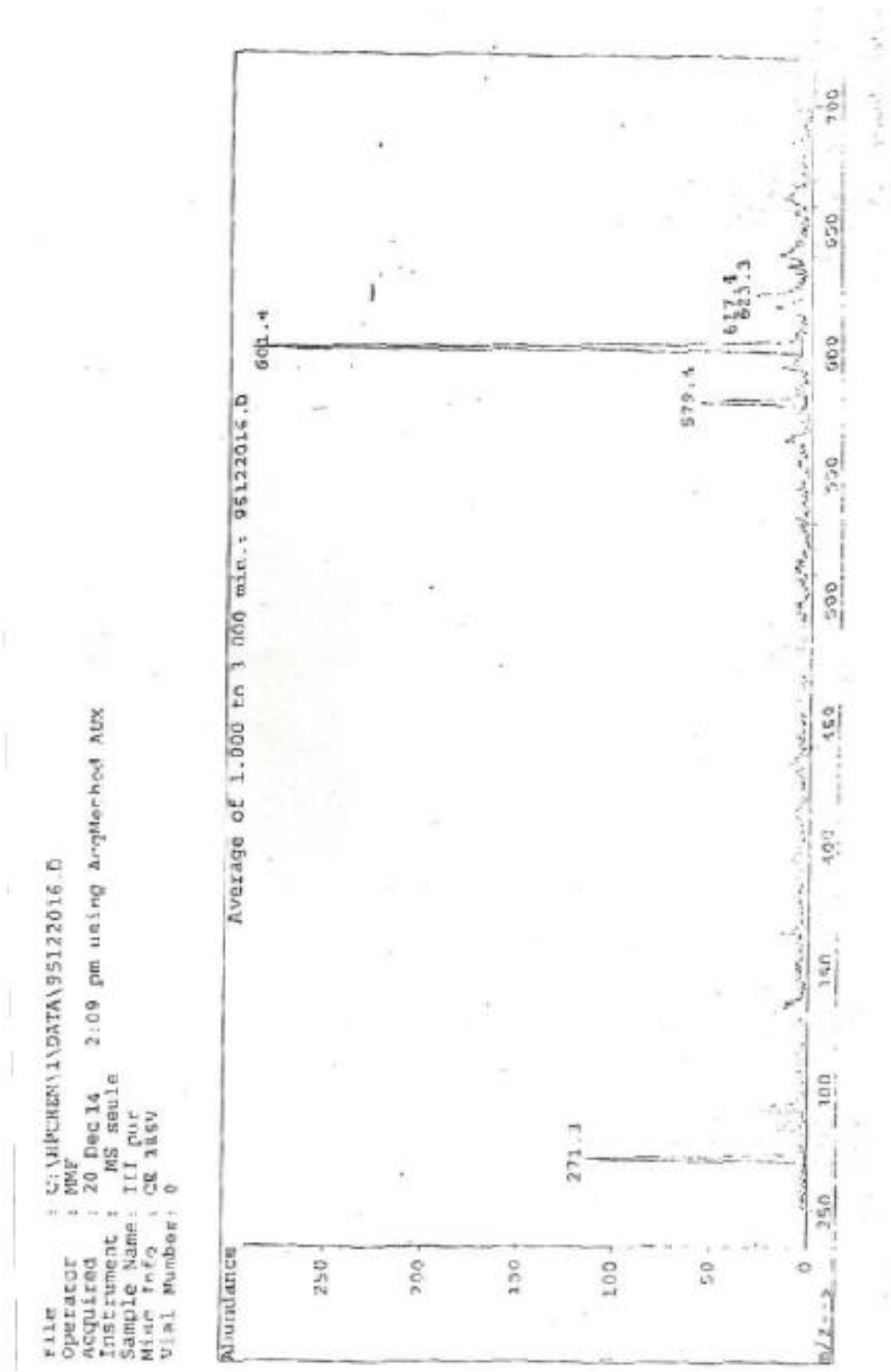
Spectrum.7 Mass spectrum of Apigenin 7- O- (6" – E- p- coumaroyl)- β- D- glucopyranoside



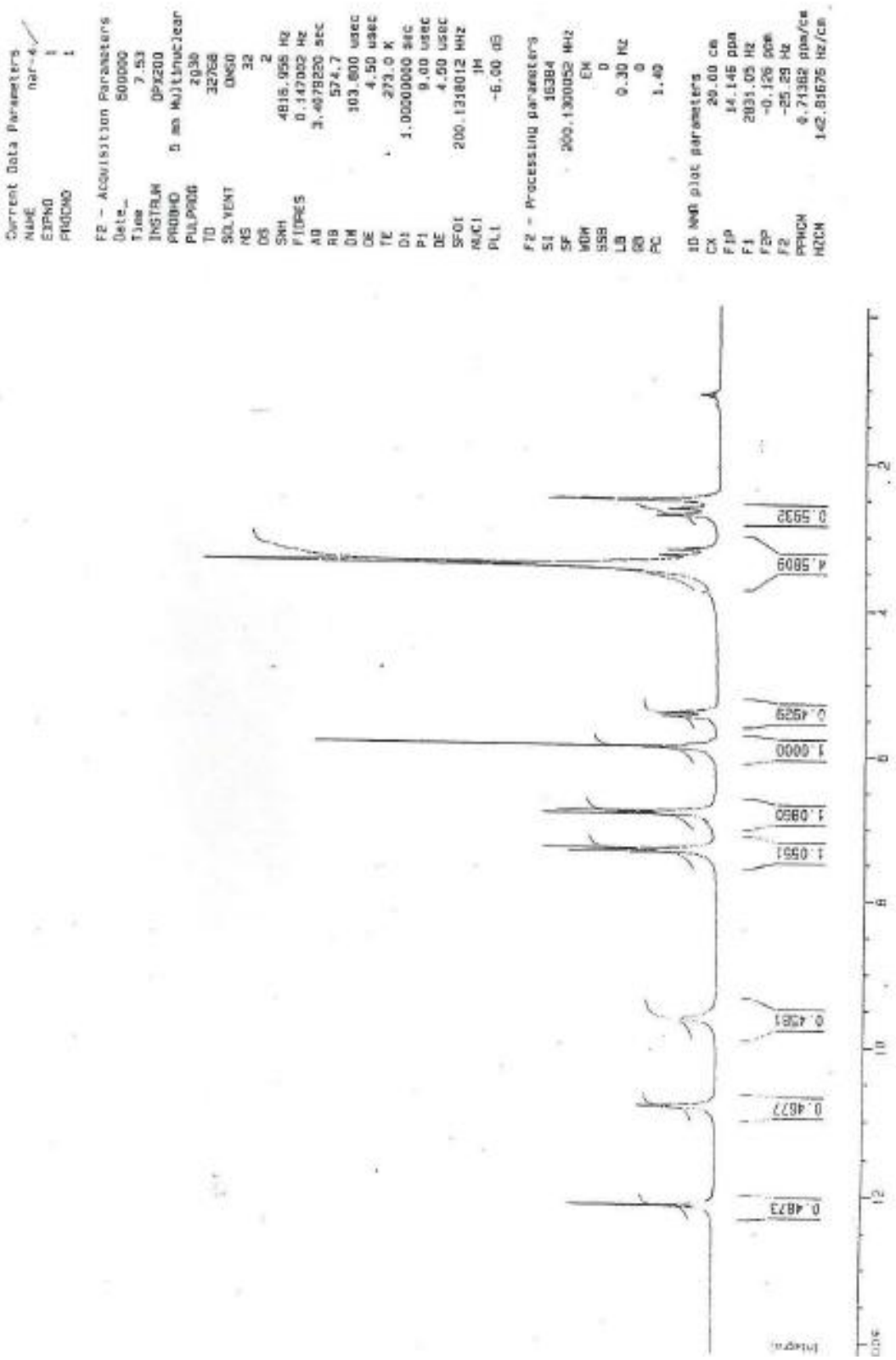
Spectrum.8 [1]H NMR spectrum of Apigenin 7- O- (3" – E- p- coumaroyl)- β- D- glucopyranoside



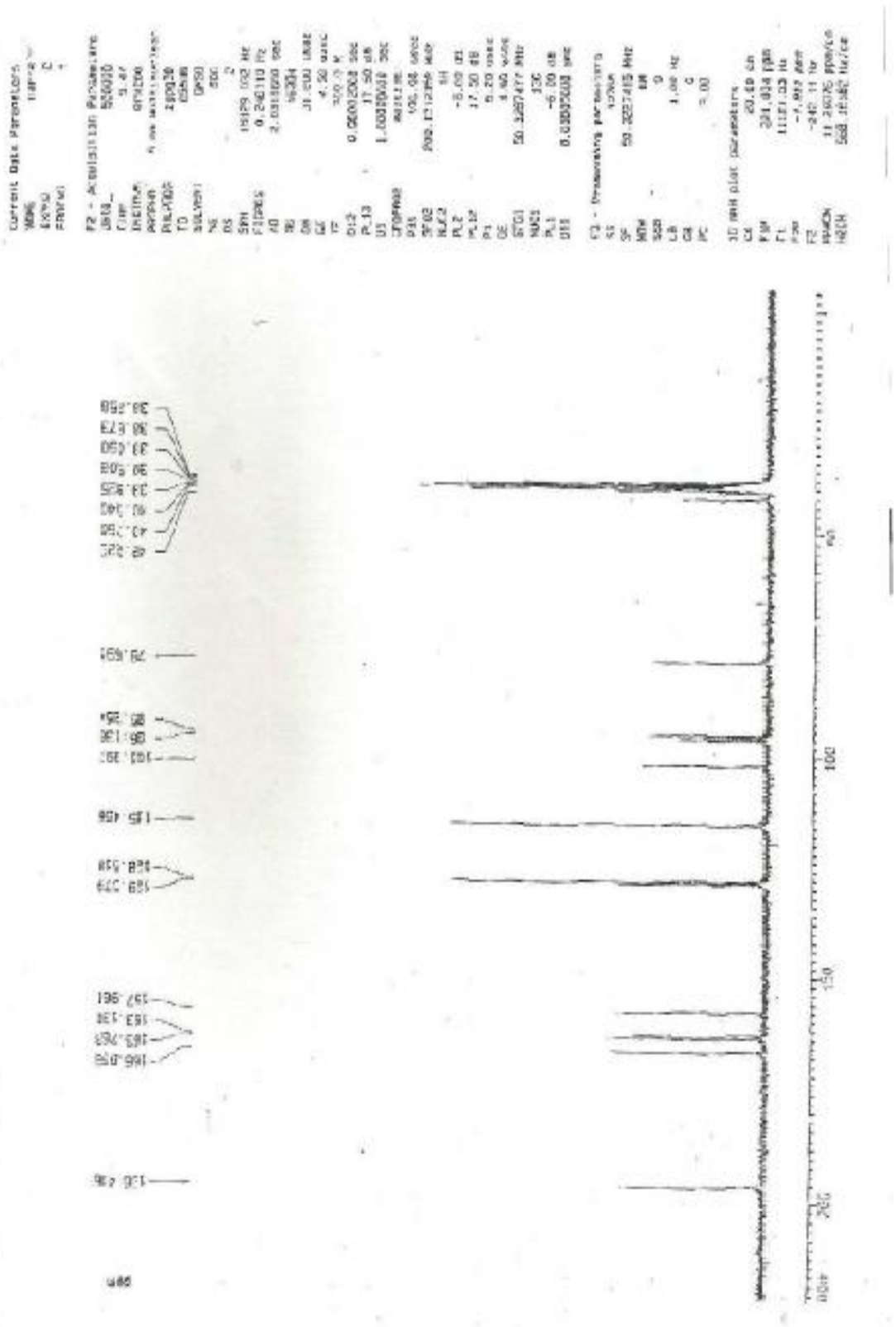
Spectrum.9 Mass spectrum of Apigenin 7- O- (3" – E- p- coumaroyl)- β- D- glucopyranoside



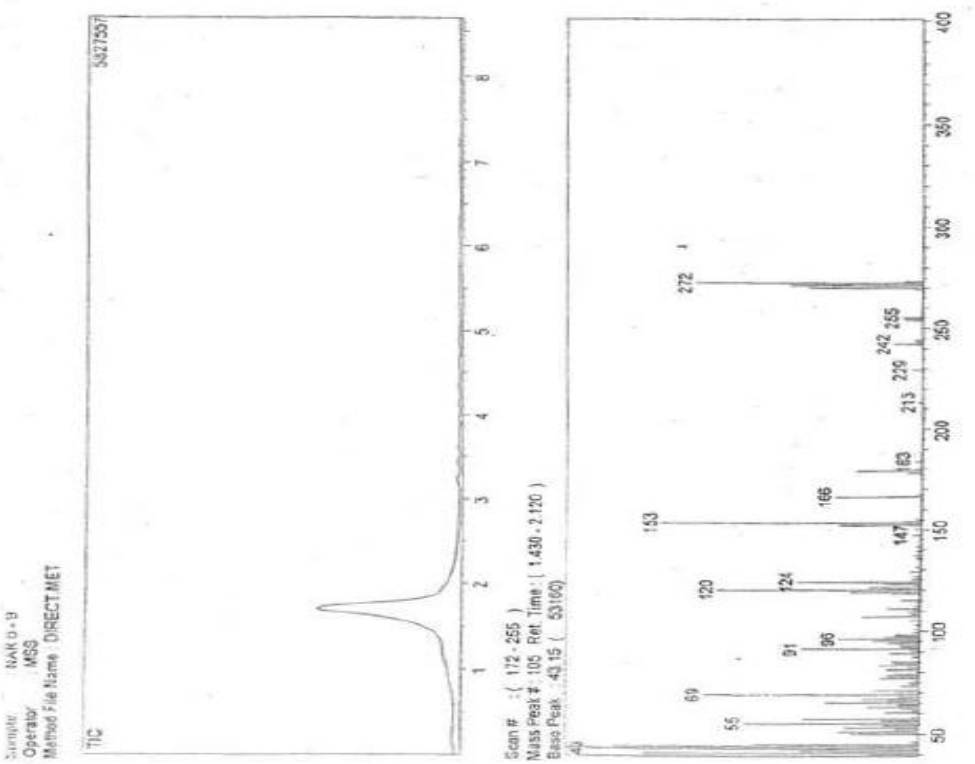
Spectrum.10 ^{1}H NMR spectrum of naringenin



Spectrum.11 ^{13}C NMR spectrum of naringenin



Spectrum.12 Mass spectrum of naringenin



Spectrum.13 EIMS spectrum of naringenin 4’ – methyl ether

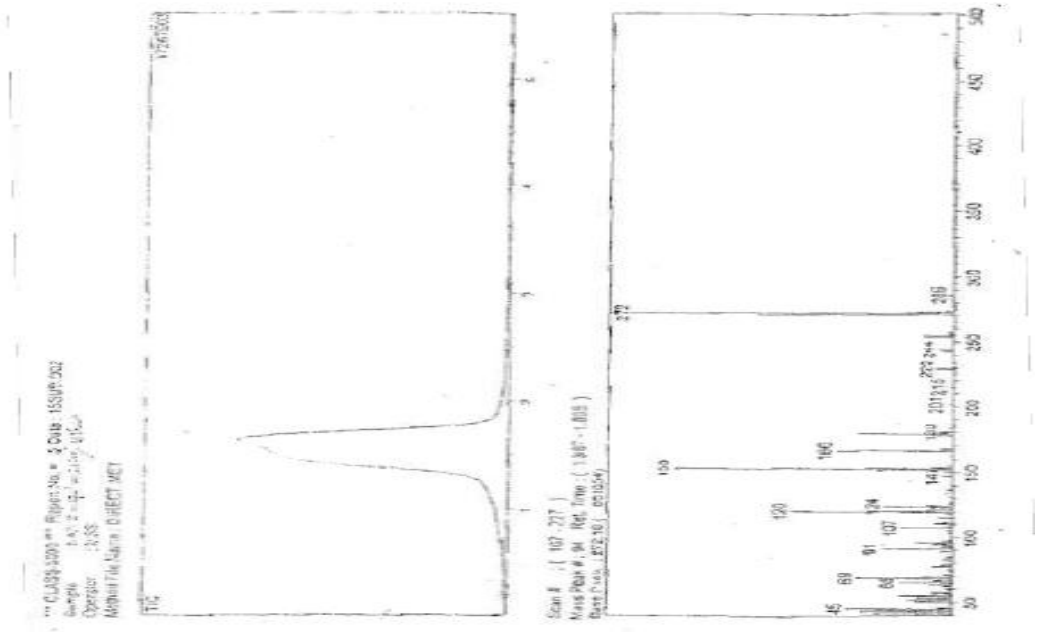


Table.1 Rf values of flavonoids of *Chrozophora rottleri*

(Rf X100 Whatman No. 1, ascending, 28±2^{0}C)

Compounds	H_2O	5% HOAc	30% HOAc	50% HOAc	BAW	Seikal	Ph OH	Forestal	t- BAW
Apigenin	0	6	53	65	91	95	95	77	93
Apigenin 7-O-methyl ether	0	4	43	61	68	92	91	68	90
Apigenin 7-O-□-Dglucopyranoside	12	13	47	70	85	77	70	80	68
Apigenin 7-O-(6''-Eparacoumaroyl) □-Dglucopyranoside	10	12	29	42	84	82	64	78	62
Apigenin 7-O-(3''-Eparacoumaroyl) □-Dglucopyranoside.	9	12	29	45	88	86	65	81	63

BAW: n BuOH: HOAc: H_2O (4:1:5) top layer
Forestal: HOAc: H_2O: Conc HCl (30:10:3)
Seikal: 27%HOAc: n BuOH (1:1)
t- BAW: t BuOH: HOAc: H_2O (3:1:3)
PhOH: Phenol saturated with water

Table.2 Rf Values of Sugar from the hydrolysis of flavone glycoside from *C. rottleri*

(Rf X 100, Whatman No: 1, ascending, 28□ □2^{0}C)

Sugar	BAW	PhOH	t-BAW	EPW	BEW	BBPW
Glucose	23	39	41	18	22	20

BEW: n BuOH: EtOH: H_2O (4:1:1)
BBPW: C6H6: nBuOH: Pyridine: H2O (1:5:3:3)
t- BAW: t BuOH: HOAc: H_2O (3:1:3)
BAW: n BuOH: HOAc: H_2O (4:1:5) top layer
PhOH: Phenol saturated with water

Table.3 Rf Values of Phenolic acid from hydrolysis of flavone glycoside from *C.rottleri*

(Rf X 100, Whatman No: 1, ascending, 28□ □2^{0}C)

Acid	H2O	5%	30%	50%	BAW	Seikal	PhOH	Forestal	t- BAW
p-coumaric acid	42	60	76	81	93	95	63	89	95

Table.4 Rf values of the polyphenolics of *C.rottleri*

(Rf X 100 (Whatman No. 1, ascending, 28 ± 2^{0}C)

Acid	H2O	5%	30%	50%	BAW	Seikal	PhOH	Forestal	t- BAW
Naringenin	0	2	35	41	94	95	68	53	9
Naringenin 4' methyl ether	2	3	35	43	90	91	66	52	93

Rf Table 4

^{1}H NMR (200MHz, DMSO-d6, □, ppm) (Spectrum- 10) 12.15 (s, 1H, OH-5), 10.81 (s, 1H, OH-7), 9.61(s, 1H, OH-4'), 7.41(d, 2H, J=8.89Hz, H- 2',6'), 6.94 (d, 2H, J=8.43 Hz, H-3', 5'), 5.88 (S, 1H, H-8), 5.46 (dd, J =2.2 & 12 Hz, 1H, H-2), 3.41 (dd, J= 12 & 15Hz, 1H, Hax–3), 2.72 (dd, J = 2.8 & 15 Hz, 1H, Heq-3).

^{13}C NMR (50 MHz, DMSO-d6, □, ppm) (Spectrum- 11)

196.42 (C-4), 166.87 (C-7), 163.77 (C-5), 163.13 (C-9), 157.96 (C-4'), 129.11(C-1'), 128.51 (C-2', 6'), 115.47 (C-3', 5'), 101. 99 (C-10), 96.14 (C-6), 95.25 (C-8), 78.69 (C-2), 42.23 (C-3).

MS (EIMS, relative intensity as %) (Spectrum- 12)

273 (MH+, 5), 272 (M+, 63), 271 (M-H, 37), 255 (M+ - OH, 5), 153 (72) 124(35) 120 (56), 43 (100).

Conversion of naringenin to chalconaringenin

Naringenin (10mg) was dissolved in 8 % aq KOH solution (3ml) and immediately acidified with 2N HCl at 0^{0}C. The reaction mixture was suspended in water and repeatedly extracted with ether.

The ether layer was washed, dried and evaporated to give a gummy residue which was then chromatographed over SiO2.

Elution with benzene afforded light yellow needles.

It was purple under UV and light orange under UV/NH_3. Co-PC with authentic chalconaringenin showed identity.

Acetylation of compound VI (naringenin triacetate)

Compound I (5mg) was dissolved in few drops of pyridine and reacted with 2ml of dimethylketone. It was kept at room temperature for 1day and dropped into broken ice, maintained for 3hr and filtered. When this white solid recrystallized from the mixture of ethylacetate and petrol it gave colourless needles with mp. 187-189^{0}C.

Methylation of compound VI (naringenin trimethyl ether)

Compound I (5mg) was dissolved in 10ml of dry dimethylketone and to this mixture 1ml methylsulphate and 1g of anhyd potassium carbonate were added and then refluxed to about 3days at 70^{0}C. The reaction product was cooled, filtered and then washed with dimethylketone. The residue obtained from dimethylketone was added to cold water. The white solid was obtained. It was then filtered, washed with cold water and then dried and recrystallized by methanol to yield homogenous colourless needles with mp. 123-125^{0}C.

Compound VII (5, 7- dihydroxy 4'- methoxy flavonone: naringenin 4'- methyl ether)

It was yellow needles getting from methanol with molecular formula $C_{16}H_{14}O_5$ and mp. 248-250^{o}C. With Mg-HCl it gave magenta red colour and pink colour with alcoholic sodiumborohydride and hydrochloric acid. Under UV it was dull violet and under UV/NH3gave yellow.

UV (□ □ max, nm)

MeOH: 287, 325sh
NaOAc: 284sh, 320
NaOAc/H3BO3: 291, 323 sh

$AlCl_3$: 303, 368
$AlCl_3$/HCl: 303, 367
NaOMe: 246, 318

Rf Table 4

MS (EIMS, relative intensity as %) (Spectrum- 13)

286 (M+, 2), 272 (MH+-CH3, 100), 180(MH+ paramethoxy phenyl, 36), 107

The phytochemical study of aerial parts of *C. rottleri* resulted in the isolation of seven compounds, one of them viz. Naringenin is reported for first time in the *C. rottleri* aerial parts in the ethanol extracts.

Traditional uses of *Chrozophora* plants are to cure skin disorders, skin burns, diarrhea, jaundice, mouth ulcer, fever, joint pain and swelling, abdominal pain, migraine, menstrual problems, wounds, and to expel intestinal worms.

The screening and investigation for phytochemicals and pharmacological studies of this plant will provide scientific evidence for their rational use in food and prevention and treatment of infectious and oxidative stress related diseases.

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