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Isolation of phytoconstituents from the stems of M*ussaenda erythrophylla*

M. Chinna Eswaraiah* and A. Elumalai

Department of Pharmacognosy, Anurag Pharmacy College, Ananthagiri (v), Kodad (M), Nalgonda (DT), Andhra Pradesh, India

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ABSTRACT

Ethyl acetate extract of the stems of Mussaenda erythrophylla ((Rubiaceae) led to the isolation of β-sitosterol, 5 hydroxy-7, 4'-dimethoxy flavones, 3- iso cumaryloxy – cyclopropane-1-oic acid and 4 -hydroxy-3-methoxy cinnamic acid. Their structures were elucidated by IR and NMR spectroscopic method. These compounds were isolated for the first time from this plant.

Key Words: *Mussaenda erythrophylla*, β-sitosterol, 5 hydroxy-7, 4'-dimethoxy flavones, 3- iso cumaryloxy – cyclopropane-1-oic acid.

INTRODUCTION

Mussaenda erythrophylla (Rubiaceae) is native to western tropical Africa, occasionally seen in gardens and parks as ornamental plant in India and is commonly known as mussenda (Telugu), nagavalli (Sanskrit) and red flag bush (English)[1]. It is a perennial, evergreen shrub with branched tap root system. The roots are useful for cough, jaundice and when chewed acts as an appetizer [2]. A number of triterpenoids and glycosides were reported. Mussaenda genus viz., contains mussaendosides U(1) and V(2) [3], mussaendosides G(1) and K(2) are two new triterpenoid saponins [4], mussaendosides A-C, M and N with cyclolanostene type aglycone [5-6] and aureusidin[7], iridoid glycosides[8]. The pharmacological activities reported from Mussaenda species were diuretic, antiphlogistic, antipyretic and effective in laryngopharyngitis, acute gastroenteritis and dysentery [9] and also anti-fertility activity [10]. It is established that plants which have anti oxidant property exert hepatoprotective [11-14] and anti diabetic actions [15-17].

MATERIALS AND METHODS

Plant materials

The stems of *Mussaenda erythrophylla* was collected in M.V.P.Colony, Visakhapatnam, in the month of April, 2006. (Voucher no.TSN/DOP/ME0406). The authentications of the plant were done by Prof. Dr. M. Venkaiah, Department of Botany, Andhra University, Visakhapatnam.

Instruments

Rotary Flash Evaporator (Medica Instrument Manufacturing Company, Mumbai), Model:Roteva Equitron, IR and NMR [18].

Source of Chemicals and Drugs

All chemicals and solvents were of the analytical grade obtained from S.D. Fine Chemical Pvt. Ltd., Mumbai, Sigma Chemical Company, U.S.A., Loba Chemic, Mumbai.

Extraction and Isolation procedure

Freshly collected plant materials (*M.erythrophylla* stem) were shade dried at room temperature and coarsely powdered in Wiely mill. The powdered material of *M. erythrophylla* stem (1kg) was successively extracted with hexane, ethyl acetate and methanol by soxhlet extraction. The crude extracts were evaporated to dryness in a rotary film evaporator.

Yield of extracts

Hexane extract - 2.5gm
Ethyl acetate extract - 30gm
Methanolic extract - 12.5 gm

Since the yield of hexane and methanolic extract was very low from all the three extracts, these extracts were not used in this study.

RESULTS AND DISCUSSION

Total two compounds were isolated. Their structures were elucidated by IR and NMR spectroscopic methods. Results of qualitative phytochemical screening of plant extracts are shown in Table-1.

Table 1: Nature of phytoconstituents present in different extracts of *M.erythrophylla* stem

Phytoconstituents	Ethyl acetate extract
Phytosterols	+
Triterpenes	+
Glycosides	-
Saponins	-
Flavonoids	+
Tannins	-
Carbohydrates	-
Alkaloids	-

+ = Present, – = Absent

Qualitative phytochemical screening of ethyl acetate extract of *M.erythrophylla* stem revealed the presence of steroids, triterpenoids and flavonoids.

Structural elucidation of compound ME-1

It was crystallized from hexane as colourless fine needles, m.p.136-138 °C. It showed positive colour reaction with LB test for steroids. The ^{1}H NMR showed peaks at 0.80-1.25 (methyl), 3.50 (1H, 3α-H) and 5.30 (1H, m, C_5-H) (400 MHz, CDCl3, Fig-1, Table-2). Based upon the above data it was identified as β-sitosterol (Faizi *et al*., 2001; Chau *et al*., 2005). It was further confirmed by comparison with authentic sample through co-TLC and mixed melting point. This compound was reported for the first time from *M.erythrophylla.*

Table-2: ^{1}H NMR spectral data of compound ME-1 (β-sitosterol)

Assignment	^{1}H NMR (400 MHz, δ, $CDCl_3$)
Methyls	0.8-1.25
3-H	3.5 (1H,m)
H-5	5.2 (1H,m)
O-H	5.0 (H,m)

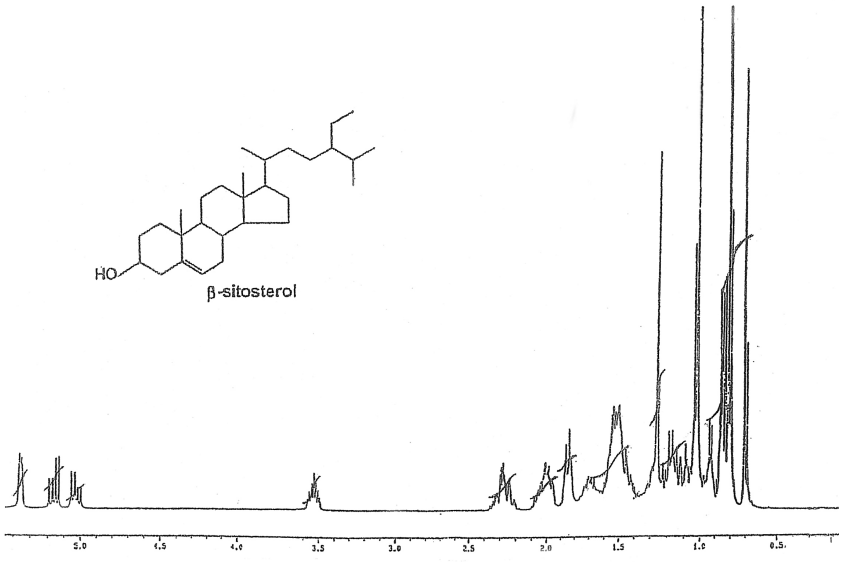

Fig 1: 1H NMR Spectrum of β-Sitosterol

Structural elucidation of compound ME-2

This compound was obtained from hexane: ethyl acetate (80:20) as yellow color crystal, m.p.254-256ºC The molecular formula $C_{17}H_{14}O_5$ was established from LC-MS data and elemental analysis. This compound showed very strong color reaction with Mg/HCL (Shinoda test) indicating flavonoid nature.

The ^{1}H NMR spectrum (400 MHz $CDCl_3$, Fig 2, Table-3) clearly reveals a singlet at δ 6.57 integrating for one proton, which can be attributed to the flavone proton (C-3H). The ^{1}H NMR spectrum also reveals a chelated hydroxyl presence of singlet at δ 12.80 integrating for one proton, a peak at δ 3.89 integrating for 6 protons is due to the presence of 2 methoxyls at 7th and

4’ position of the flavone skeleton. Two doublets at δ 6.48 and 6.37 each integrating for one proton with coupling constant 2 Hz are due to the protons at 6th and 8th position of the aromatic ring respectively. The spectrum also exhibited two doublets at δ 7.840 and 7.01 each integrating for two protons with a coupling constant value of 9 Hz in each case (ortho coupling). The first peak due to the protons at 2’ and 6’ position and second one is due to the protons at 3’ and 5’ position of aromatic nucleus.The ^{13}C NMR spectrum (100 MHz $CDCl_3$, Fig-3, Table-3) shows two signals at δ 55.50 and 55.75 indicating the presence of two methoxyl groups a carbonyl carbon at δ 182.44. Thus the compound is 5- hydroxy-7, 4’-dimethoxy flavone. This is further supported by a M+H ion at 299, in its mass spectrum. This compound was first time reported from *M.erythrophylla*.

Table-3: ^{1}H NMR and ^{13}C NMR Spectral data of compound ME-2 (5 hydroxy-7, 4’-dimethoxy flavone)

Carbon No.	^{1}H NMR (400 MHz, δ, $CDCl_3$)	^{13}C NMR (100MHz, δ, $CDCl_3$)
1		
2		128.04
3	6.57 (1H, s)	98.03
4		182.44
5		160.2
6	6.48 (1H, d)	98.03
7		165.48
8	6.37 (1H, d)	92.64
9		162.63
10		104.42
1’		
2’ and 6’	7.84 (1H, dd, *J*= 9Hz)	128.04
3’ and 5’		
4’		
OCH_3	3.84 (6H, s)	55.75

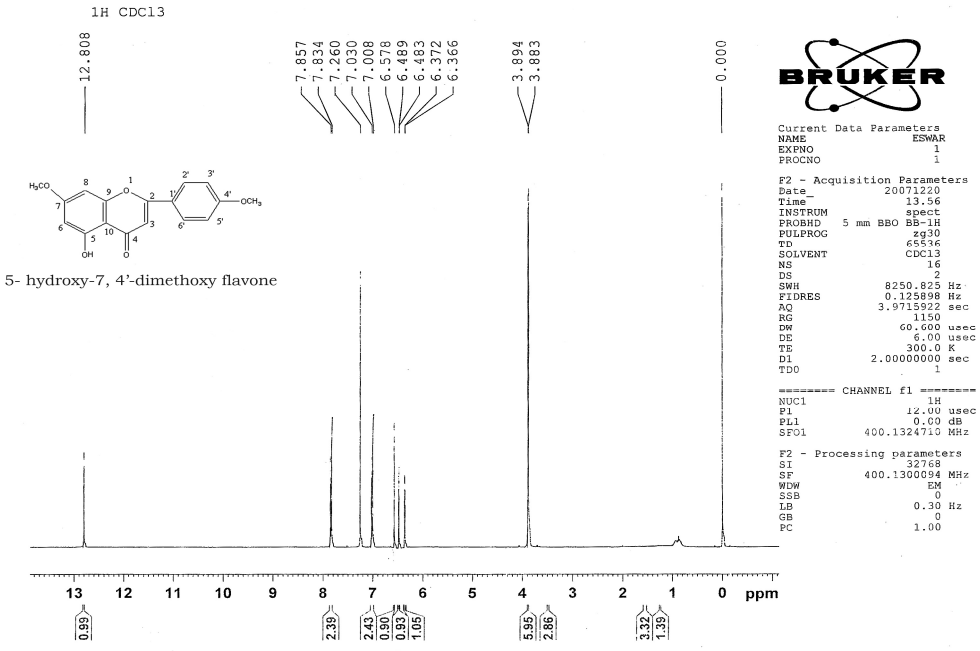

Fig 2: ^{1}H NMR Spectrum of 5-hydroxy-7, 4’-dimethoxy flavone

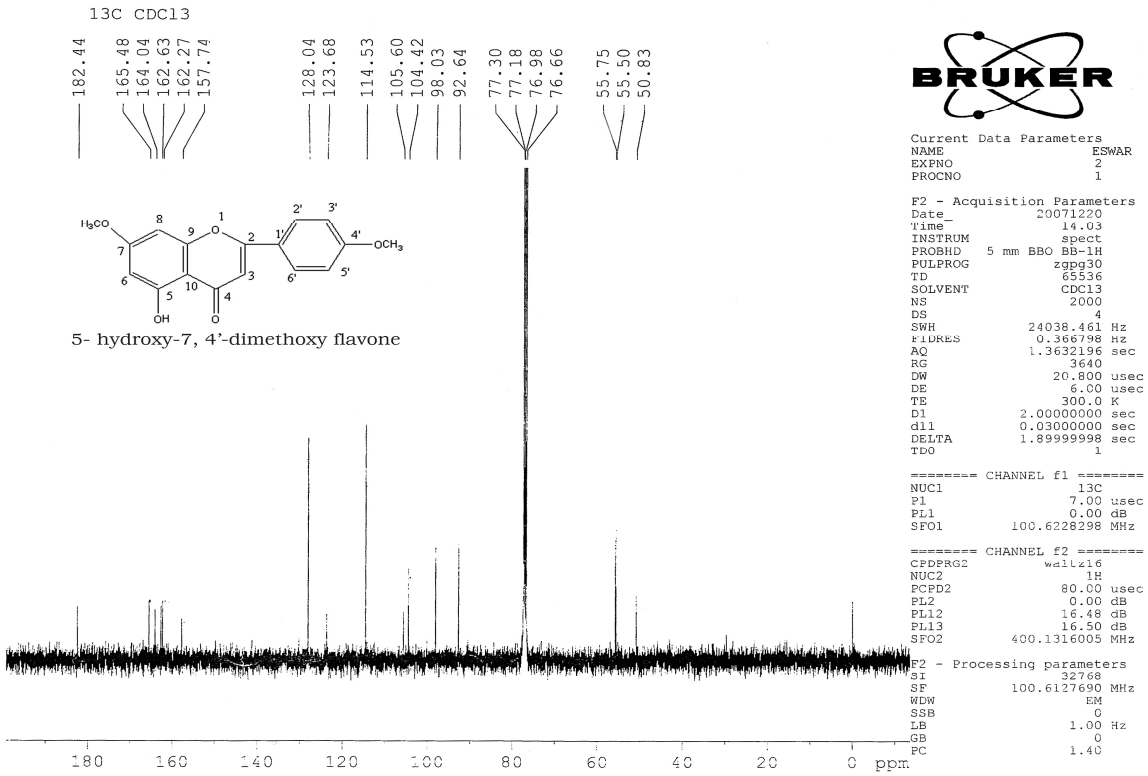

Fig. 3: ^{13}C NMR Spectrum of 5-hydroxy-7, 4'-dimethoxy flavone

Structural elucidation of compound ME-3

This compound was obtained from hexane: ethyl acetate (60:40) as pale brown color solid. The molecular formula $C_{13}H_{10}O_5$ was established from LC-MS data, m.p. 258-260ºC the molecular weight of the compound is 246**.** The IR spectrum (Fig.41) shows the presence of –OH and C=O group, by exhibiting absorption bands at 3438 cm^{-1} and 1746 cm^{-1} respectively.

^{1}H NMR spectra (400MHz, DMSO, Fig.4, Table-5) shows a triplet signal at δ 1.23 for two protons for a methylene group, one proton multiplet at δ 3.08 indicating a proton under oxygen function and a multiplet at δ 2.30 for a proton adjacent to a carbonyl group. Apart from this it exhibits five signals each for one proton in the aromatic region. Consistent with the ^{1}H NMR the ^{13}C NMR data shows (100MHz, DMSO, Fig5, Table-5) three signals in the aliphatic region at δ 45.38, 20.81 and 8.38. The upfield signal at δ 8.38 suggests the presence of a cyclopropane ring system. The two downfield signals at δ 168.71 and 159.64 were characteristic of carbonyl group. Further it shows the presence of 8 aromatic carbon atoms in the molecule. From the above spectral data the structure of compound ME-3 may be proposed as 3- iso coumaryloxy – cyclopropane-1-oic acid and also supported by its mass spectral fragmentation. This compound was reported for the first time from *M.erythrophylla*.

Fig-4: Mass Spectral Fragmentation of ME- 3:

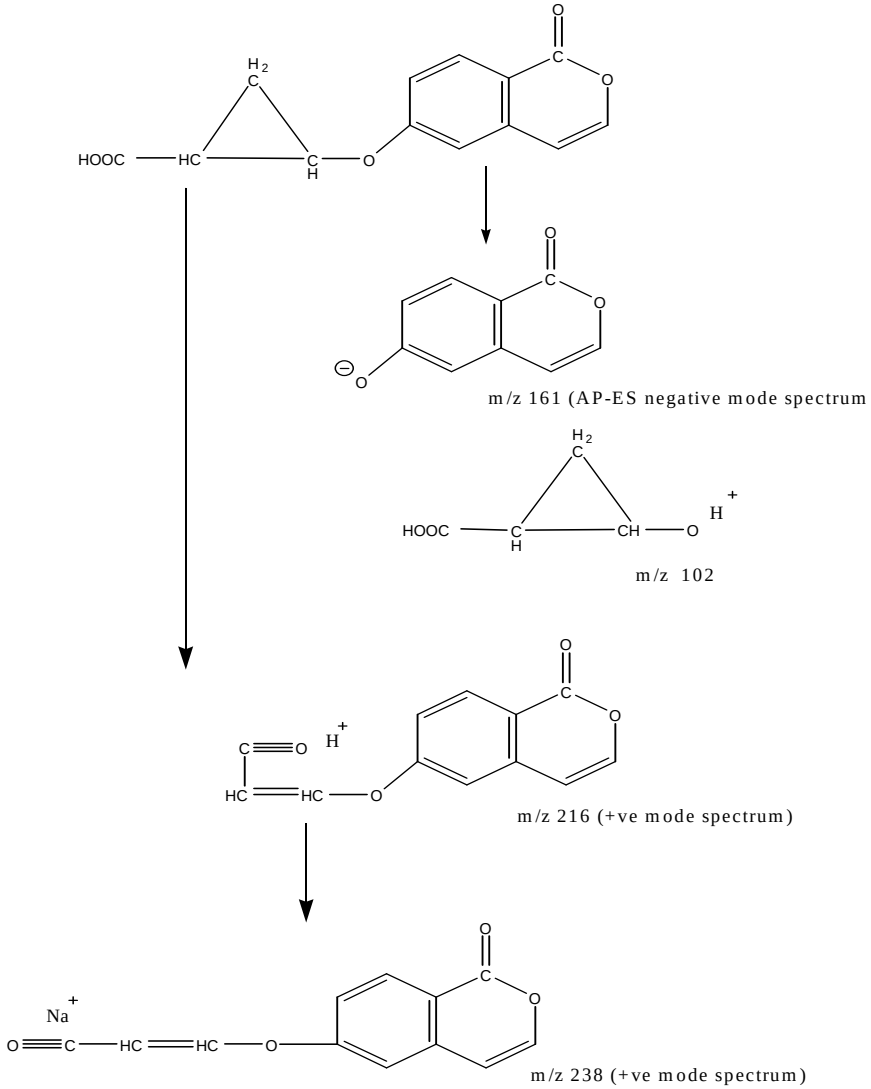

Table – 4: ^{1}H NMR and ^{13}C NMR spectral data of Compound ME-3 (3- iso cumaryloxy – cyclopropane-1-oic acid)

Position	^{1}H NMR (400MHz, δ, DMSO)	^{13}C NMR, (100MHz, δ, DMSO)
1	-	159.74
3	7.79 (d, *J*= 8Hz)	143.76
4	6.49 (d, *J*= 8 Hz)	110.03
5	7.28 (d, *J*=2 Hz)	115.47
6	-	154. 06
7	7.17 (dd, *J*= 7.0, 2.0 Hz)	118.58
8	8.10 (d, *J*= 7 Hz)	129.28
9	-	116.61
10	-	152.88
1’	3.08 (m)	45.38
2’	2.38 (m)	20.81
3’	-	168.71
4’	1.23 (t)	8.38

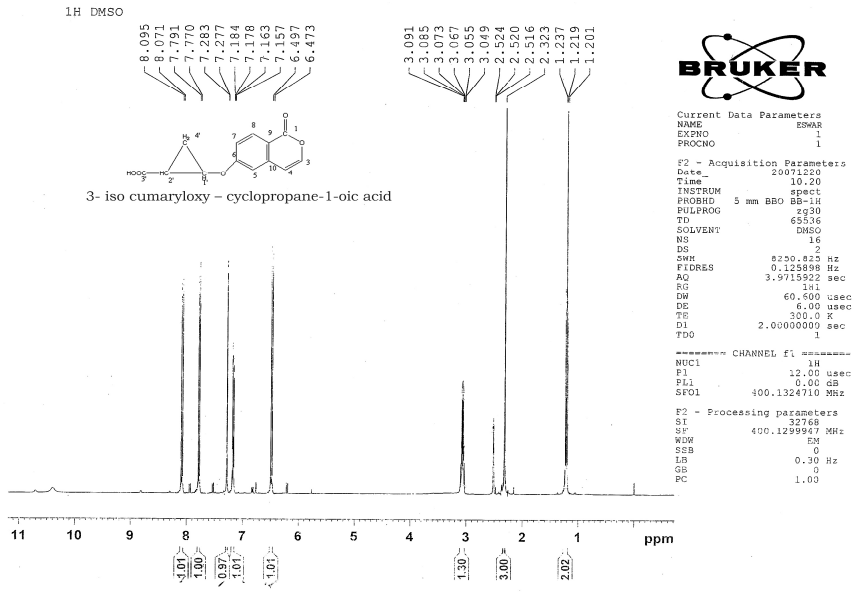

Fig-5: ^{1}H NMR Spectrum of 3-iso cumaryloxy-cyclopropane-1-oic acid

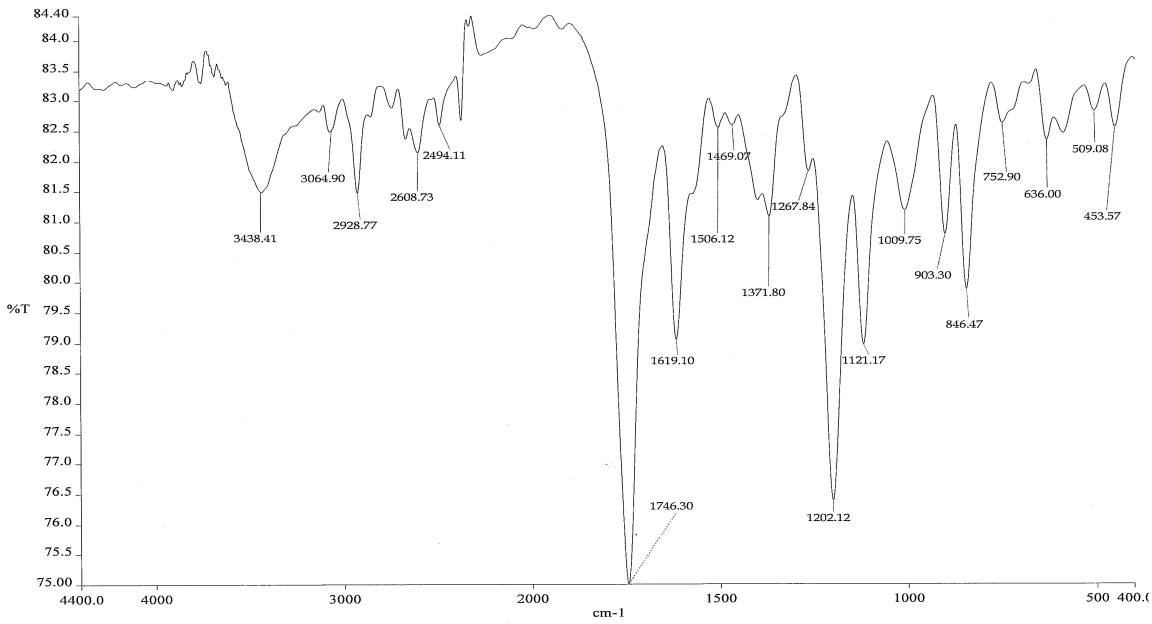

Fig-6: ^{13}C NMR Spectrum of 3-iso cumaryloxy-cyclopropane-1-oic acid

structural elucidation of compound ME-4:

This compound was obtained from hexane: ethyl acetate (50:50) as colorless fine crystals, m.p 169-171 °C. The molecular formula $C_{10}H_{10}O_5$ was established from LC-MS data. The IR spectrum (Fig.45) showed a broad band at 3434.60 cm^{-1} due to the phenolic hydroxyl group. The characteristic absorption band of α, β unsaturation system was observed at 1680 cm^{-1}. The bands located at 1609 cm^{-1}, 1514 cm^{-1}, 1427 cm^{-1}, 1274 cm^{-1}, 942 cm^{-1} corresponding to aromatic moiety.

The ^{1}H NMR spectrum (400MHz, DMSO, Fig.8, Table-6) of ME showed a pair of AB doublet (J=16 H_z) at δ 7.45 and 6.85 consistent with trans olefinic protons of α, β-unsaturated system. It also exhibited one methyl, one hydroxyl and carboxyl signals at δ 3.37, 9.58 and 12.11 respectively. The signals at δ 7.09 (1H, d, 8.0 Hz) and 6.82 (1H, d, 8.0 Hz) corresponds to C-5, C-6 protons respectively. The signals at 7.27 corresponding to C-2 protons. This is further confirmed by ^{13}C NMR spectral (100MHz, DMSO, Fig-9) data 1C.123.0, 2C.144.42, 3C.147.91, 4C.149.09, 5C.115.54, 6C.115.64, 7C.125.76, 8C.111.26, 9C.167.90.

The above spectral data is in good agreement with that of 4 –hydroxy-3-methoxy cinnamic acid and the identity was further confirmed by comparison with authentic sample (m.p. and Co-TLC). This compound was reported for the first time from *M.erythrophylla.*

Table-5: ^{1}H NMR and ^{13}C NMR spectral data of ME-4 (4 -hydroxy-3-methoxy cinnamic acid)

Position	^{1}H NMR δ (400MHz, DMSO)	^{13}C NMR δ (100MHz, DMSO)
1		123
2	7.27	144.42
3	3.37	147.91
4	9.58	149.09
5	7.09 (1H, d, J=8.0 Hz)	115.54
6	6.82 (1H, d, J=8.o Hz)	115.64
7		125.76
8		111.26
9		167.90
C=O	12.11	

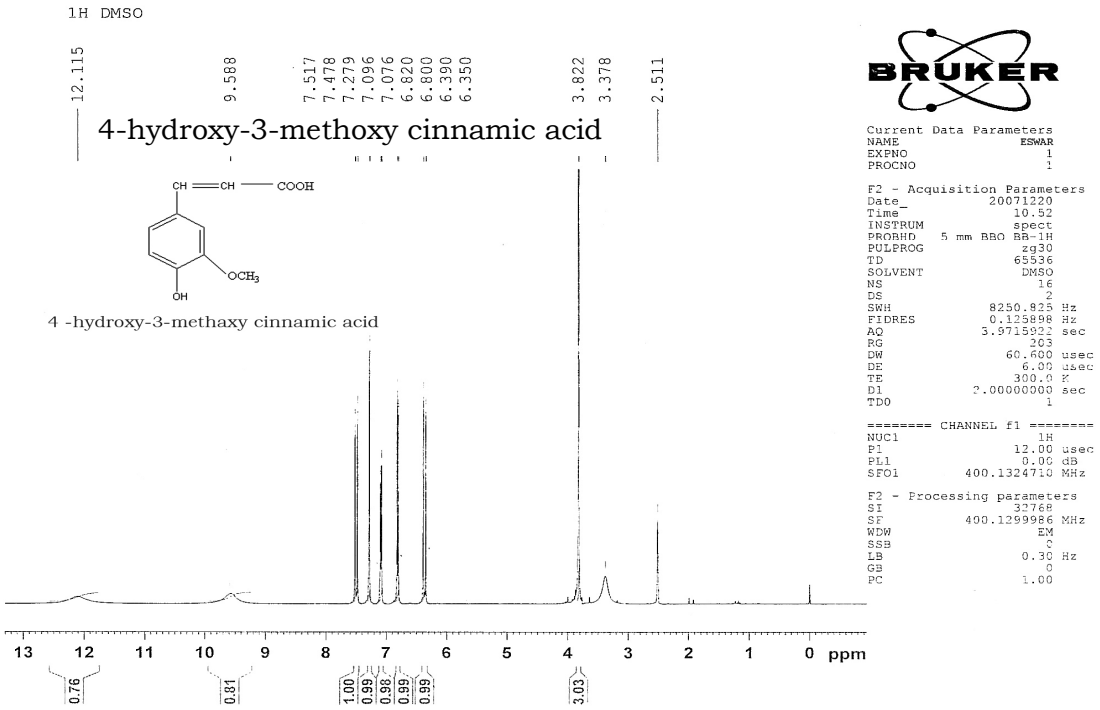

Fig-7: ^{1}H NMR Spectrum of 4- hydroxyl-3-methoxy cinnamic acid

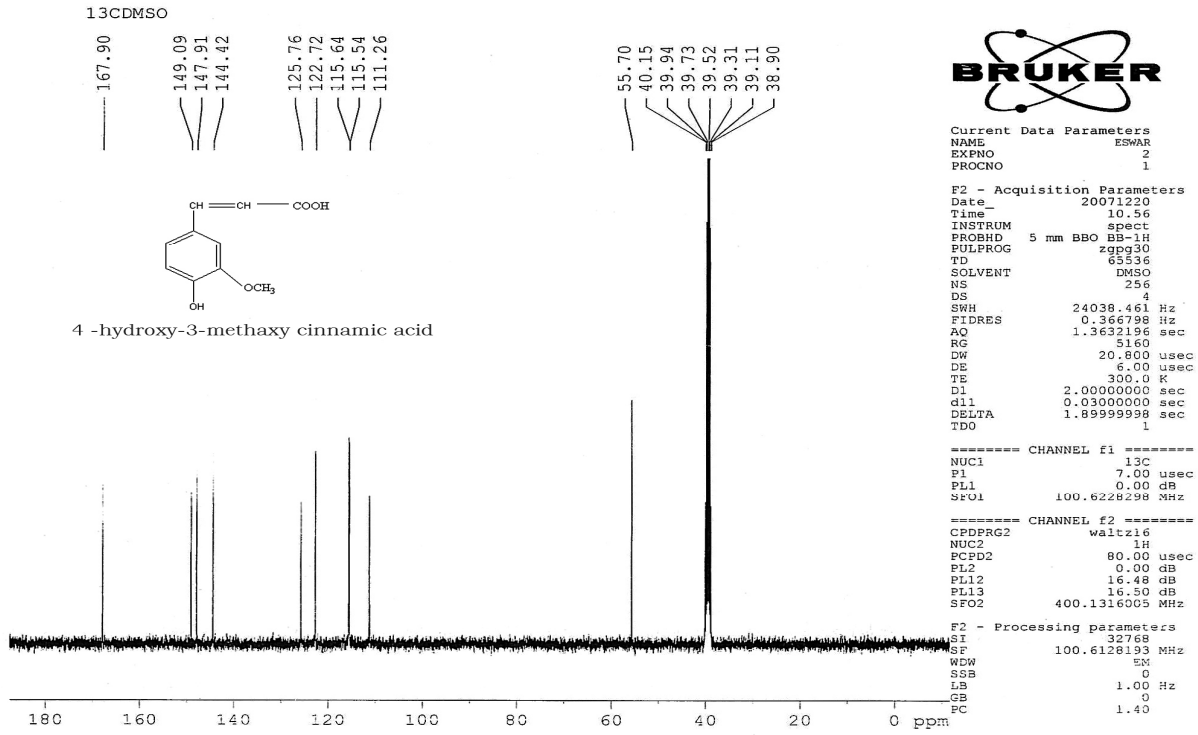

Fig-8: ^{13}C NMR Spectrum of 4- hydroxyl-3-methoxy cinnamic acid

Compound ME-1 (β-sitosterol)

Fractions 15-25 of ethyl acetate extract were combined as they were homogenous on TLC and crystallisation from n-hexane gave colourless needle .m.p.136-138°C.

Found : C, 83.35%; H, 10.28%.
Calculated for $C_{36}H_{54}O_2$: C, 83.39%; H, 10.42%
R_f : 0.8 Hexane: ethyl acetate (8:2)
Yield : 50mg
^{1}H NMR : Fig-1; Table-2.

Compound ME-2 (5 hydroxy-7, 4'-dimethoxy flavone)

Fractions (35-45) of ethyl extract were combined as they showed similar single spot in TLC, and crystallised from chloroform and obtained as a yellow color solid, m.p. 254-256 ºC.

Found : C, 68.27%; H, 4.72%; O, 26.79%
Calculated for C17H14O5 : C, 68.22%; H, 4.68%; O, 26.75%
R_f : 0.8 Hexane: ethyl acetate (7.5:2.5)
Yield : 25mg
^{1}H NMR and ^{13}C NMR : Fig-2 and 3; Table-3.

Compound ME-3 (3- iso cumaryloxy – cyclopropane-1-oleic acid)

Fractions (45-55) of extract were combined as they showed similar single spot in TLC, and obtained as a pale brown color solid, m.p. 258-260 ºC.

Found : C, 63.46%; H, 40. 72%; O, 32.58%
Calculated for $C_{13}H_{10}O_5$: C, 63.41%; H, 40.65%; O, 32.52%
R_f : 0.6 Hexane: ethyl acetate (4:6)

Yield : 40mg
IR (KBr.Cm^{-1}) : V $_{max}$ 3438 cm^{-1}, 1746 cm^{-1}
^{1}H NMR and ^{13}C NMR : Fig-5 and 6; Table-4

Compound ME-4 (4 -hydroxy-3-methoxy cinnamic acid)
Fractions (55-65) of extract were combined as they showed similar single spot in TLC, and crystal is from methanol and obtained as colorless fine crystallised, m.p. 169-171 °C.
Found : C, 57.16%; H, 47.74%; O, 38.25%
Calculated for $C_{10}H_{10}O_5$: C, 57.14%; H, 47.61%; O, 38.09%
R_f : 0.56 Hexane: ethyl acetate (3:7)
Yield : 20mg
IR (KBr.Cm^{-1}) : V $_{max}$ 3434.60, 1680, 1609, 1514, 1427 cm^{-1},
^{1}H NMR and ^{13}C NMR : Fig-7 and 8; Table-5.

The isolated compounds from *Mussaenda erythrophylla* can be use by pharmaceutical firms for drug formulation

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