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Research Article

PHYTOCHEMICAL INVESTIGATION OF CAYRATIA TRIFOLIA (LINN.) DOMIN STEM Rajinder Mann *, Sumitra Singh and Surendra Kr. Sharma Department of Pharmacognosy, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India *Corresponding Author Email: rajindermannhcp@gmail.com

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ABSTRACT

In the present investigation the methanol extract of *Cayratia trifolia* (Linn.) Domin stem was used for the isolation of phytoconstituents by using column chromatography. The identification of the isolated constituents was done by using spectroscopicanalysis. Five new compounds namely are n-tetradecanyl n-octadec-9, 12-dienoate, n-tridecanyl n-octadec-9, 12-dienoate, n-tetradecanyl n-octadec-9, 12-dien

Keywords: Cayratia trifolia (Linn.) Domin, Vitaceae, n-tetradecanyl n-octadec-9, 12-dienoate, n-tridecanyl n-octadec-9, 12-dienoate, n-tetradecanyl n-octadec-9, 12-dienoate, n-tetradecanyl n-octadec-9-enoate and n-hexadecanyl n-octadec-9-enoate.

INTRODUCTION

The medicinal values of plants are dictated by their phytochemical and other chemical constituents¹. Plant extracts contain many chemical compounds which are biologically active within the human body². Plant-derived substances have recently become of great interest owing to their versatile applications³. Scientific studies on a number of medicinal plants indicated that promising phytochemical compounds can be developed for many health problems⁴. Still most of the plants carry a large number of unidentified compounds which can be really useful for making new drugs and for the identification of lead compounds. The present study is aimed to isolate and characterize few phytoconstituents from the methanolic extract of Cayratia trifolia (Linn.) Domin. Cayratia trifolia (Linn.) Domin (Vitaceae) is a perennial climber, commonly known as fox grape in English, Amalbel, Ramchana in Hindi and Amlavetash in Sanskrit, found in India, Asia and Australia.⁵. The plant is found in hilly regions as well as the hotter part of India from Jammu and Rajasthan to Assam. The plant have trifoliated leaves with (2-3cm) long petioles and ovate to oblong-ovate leaflets. Flowers are small greenish white and brown in colour. Fruits are fleshy, juicy, spherical, about 1 cm in diameter of dark purple or black color. The roots of the plant are used as poultice on boils. Infusion of seeds along with extract of tubers is traditionally given orally to diabetic patients to check sugar level of blood. Whole plant is used as diuretic, in tumors, neuralgia and splenopathy. The paste of tubers is applied on the affected part in the treatment of snake bite. It is reported to possess antiviral, antibacterial, antiprotozoal, hypoglycemic, anticancer and diuretic activity etc⁶.

MATERIALS AND METHODS

The stem of *Cayratia trifolia* (Linn.) Domin was collected from kurukshetra, Haryana in the month of October 2010 and authenticated by Dr. H.B. Singh, Head Raw Material Herbarium

& Museum, New Delhi vide Ref. NISCAIR/RHMD/Consult-2010-11/1667/265. A voucher specimen has been retained in Department of Pharmaceutical Science, Guru Jambheshwar University of Science & Technology, Hisar. The plant material (1kg) was air-dried at room temperature (30-40°C)

Extraction of Plant Materials

The dried powdered stem of *Cayratia trifolia* (3 kg) were subjected to hot continuous extraction with methanol using soxhlet apparatus for 72 hours. The liquid extract was concentrated by distillation followed by drying and kept in desiccators. The stem extract (337.6 g, 16.88%) of *Cayratia trifolia* of brownish black colour sticky mass was obtained. The development and elution of the column was carried out with successive series of solvents in various combinations viz. petroleum ether, petroleum ether : chloroform chloroform, chloroform : methanol and methanol.

RESULTS

Compounds isolated from *Cayratia trifolia* (Linn.) Domin (Vitaceae) stem by column chromatography were

CT-1

Elution of the column with Petroleum ether : Chloroform (1:1) gave yellowish crystal of compound **CT-1**, recrystallized from acetone : methanol (1:1) 265 mg (0.0 75% yield) of R_f 0.68, m.p. 101-102°C.

UV λ_{max} (MeoH): 210 nm

IR v_{max} **(KBr):** 2924, 2854, 1736, 1651,1458, 1373, 1250, 1173, 987, 725 cm⁻¹

¹**H-NMR (CDCl₃)** : δ 5.33 (2H, m, H-9, H-10), 5.31 (2H, m, H-12, H-13), 3.72 (2H, t, J = 7.2 Hz, H₂-1), 2.50 (2H, m, H₂-11), 2.27 (2H t, J = 7.2 Hz, H₂-2), 2.17 (2H, m, H₂-8), 2.01 (2H, m, H₂-14), 1.62 (2H, m, CH₂), 1.48 (2H, m, CH₂), 2.21 (34H, brs,

17 x CH₂), 0.86 (3H, t, J = 6.5 Hz, Me-18), 0.82 (3H, t J = 6.2 Hz, Me-14).

TOFMS m/z (rel. int) : 476 $[M]^+$ (C₃₂ H₆₀ O₂) (5.3).

CT-2

Elution of the column with chloroform (100) gave reddish yellow crystal of compound **CT-2**, recrystallized from acetone : methanol (1:1) 277 mg (0.079 % yield) of R_f 0.56, m.p. 90-92°C. **UV** λ_{max} (MeoH): 210 nm

IR v_{max} (**KBr**): 2924, 2854, 1736, 1620, 1458, 1373, 1265, 1160, 1034, 725 cm⁻¹

¹**H-NMR (CDCl₃)** : δ 5.34 (1H, m, H-9), 5.32 (1H, m,H-10), 5.31 (1H, m, H-12), 5.29 (1H, m, H-13), 3.73 (2H, m, H₂-1), 2.50 (2H, m, H₂-11), 2.27 (2H, t, *J* = 7.5 Hz, H₂-2), 2.19 (2H, m, H₂-8), 2.01 (2H, m, H₂-14), 2.63 (2 H, m, CH₂), 1.48 (2H, m, CH₂), 1.23 (32H, brs, 16 x CH₂), 0.86 (3 H, t, *J* = 6.5 Hz, Me₂-18), 0.83 (3H, t *J* = 6.3 Hz, Me-13').

TOFMS m/z (rel. int) : $462 [M]^+ (C_{31} H_{58} O_2) (8.1)$.

CT-3

Elution of the column with chloroform : methanol (19:1) gave yellowish brown amorphus compound **CT-3**, recrystallized from acetone : methanol (1:1) 252 mg (0.072 % yield) of R_f 0.71, m.p. 110-112°C.

UV λ_{max} (MeoH): 209 nm

IR v_{max} (**KBr**): 2924, 2854, 1723, 1628, 1458, 1373, 1265, 1180, 1041, 725 cm⁻¹

TOFMS m/z (rel. int) : $504 [M]^+ (C_{34} H_{64} O_2) (25.4)$.

CT-4

Elution of the column with chloroform : methanol (3:1) gave brown resinous compound **CT-4**, recrystallized from acetone : methanol (1:1) 237 mg (0.067 % yield) of R_f 0.78, m.p. 95-97°C. **UV** λ_{max} (MeoH): 205 nm

IR v_{max} (KBr): 2916, 2854, 1721, 1654, 1458, 1427, 1281, 1227, 1173, 1003, 717 cm⁻¹

¹**H-NMR (CDCl₃)** : δ 5.31 (1H, m, H-9), 5.12 (1H, m,H-10), 3.73 (2H, m, H₂-1), 2.27 (2H, t, *J* = 7.2 Hz, H₂-2), 2.15 (2H, m,

H₂-8), 2.01 (2H, m, H₂-11), 1.64 (2 H, m, CH₂), 1.48 (2H, m, CH₂), 1.22 (44H, brs, 22 x CH₂), 0.85 (3 H, t, J = 6.3 Hz, Me-18), 0.82 (3H, t J = 6.5 Hz, Me-14).

TOFMS m/z (rel. int) : $478 [M]^+ (C_{32} H_{62} O_2) (41.8)$.

CT-5

Elution of the column with chloroform : methanol (1:1) gave brown resinous compound CT-5, recrystallized from acetone : methanol (1:1) 234 mg (0.066 % yield) of R_f 0.76, m.p. 103-104°C.

UV λ_{max} (MeoH): 206 nm

IR v_{max} **(KBr):** 2920, 2850, 1722, 1651, 1404, 1260, 1187, 1103, 1041, 721 cm⁻¹

TOFMS m/z (rel. int) : 506 $[M]^+$ (C₃₄ H₆₆ O₂) (11.2).

DISCUSSION

CT-1

Compound CT-1 named n-tetradecanyl n-octadec-9, 12dienoate, was obtained as yellowish crystal from Petroleum ether : Chloroform (1:1) eluant. It had IR absorption bands for stretching for ester function (1736 cm⁻¹), unsaturation (1651 cm⁻¹) ¹) and long aliphatic chain (725 cm⁻¹). On the basis of mass spectra, the molecular ion peak of compound CT-1 was determined at m/z 476 constituent to the molecular formula of an ester $(C_{32}H_{60}O_2)$. The ¹H-NMR spectra of compound CT-1 showed two-proton multiplet at δ 5.33 and 5.31 were assigned at position H-9, H-10, H-12 and H-13 respectively. A two proton triplet was appeared at δ 3.72 (J =7.2 H_z) and δ 2.27 (J =7.2 H_z). A two-proton multiplet at position at H₂-11, H₂-8 and H₂-14 protons at δ 2.50, 2.17 and 2.01. The methyline group (-CH₂) showed two proton multiplet at δ 1.62 and δ 1.48. The broad peaks of methylene group (17 x CH_2) were found at δ 2.21. The terminal methyl group showed three proton triplet at δ 0.86 (J =6.5 H_Z) and δ 0.82 (J =6.2 H_Z). On the basis of these evidences, the structure of compound CT-1 has been characterized as *n*-tetradecanyln-octadec-9, 12-dienoate.



n-tetradecanyl n-octadec-9, 12-dienoate

CT-2

Compound **CT-2**named **n-tridecanyl n-octadec-9, 12dienoate,**was obtained as reddish yellow crystal from chloroform (100) eluant. It had IR absorption bands for stretching for ester function (1721 cm⁻¹), unsaturation (1620 cm⁻¹) and long aliphatic chain (725 cm⁻¹). On the basis of mass spectra, the molecular ion peak of compound **CT-2** was determined at m/z 462 constituent to the molecular formula of a ester ($C_{31}H_{58}O_2$). The ¹H-NMR spectra of compound **CT-2** showed one-proton multiplet at δ 5.34, 5.32, 5.31 and 5.29 was assigned at position H-9, H-10, H-12 and H-13 respectively. A two proton multiplet at δ 3.73 and 2.50 was ascribed to the methyleneprotons. A two- proton triplet is appeared at δ 2.27 (*J* =7.2 H_Z). A two- proton multiplet at position at H₂-8 and H₂-14 protons at δ 2.19 and 2.01. The methyline group (-CH₂) showed two proton multiplet at δ 1.63 and δ 1.48. The broad peaks of methylene group (16 x CH₂) were found at δ 1.23. The terminal methyl group showed three proton triplet at δ 0.86 (*J* =6.5 H_Z) and δ 0.83 (*J* =6.3 H_Z). On the basis of these evidences, the structure of compound **CT-2** has been characterized as **n-tridecanyl n-octadec-9, 12-dienoate.**



n-tridecanyl n-octadec-9, 12-dienoate

CT-3

Compound **CT-3** named **n-hexadecanyl n-octadec-9**, **12-dienoate**, was obtained as yellowish brown amorphus mass from chloroform: methanol (19:1) eluant. It had IR absorption bands for stretching for ester function (1723 cm⁻¹), unsaturation (1628 cm⁻¹) and long aliphatic chain (725 cm⁻¹). On the basis of

mass spectra, the molecular ion peak of compound CT-3 was determined at m/z 504 constituent to the molecular formula of a ester ($C_{34}H_{64}O_2$). On the basis of spectra, the structure of compound CT-3 has been characterized as **n-hexadecanyl n-octadec-9**, 12- dienoate.



n-hexadecanyl n-octadec-9, 12- dienoate

CT-4

Compound **CT-4** named **n-tetradecanyl n-octadec-9enoate**,was obtained as brown resinous from chloroform: methanol (3:1) eluant. It had IR absorption bands for stretching for ester function (1721 cm⁻¹), unsaturation (1654 cm⁻¹) aromaticity (1458 cm⁻¹) and long aliphatic chain (717 cm⁻¹). On the basis of mass spectra, the molecular ion peak of compound **CT-4** was determined at m/z 478 constituent to the molecular formula of a ester ($C_{32}H_{62}O_2$). The ¹H-NMR spectra of compound **CT-4** showed one-proton multiplet at δ 5.31, 5.12 and 3.73 was assigned at position H-9, H-10 and H-1 respectively. A two proton triplets at δ 2.27 was ascribed to the OCH₂ protons. A two- proton multiplet at δ 2.15, and 2.01 was position at H₂-8' and H₂-11' protons. The methyline group (-CH₂) showed two proton multiplet at δ 1.64 and δ 1.48.. The broad peaks of methylene group (22 x CH₂) were found at δ 1.22. The terminal methyl group showed three proton triplet at δ 0.85 (*J* =6.3 H_Z) and δ 0.82 (*J* =6.5 H_Z). On the basis of these evidences, the structure of compound **CT-4** has been characterized as **n-tetradecanyl n-octadec-9-enoate**.



n-tetradecanyl n-octadec-9-enoate

CT-5

Compound **CT-5** named **n-hexadecanyl n-octadec-9enoate**,was obtained as brown resinous mass from chloroform: methanol (1:1) eluant. It had IR absorption bands for stretching for ester function (1722 cm⁻¹), unsaturation (1651 cm⁻¹) and long aliphatic chain (721 cm⁻¹). On the basis of mass spectra, the molecular ion peak of compound **CT-5** was determined at m/z 506 constituent to the molecular formula of a ester ($C_{34}H_{66}O_2$). On the basis of spectra, the structure of compound **CT-5** has been characterized as **n-hexadecanyl n-octadec-9-enoate.**



n-hexadecanyl n-octadec-9-enoate

Table 1: Physical constants and nomenclature of the phytoconstituents isolated from Cayratia trifolia L. Domin. Stem

Code No.	Name	Molecular formula/ M. wt.	Column Fraction; Rf value	% Yield	Melting point (°C)
CT-1	n-tetradecanyl n-octadec-9, 12- dienoate	C ₃₂ H ₆₀ O ₂	1:1 (P:C); 0.68	265 mg (0.0 75	101-103
CT-2	n-tridecanyl n-octadec-9, 12- dienoate	$C_{31}H_{58}O_2$	100 (C); 0.56	277 mg (0.079)	90-92
CT-3	n-hexadecanyl n-octadec-9, 12- dienoate	$C_{34}H_{64}O_2$	19:1 (C:M);0.71	252 mg (0.072	110-112
CT-4	n-tetradecanyl n-octadec-9-enoate	$C_{32}H_{62}O_2$	3:1 (C:M);0.78	237 mg (0.067	95-97
CT-5	n-hexadecanyl n-octadec-9-enoate	$C_{34}H_{66}O_2$	1:1 (C:M);0.76	234 mg (0.066)	103-104

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