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Monograph on quality standards of *Viscum angulatum* B. Heyne ex DC.

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The genus *Viscum* (Viscaceae) comprise of several species of hemi-parasitic plants with medicinal properties. Commonly known as mistletoes, these plants grow on other trees with the partial parasitic association. *Viscum angulatum* B.Heyne ex DC. is one such less explored leafless mistletoe of Asian countries with medicinal claims. In Ayurveda (*Bandaka*) and Siddha (*Pulluri/Pulluruvi*), many botanicals from mistletoe families have been attributed with medicinal properties. The objective of the current study is to develop a monograph on quality standards of *V. angulatum* occurring in high altitude hills of Western Ghats. Aerial parts of *V. angulatum* were collected and authenticated and preserved in FAA for microscopic studies and some quantity of the plant material was shade-dried and coarsely powdered. Successive extracts were subjected to chromatography and isolation - characterization of the major compounds. Leaf-less quadrangular stem was found to be a diagnostic macroscopic feature of this species. The preliminary phytochemical investigation of extracts showed presence of alkaloids, carbohydrates, coumarins, flavonoids, phytosterols, triterpenoids, saponins and tannins. HPTLC fingerprint of *n*-hexane and ethyl acetate extracts has been obtained for identification of extracts. The ethyl acetate extract yielded 10-hydroxyoleoside dimethyl ester which can be used as a marker compound for routine quality check of *V. angulatum* growing on *Mussaenda hirsutissima*. The set of data obtained in the present investigation can serve as a standard for the identification as well as further studies. These results on standards of this plant are the first report so far.

Keywords: Herbal fingerprint, Macro-microscopy, Mistletoes, Physico-chemical, Seco-iridoids.

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Introduction

Mistletoes (Santalaceae) are hemi-parasitic plants, producing parasitic roots on the aerial shoots of other higher plants; except for the genera *Nuytsia, Atkinsonia*, and *Gaiadendron*[1]. Six Indian mistletoes, two from *Loranthus* and four from *Viscum* are medicinally important. Though medicinal values are attributed to many Indian mistletoes, many are unexplored in detail for their therapeutic potential to the extent of *Viscum album* L. (European mistletoe)[2].

V. angulatum (syn. *Aspidixia angulata* Tiegh.) is a hemi-parasitic shrub growing on host plants like *Olea dioica, Flacourtia indica*, etc. with smooth and four-angled leaf-less branches having broadened internodes[3]. The mistletoe is used traditionally in Asian countries against hypertension. A few studies revealed the phytochemical profiling of the plant and some bioactive compounds isolated from *V. angulatum* are flavonoids and phenolic glycosides like pinocembrin 7-o-apiosyl (1→5) apiosyl (1→2)-*β*-D-glucopyranoside, 2',3',4',3' -tetramethoxy-1,3-diphenylpropane 5',4' -di-o-*β*-D-glucopyranoside and rhamnocitrin 3-o-apiosyl (1→5) apiosyl (1→2)-[α-L-rhamnopyranosyl (1→6)]-*β*-D-glucopyranoside,[4] which impart the antitumor potential to the plant. A distinctive antioxidant protein, VanPrx (A novel cationic peroxidase) has also been purified from *V. angulatum*[5]. Kuttan, *et al.*[6] reported selective cytotoxicity of *V. angulatum* in transformed cells and no toxicity was observed to normal cells. They also showed anti-tumor and anti-inflammatory activity of the plant and revealed the potential effect than other species of *Viscum*. It showed significant antioxidant and high cytotoxic activities on breast cancer cells (MDA-MB-231)[7]. The role of host plant in determining the medicinal efficacy of *Viscum* has been analyzed as mistletoes depend on its host for water and other nutrition[7]. The diuretic activity of *V. angulatum* has been studied by Jadhav *et al.*[8].

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Concerning these medicinal properties, detailed pharmacognostical studies to explore the quality standards of this species of mistletoe have been performed and reported in this paper. The present study has unveiled the pharmacognostic characters of this mistletoe species. The literature survey suggests that other than *V. album*, other Indian mistletoes belonging to the genus *Viscum* are less explored for their medicinal potential.

Materials and Methods

Materials

The fresh aerial part of *V. angulatum* growing as hemi-parasite on *Mussaenda hirsutissima* (Hook.f.) Hutch. ex Gamble was collected in October 2013, from Kundadri Hills (13°33'27"N 75°10'13"E) of Shimoga District of Karnataka, India. It was identified and authenticated by comparison with the botanical description mentioned in Floras[3] at the Pharmacognosy Laboratory of SDM Centre for Research in Ayurveda & Allied Science Udupi. The fresh aerial parts were used for the study of macroscopic and microscopic characterization. Collected aerial parts were shade-dried and coarsely powdered. The coarse powder was used for the physicochemical analysis followed by extraction, HPTLC and isolation of major compound.

Methods

The aerial parts were examined macroscopically as per standard procedures. Thin hand sections were cut using a blade, stained as per standard methodology[9], and then examined microscopically. Photomicrographs of the microscopic sections were captured with the help of the ZEISS Axio Lab. A1 microscope fitted with ZEISS Axiocam ERc 5s with the help of the ZEISS AxioVision software. Percentage of total ash, acid-insoluble ash, water-soluble ash, ethanol and water-soluble extractive, and loss on drying was performed as per standard protocol[10,11]. A preliminary phytochemical investigation was done to detect the presence of carbohydrate, triterpenoid, steroid, tannins, glycosides, flavonoids, and coumarin in ethyl acetate and ethanol extracts[12].

HPTLC of the *n*-hexane and ethyl acetate extract was carried out using CAMAG Linomat 5 applicator, the separation was obtained using toluene: ethyl acetate (9:1) and chloroform: methanol (8:2) as mobile phase, respectively, on Aluminium HPTLC Silica gel 60 F_{254} plates. The R_f values were determined from the photo-documentation performed using CAMAG TLC Visualizer 2 photo-documentation cabinet and the plates were scanned under 254, 366, 540, and 620 nm using CAMAG TLC Scanner 4[13,14].

The column was slurry packed with silica using *n*-hexane. Ethyl acetate extract prepared using Soxhlet extractor was loaded to the column and completely defatted with *n*-hexane (VA1). The column was washed with 10 mL of methanol followed by evaporation of the fraction using IKA RV 10 digital vacuum Rotary evaporator. The residue (VA2) so obtained was subjected to further column chromatography. A column was slurry packed with silica using chloroform. VA2 was loaded to the column and eluted with chloroform. Gradually polarity was raised by mixing methanol to chloroform. At 11% methanol, a single compound (VA3) was eluted. It was crystallized by using diethyl ether followed by characterization using HPTLC, UV and NMR. The ^{1}H, $^{13}C\{^{1}H\}$ and DEPT 135 NMR spectra for the isolated compound were recorded in DMSO-d_6 solution on Bruker Avance III NMR spectrometer at 400.22 (^{1}H) and 100.63 (^{13}C) MHz. The compound (10-hydroxyoleoside dimethyl ester) post identification was used for co-TLC with the extract.

Results

Macroscopy

Stems slender, angular, branches numerous, more than 2 or rarely decussate at nodes and dichasially branched; leaves scaly, mostly not visible, up to 0.5 mm long, sometimes prophylls were seen at the base of the branches; flowers found as solitary or in groups either with all female flowers or with a single female flower surrounded by male flowers; four triangular perianth lobes in male flower, stamens four, epiphyllous with sessile anthers; the ovary is obovoid, style is short, monoecious, lateral sessile, 1 or 3-flowered; the berry is yellow, baccate, sub-globose to globose, with persistent bracts at the base (Fig. 1).

Microscopical characteristics

TS of mature stem shows an angular outline, the outermost layer is the epidermis followed by the middle cortex, vascular bundles, and the innermost pith. The epidermis is made up of tangentially elongated cells with thin cross walls and covered by a thick cuticle. The cortex is formed of parenchyma cells, filled with contents like refractive drops and

Fig. 1 — Macroscopic features of fresh and dried *Viscum angulatum*, a) Photograph from natural habitat, b) Dichotomously branched aerial portion, c) A branch, d) Fruits, e) Quadrangular stem without leaves, f) Dried stem, g) Coarse powder, and h) Fine powder.

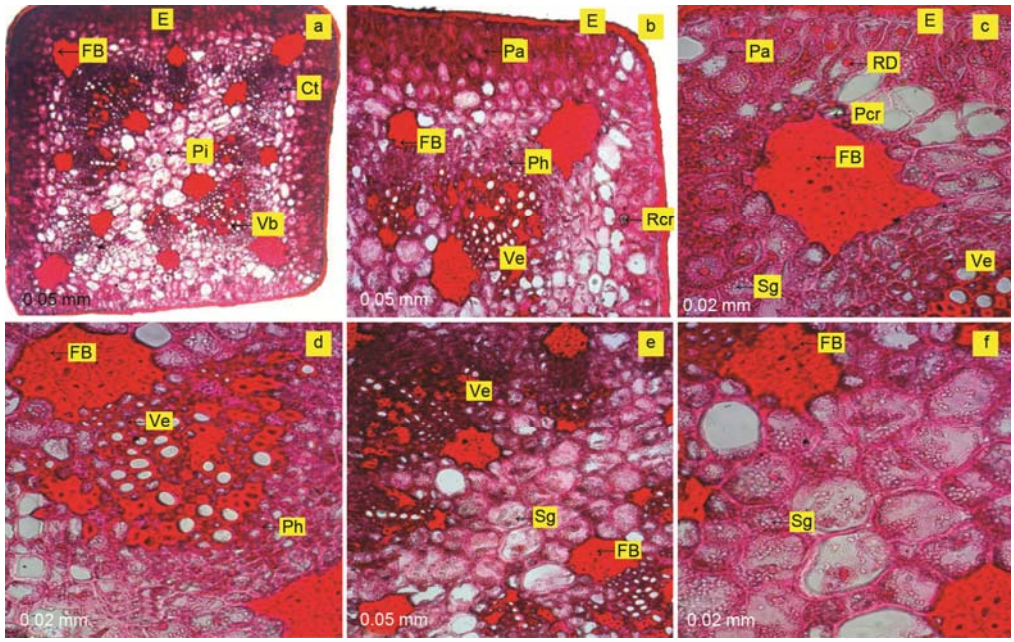


Fig. 2 — Microscopic features of young (quadrangular) stem of *Viscum angulatum*, a) TS of stem, b) A portion of TS enlarged, c) Epidermis palisade and fibre bundle, d) Vascular bundle, e) Central region showing pith, and f) Pith parenchyma with starch grains.
Abbreviation: Ct – Cortex, E – Epidermis, FB – Fibre bundle, Pa – Parenchyma, Pcr – Prismatic crystals, Ph – Phloem, Pi – Pith, Rcr– Rosette crystals, RD – Refractive drops, Sg – Starch grains, Ve – Vessels.

starch grains, without inter-cellular spaces. Calcium oxalate crystals of both prismatic and rosette types occur in the cortical parenchyma. Patches of the thick-walled, narrow-lumened group of fibres and highly pitted stone cells are found in the cortex. Vascular bundles are distributed towards the inner cortex formed of xylem and phloem. Phloem is composed of usual elements with patches of stone cells as described in the cortex. Cambium is 2 to 3 layered, obscure, separates the inner dense core of xylem from outer tissues. Xylem composed of vessels, tracheids, parenchyma, and xylem fibres. Xylem vessels are arranged in radial rows of vessels associated with tracheids. Xylem parenchyma forms uni- to bi-seriate rays. A patch of thick-walled bundles of pericyclic fibres are present above phloem tissues and also below the xylem of each bundle. Pith consists of loosely arranged reticulate parenchyma which contains starch grains and granular contents as in cortex (Fig. 2 & 3). Microscopically, quadrangular stem with evenly distributed 12 fibre/sclereid groups was found to be the distinguishing characteristics of young stems which turn round on maturity.

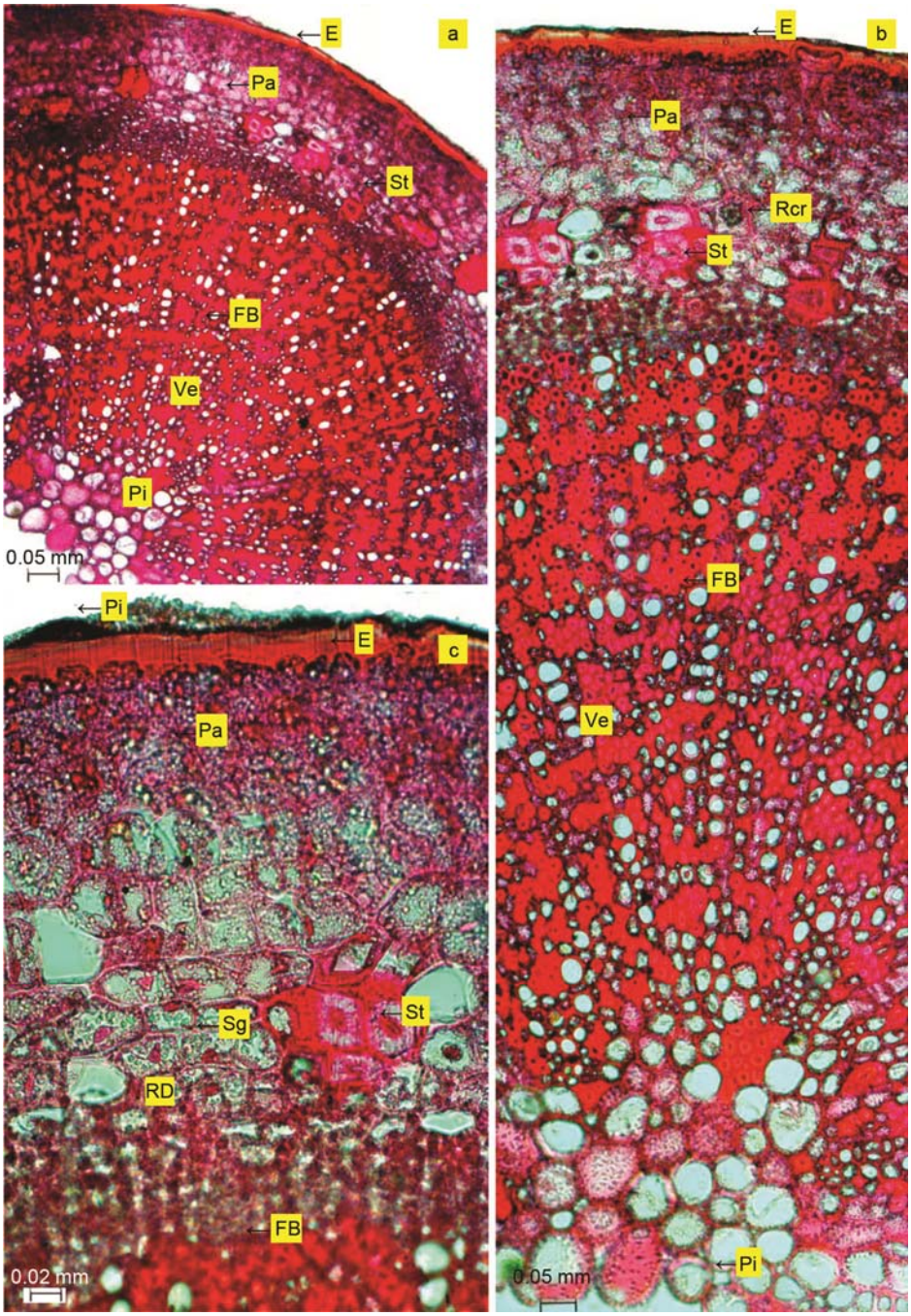


Fig. 3 — Microscopic features of matured (round) stem of *Viscum angulatum* Heyne ex DC, a) TS of stem, b) A portion of TS enlarged, and c) Epidermis, cortex and phloem.

Abbreviations: E – Epidermis, FB – Fibre bundle, Pa – Parenchyma, Pcr – Prismatic crystals, Ph – Phloem, Pi – Pith, Rcr– Rosette crystals, RD – Refractive drops, Sg – Starch grains, St- Stone cell, Ve – Vessels.

Maceration analysis

A macerate of stem shows characters such as epidermis in surface view, parenchyma cells with refractive drops, thick-walled stone cells often sclereidal in nature; bordered pitted and reticulate vessels attached to pitted xylem parenchyma cells, pitted tracheids attached to xylem fibres; thick-walled fibres, prismatic and rosette crystals (Fig. 4).

A comparative account on macroscopical and microscopical features of *V. angulatum* with *V. orientale*[14] and *V. album*[15] is presented in Table 1.

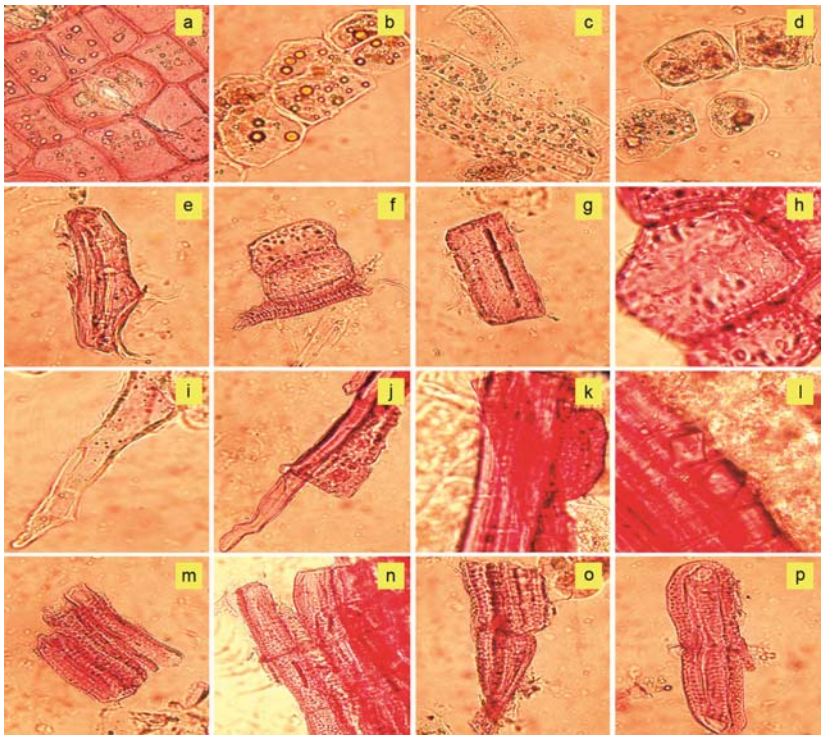


Fig. 4 — Microscopic features of macerated stem of *Viscum angulatum*, a) Epidermal cells of stem with paracytic stomata, b) Parenchyma with refractive drops, c-d) Parenchyma with starch grains, e-h) Pitted stone cells, i) Unicellular trichome, j) Thick walled fibre, k) Fibre bundle, l) Crystal fibre, and m-p) Bordered pitted vessels

Table 1 — Comparative account on macro- micro-morphology of some medicinal *Viscum* sp

	Character	*V. orientale*[14]	*V. album*[15]	*V. angulatum*
Macroscopy	Branches	Dichotomous, often unequal, terate or angled and grooved	Dichotomous, terate, lower ones whorled	Slender, angular; branches numerous, more than 2 or rarely decussate at nodes
	Leaf	Opposite, whorled, obscurely petioled, equally elliptic or elliptic-lanceolate, obtuse	Opposite, whorled, sessile, obovate-cuneate, tip rounded	Scaly, mostly not visible, up to 0.5 mm long, sometimes prophylls is seen at the base of the branches
	Flower	Monoecious, axillary sessile fascicles	Dioecious, fascicled, terminal in the forks of branches	Monoecious, lateral sessile, 1 or 3-flowered
	Fruit	Purple, minutely copiously dotted, globose	White, long, ellipsoid	Yellow, baccate, subglobose to globose, with persistent bracts at base
Microscopy	Stem	Tangentially elongated and the		
	Epidermal cells	cells are divided by thin cross walls	-Do-	-Do-
	Stomata	Paracytic type – large	-Do-	-Do-
	Guard cells	Located in pits, auxiliary cell not papillate	Located in pits and partly covered by papilliform auxiliary cells	Located in pits and partly covered by papilliform auxiliary cells
	Starch grains	Simple	-Do-	-Do-
	Calcium oxalate crystals (Prismatic)	Diameter 25 to 30 µm	Diameter 30 to 40 µm	Diameter 20 to 30 µm
	Pith	Intact	Partly hollow	Intact
	Parenchyma of pith and rays	Rays pitted, pith cells not pitted	Greatly thickened and pitted	Rays pitted, pith cells without pits
	Leaves	Cells of the epidermal layer are		NA
	Epidermal layer	covered by a thick cuticle	-Do-	
	Stomata	Paracytic type present on both sides of the leaves	-Do-	NA
	Starch granules	Simple; 5 and 8 µm diameter	Simple; 8 and 15 µm length	NA

Table 2 — Physico-chemical parameters of aerial part of *Viscum angulatum* B. Heyne ex DC.

Parameter	% w/w (Mean±SEM)
Total ash	7.879±0.026
Acid-insoluble ash	0.15 ± 0.01
Water-soluble ash	4.93±0.127
Loss on drying	9.047±0.020
Ethanol- soluble extractive	10.357±0.043
Water-soluble extractive	32.347±0.447
Successive extractive values	
a. *n*-Hexane	4.25±0.02
b. Chloroform	3.21±0.01
c. Ethyl acetate	3.78±0.01
d. Ethanol	8.37±0.01
Total	14.46 ± 0.04

Table 3 — Results of preliminary phytochemical tests for aerial part of *Viscum angulatum* B.Heyne ex DC.

Test	n-Hexane	Chloroform	Ethyl acetate	Ethanol
Alkaloids	+	+	-	-
Carbohydrates/ glycosides	-	+	+	+
Coumarins	+	-	-	-
Flavanoids	-	-	+	+
Phenols	-	-	+	+
Steroids	+	+	+	+
Saponins	-	-	-	+
Tannins	-	-	+	+
Terpenoids	+	+	+	+

Physicochemical constants

The physicochemical constants such as ash value, acid insoluble ash, water-soluble ash, loss on drying, total ethanol and water-soluble extractive are presented in Table 2.

Preliminary phytochemical screening

The preliminary phytochemical investigation of *n*-hexane, chloroform, ethyl acetate, and ethanol extracts of aerial parts showed the presence alkaloid, carbohydrate, coumarin, flavonoids, steroid, saponin, tannin, and terpenoid in various extracts (Table 3).

High-performance thin-layer chromatography (HPTLC)

HPTLC of *n*-hexane extract alone and ethyl acetate extract with marker compound was carried out using toluene: ethyl acetate (9:1) chloroform: methanol (4:1) as mobile phase respectively. R_f values and colour of the spots were recorded (Table 4 and 5) by TLC photo-documentation (Fig. 5). HPTLC densitometric scan of the plate developed for *n*-hexane and ethyl extracts showed numerous bands under 254, 366, and 540 nm (white light) after derivatization. The ethyl acetate extracts showed the presence of a major peak at R_f 0.58 which was isolated and identified as 10-hydroxyoleoside dimethyl ester subsequently. The UV scan of the spot at R_f 0.58 showed λ_{max} at 240 nm (Fig. 5) in both extract and isolate. The NMR spectral details of 10-hydroxyoleoside dimethyl ester are given in

Table 4 — R_f values of TLC of *n*-hexane extract of aerial part of *Viscum angulatum* B.Heyne ex DC.

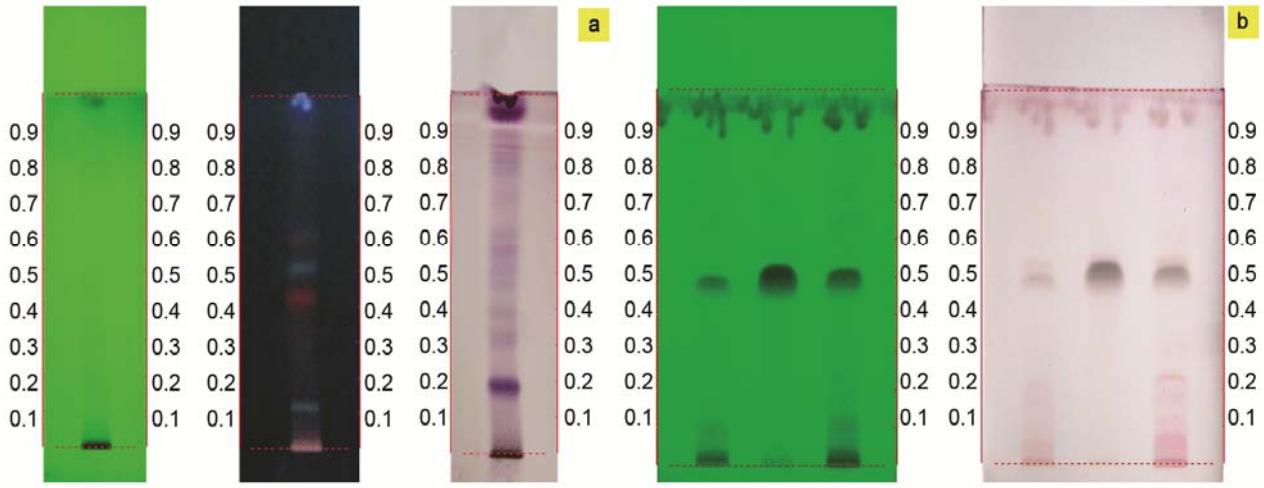
At 254 nm	At 366 nm	Post Derivatization
-	0.04 (F L pink)	-
-	0.13 (F Violet)	-
0.19 (L Green)	-	0.19 (D Violet)
-	0.21 (F L Violet)	-
0.30 (L Green)	0.30 (F L Violet)	0.30 (L Violet)
-	-	0.34 (L Violet)
-	-	0.40 (L Violet)
-	0.44 (F Red)	-
-	-	0.47 (L Violet)
0.49 (L Green)	-	-
-	0.52 (F Violet)	0.52 (L Violet)
-	-	0.58 (Violet)
-	0.61 (F L Red)	-
-	-	0.63 (L Violet)
-	-	0.69 (L Violet)
-	0.71 (F L Red)	-
-	-	0.73 (L Violet)
0.78 (L Green)	-	-
-	-	0.82 (L Violet)
-	0.86 (F L Violet)	0.86 (L Violet)
-	0.91 (F L Violet)	-

F- Fluorescent; L – Light; D - Dark

Table 5 — R_f value of TLC of ethyl acetate extract of aerial part of *Viscum angulatum B.* Heyne ex DC.

At 254 nm	At 366 nm	Post Derivatization
0.04 (Green)	-	0.04 (L Pink)
-	-	0.07 (L Pink)
0.12 (L Green)	-	0.12 (L Brown)
-	-	0.19 (L Pink)
-	-	0.23 (L Pink)
0.25 (L Green)	-	-
-	0.32 (F L Green)	-
0.35 (L Green)	-	-
0.52 (L Green)	-	0.52 (D Violet)
-	0.53 (F L Violet)	-
-	-	0.55 (L Violet)
-	0.58 (F L Violet)	-
0.67 (L Green)	-	-
-	0.70 (F L Violet)	-
0.74 (L Green)	-	-

F- Fluorescent; L – Light; D - Dark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b
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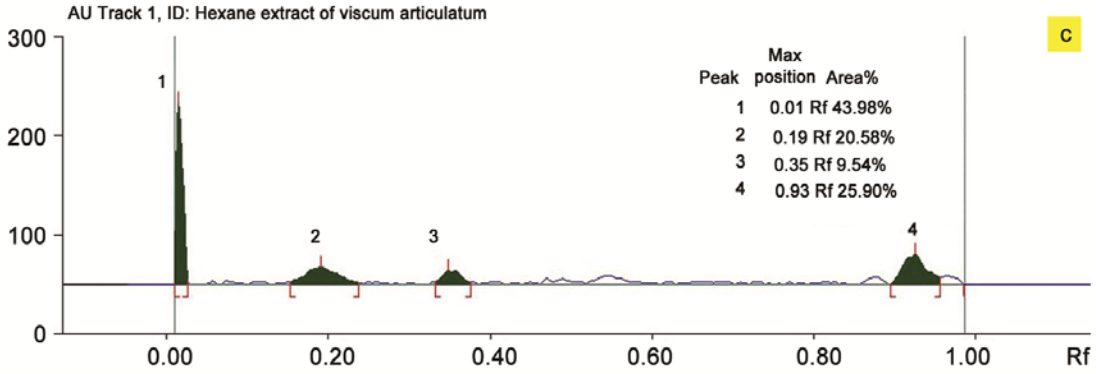
0.6
0.5
0.4
0.3
0.2
0.1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Max
Peak position Area%
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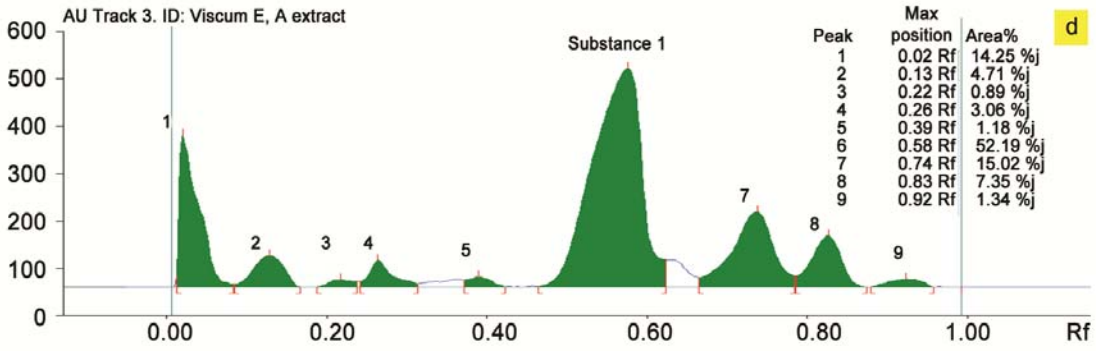
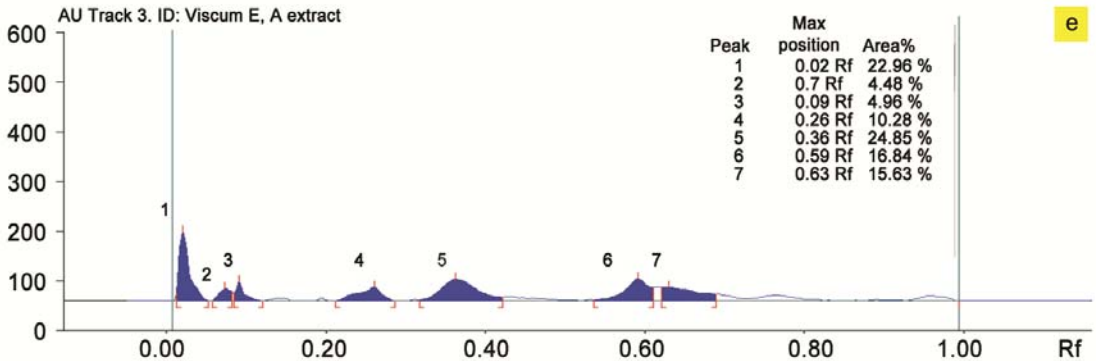
2 0.19 Rf 20.58%
3 0.35 Rf 9.54%
4 0.93 Rf 25.90%
300
200
100
0
0.00
0.20
0.40
0.60
0.80
1.00
Rf


AU Track 3. ID: Viscum E, A extract
d
Substance 1
Max
Peak position Area%
1 0.02 Rf 14.25 %j
2 0.13 Rf 4.71 %j
3 0.22 Rf 0.89 %j
4 0.26 Rf 3.06 %j
5 0.39 Rf 1.18 %j
6 0.58 Rf 52.19 %j
7 0.74 Rf 15.02 %j
8 0.83 Rf 7.35 %j
9 0.92 Rf 1.34 %j
600
500
400
300
200
100
0
0.00
0.20
0.40
0.60
0.80
1.00
Rf


AU Track 3. ID: Viscum E, A extract
e
Max
Peak position Area%
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2 0.7 Rf 4.48 %
3 0.09 Rf 4.96 %
4 0.26 Rf 10.28 %
5 0.36 Rf 24.85 %
6 0.59 Rf 16.84 %
7 0.63 Rf 15.63 %
600
500
400
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200
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0.00
0.20
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0.60
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1.00
Rf

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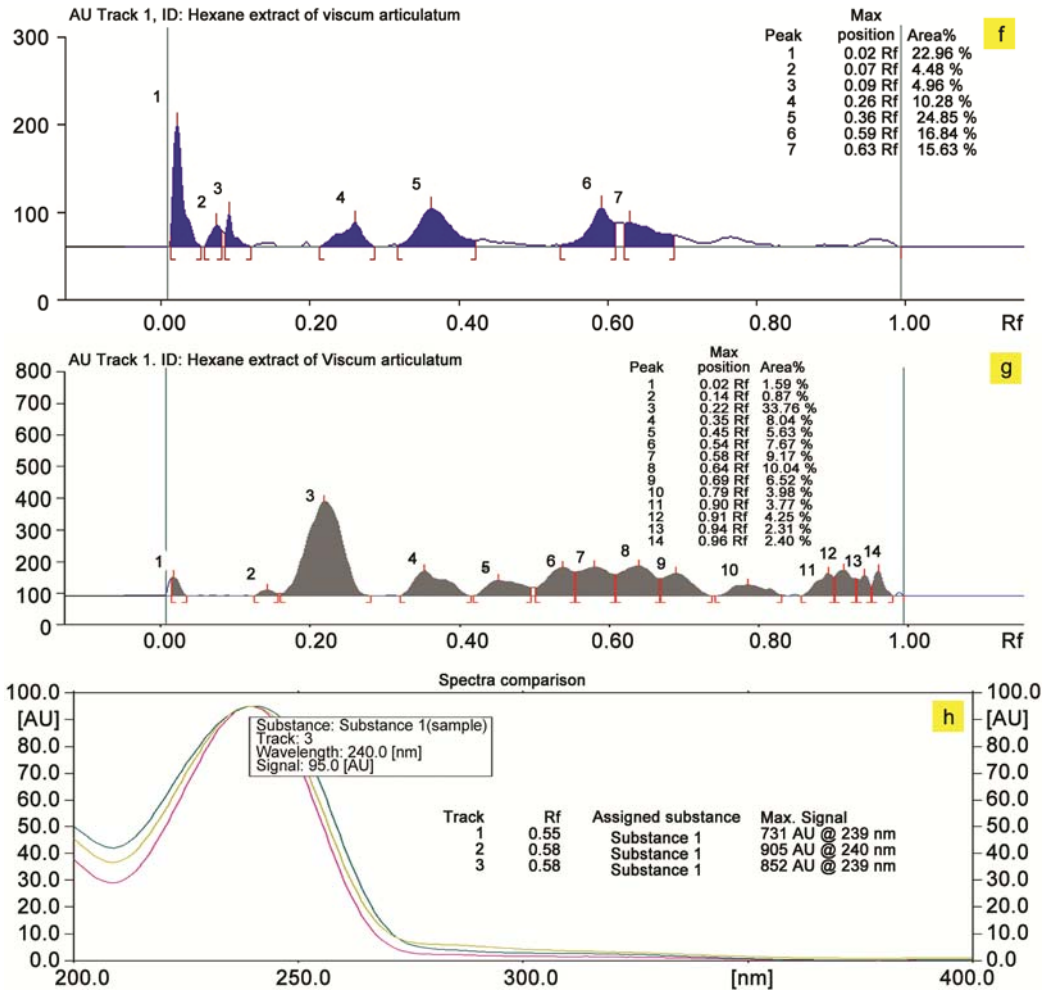
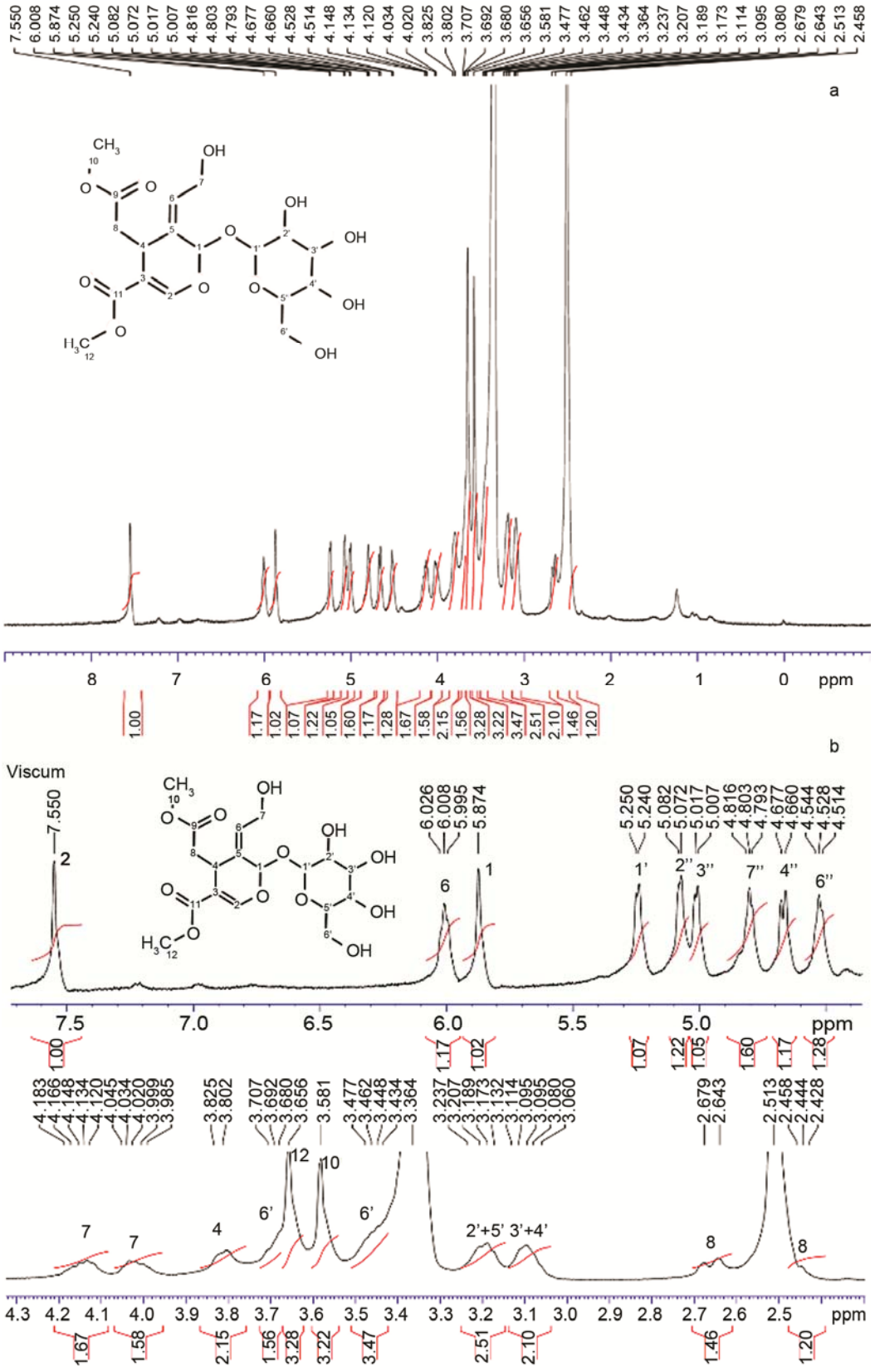


Fig. 5 — HPTLC fingerprint of extracts of *Viscum angulatum* Heyne ex DC, a) n-Hexane extract, b) Ethyl acetate extract with marker compound, c) n-Hexane extract at 254 nm, d) Ethyl acetate extract at 254 nm, e) n-Hexane extract at 366 nm, f) Ethyl acetate extract at 366 nm, g) n-Hexane extract at 540 nm, and h) Super imposable spectra of marker compound.

Table 6 and Fig. 6. The NMR confirmed the structure of the compound as 10-hydroxyoleoside dimethyl ester an iridoid glycoside[16].

The isolated compound was obtained as a greenish-brown gummy material. The NMR spectra were recorded in DMSO-d_6 as solvent (Fig. 6). ^{1}H NMR spectrum showed twenty signals in the region of 2.45 to 7.55 ppm in which two signals appeared as singlet with proton count of three at 3.65 and 3.58 ppm indicated the presence of two ester methyl groups. The singlet at 7.55 and 5.87 ppm indicated olefinic protons present in the molecule which is characteristic of a secoiridoid moiety[17]. A series of signals appeared between 4.51 and 3.51 ppm indicated that a glycoside moiety present in the molecule. ^{13}C NMR spectrum showed 18 signals in the region of 31.23 to 171.55 ppm indicating the number of carbons present in it. The resonances at 171.55 and 166. 56 ppm confirmed the presence of two ester carbonyl carbons in the compound. The resonance at 51.77 and 51.95 ppm indicated two methoxy methyl carbons. In DEPT 135 spectrum, three negative signals appeared at 40.81, 57.62 and 61.53 ppm which was assigned to CH_2 carbons attached with ester, hydroxyl and sugar unit respectively. The disappearance of resonances at 171.55 & 166.56 ppm and 128.63 & 108.00 ppm in DEPT 135 spectra were assigned to quaternary carbons of two ester carbonyls and two olefinic carbons of secoiridoid unit respectively. The ^{1}H, ^{13}C and DEPT 135 NMR resonance assignments to individual protons and carbons of the isolated compound are given in Table 6. Based on the results of the above analysis and comparison with literature[18], the molecule was identified as a 10-hydroxy oleoside dimethyl ester.



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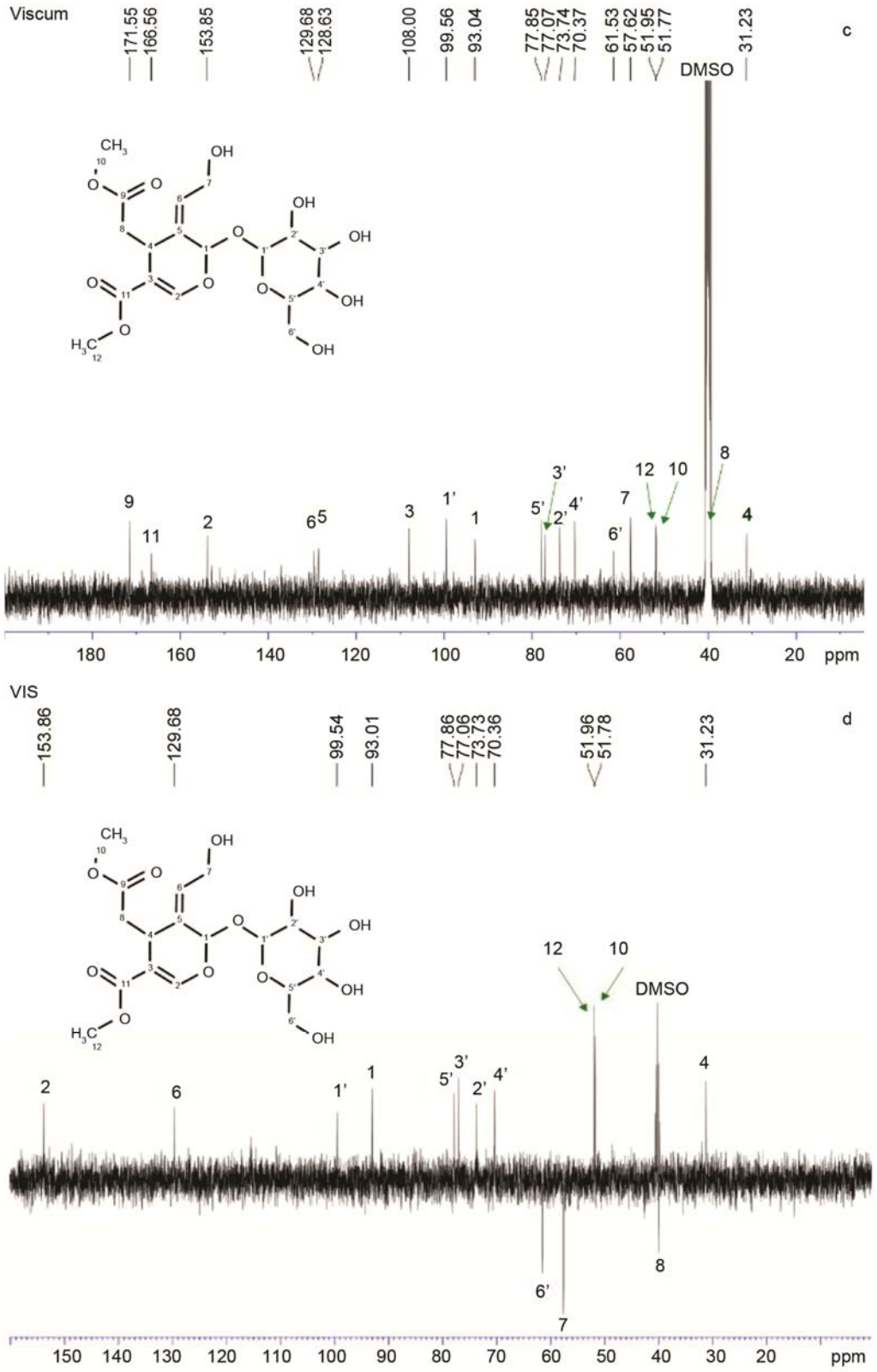

Fig. 6 — NMR Spectra with assignments of the isolated compound from *Viscum angulatum* B. Heyne ex DC. In DMSO-d_6, a) ^{1}H NMR Spectrum, b) ^{1}H NMR expansion Spectra, c) ^{13}C {^{1}H} NMR Spectrum, d) DEPT 135 NMR Spectrum

Table 6 — ^{1}H, ^{13}C and DEPT 135 NMR assignments of the isolated compound from aerial part of *Viscum angulatum* B. Heyne ex DC. in DMSO-d_6

Carbon No.	δ_C ppm	δ_H (No. of H's, Multiplicity, Coupling constant) ppm	DEPT 135	XH_n	^{1}H NMR Assignment
1	93.04	5.87 (1H, s)	+	CH	H-1
2	153.85	7.55 (1H, s)	+	CH	H-2
3	108.00	-	0	C	-
4	31.23	3.79 - 3.83 (1H, m)	+	CH	H-4
5	128.63	-	0	C	-
6	129.68	6.01 (1H, t, *J* = 6 Hz)	+	CH	H-6
7	57.62	3.97 - 4.05 (1H, m), 4.11 - 4.19 (1H, m)	–	CH_2	H-7
8	40.81*	2.41 - 2.47 (1H, m), 2.63 - 2.69 (1H, m)	–	CH_2	H-8
9	171.55	-	0	C	-
10	51.77	3.58 (3H, s)	+	CH_3	H-10
11	166.56	-	0	C	-
12	51.95	3.66 (3H, s)	+	CH_3	H-12
1'	99.56	5.24 (1H, d, *J* = 4 Hz)	+	CH	H-1'
2'	73.74	3.16 - 3.24 (2H, m)	+	CH	H-2'
3'	77.07	3.04 - 3.14 (2H, m)	+	CH	H-3'
4'	70.37	Merged with 3'	+	CH	H-4'
5'	77.85	Merged with 2'	+	CH	H-5'
6'	61.53	3.43 - 3.48 (1H, m), 3.67 - 3.71 (1H, m)	–	CH_2	H-6'
7'	-	4.80 (1H, t, *J* = 5 Hz)	n.a.	OH	H-7'
2"	-	5.08 (1H, d, *J* = 4 Hz)	n.a.	OH	H-2"
3"	-	5.01 (1H, d, *J* = 4 Hz)	n.a.	OH	H-3"
4"	-	4.67 (1H, d, *J* = 7 Hz)	n.a.	OH	H-4"
6"	-	4.53 (1H, t, *J* = 5 Hz)	n.a.	OH	H-6"

(+ = Positive; – = Negative; 0 = Disappeared, n.a. = Not applicable)
(* resonance merged with DMSO-d_6 distinguished with DEPT 135)

Discussion

The order Santalales is characterized by mistletoes and related plants with strong ethnopharmacological records. The genus *Viscum* comprises ethnomedicinally valuable plants that are used in various traditional systems and folk medicine for treating diseases. The paste of *V. angulatum* branches wrapped in cloth and boiled in water is applied for inflammation in the body and face, after severe jaundice[19]. A preparation of paste of *V. articulatum* is used for fever, blood disease, ulcer, epilepsy, and biliousness[17]. *V. monoicum* is characterized by all its parts having medicinal values: seed decoction is used in fever, jaundice, typhoid, and stomach disorders; leaf extract with turmeric acts as antifungal agents and paste of leaf is applied in hip joints for inflammation[19].

Since all the species of *Viscum* having specific biological activities the identification of each species will contribute to natural product discovery and it will also help in quality assurance of desired medicinal properties obtained from respective species. As most of the species are having similar morphology, it is difficult to distinguish species in their dried or powdered form when the raw drug is intended for much herbal drug preparation. The identification of crude drugs is the foremost step in herbal drug research as every herbal drug requires a standard for proper authentication. To be an expert in natural product's knowledge in taxonomical, morphological and anatomical aspects in addition to their phytochemistry is essential. The multidisciplinary approach of pharmacognosy is inevitable for the standardization of herbal materials.

V. angulatum is an unexplored Indian mistletoe with valuable medicinal activities. Mistletoes are regarded as important therapeutic plants worldwide. Only *V. album*, belonging to the genus *Viscum* is explored for its medicinal potential in detail so far. The present study has recorded the pharmacognostic characters of *V. angulatum* parasitic on host plant *M. hirsutissima* (Hook. f.) Hutch.ex Gamble. The macroscopic and microscopic characters aid in the authentication of the plant. Diagnostic distinguishing

features have been compared with other mistletoes like *V. album* and *V. orientale*. Physico-chemical pharmacopoeial standards for this plant have been derived as per standard methods. Preliminary phytochemical screening and HPTLC fingerprint profiling along with isolation of the major compound have also been recorded for the identification of the *V. angulatum* extract.

Helicanthes elastica (Desr.) Danser, mango mistletoe is a one such less studied Indian mistletoe species growing widely on mango trees, and other host species[20]. The pharmacognostic study revealed the presence of calcium oxalate crystals and trichosclereids in the lamina as diagnostic characters of *H. elastica*[21]. An earlier study on Iranian species, *Loranthus grewingkii*, and *Lycopus europaeus* differentiated both for their type and distribution of calcium oxalate crystals. *L. grewingkii* shown to have irregular, glandular as well as platelet crystalloid wax structures while it was smooth in L. *europaeus*[22]. An Argentinan mistletoe *Ligaria cuneifolia* Tiegh was found to be effective as a substitute for European mistletoe V. *album* L. due to its immune-modulating power and antitumoral effects[21]. Scattered, irregular, ramified, branched stone cells with calcium oxalate crystals and paracytic stomata were reported in the lamina of *L. cuneifolia*[23].

Phytochemical compounds present in plants are responsible for the therapeutic efficacy of medicinal herbs. Several studies reported the presence of bioactive compounds like flavonoids, phenols, lectins, sterols, triterpenes, in mistletoes[22,23]. The preliminary phytochemical analysis records the presence of important phytoconstituents like alkaloids, carbohydrates, coumarin, flavonoids, saponins, tannins, triterpenes and phytosterols in various extracts of *V. angulatum*. Chemical fingerprinting is found to be one of the most effective quality assurance tools for authentication of plants or drugs. Variations of chemical compounds present in plants can be successfully assessed by using HPTLC. The chromatographic prints of *V. angulatum* will provide efficient data to identify the species along with other pharmacognostic records.

Iridoids and their glycosides have been reported from other species of *Mussaenda*[24]. As this mistletoe was collected from a *Mussaenda sp* the occurrence can be connected to the host plant *M. hirsutissima*. Though mistletoes have host specificity to some extent, they were reported to be growing on several host species[25]. Phytochemicals of host species always get mixed with the chemicals of hemiparasite growing on it, as reported by a study employing HPTLC[13]. The finding suggested that while collecting mistletoe its host species must also be considered to obtain the desired effect.

Conclusion

The present work will serve as a quality standard document for the detection of this less recognized Indian mistletoe *V. angulatum*. The ethyl acetate extract yielded 10-hydroxyoleoside dimethyl ester which can be used as a marker compound for routine quality check of *V. angulatum* growing on *Mussaenda hirsutissima*. The finding will help researchers in further research on this unexplored medicinal plant.

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