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Phytochemical examination of compounds from Mango mistletoe – *Helicanthus elastica* (Desr.) Danser

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The whole plant of *Helicanthus elastica* growing on mango tress has been subjected for detailed phytochemical investigations. The alcoholic extract has been fractionated into *n*-hexane, ethyl acetate and *n*-butanol soluble portions. Composition of *n*-hexane soluble portion has been analysed using GC-MS and ethyl acetate soluble portion is chromatographed using a column. Isolated compounds have been identified using IR and NMR data. Of 28 compounds eluted by GLC, 9 have been identified by comparison of mass spectrum with that available in GC-MS libraries. Column chromatography yield 7 compounds *viz*. friedelin, epifriedelinol, β-amyrin, β-sitosterol, ethyl gallate, gallic acid and β-sitosterol-3-O-β-D glucopyranoside. All these compounds are reported for the first time from this plant and they can be used as chemical markers for *H. elastica*.

Keywords: Chemical marker, GC-MS, isolation, loranthaceae, Mango mistletoe

The study of chemical markers is applicable to many research areas, including authentication of genuine species, search for new resources or substitutes of raw materials, optimization of extraction and purification methods, structure elucidation and purity determination. Systematic investigations using chemical markers may lead to discoveries and development of new drugs. In India, six mistletoes, two belonging to the genus *Loranthus* and four to the genus *Viscum* are considered to be medicinal. This group of plants is therapeutically important, but there are no detailed phytochemical investigations in report. *Helicanthus elastica* (Desr.) Danser. (Syn. *Loranthus elasticus* Desr.) – Loranthaceae is one among the common Indian mistletoes which has not been studied in detail. It is a dichotomously branched, glabrous; pendulous shrub with swollen joints, the young branches being green. It is used to prevent abortion; also in vescical calculi and kidney affections[1]. The plant is reported to be good antimicrobial[2] and antioxidant agent[3]. It is also a good source of many nutritional supplements[4]. In the current study whole plant of *H. elastica* growing on very common host mango (*Mangifera indica*)[5] is subjected to detailed phytochemical investigation.

Results and Discussion

Preliminary phytochemical tests

Phytochemicals present in successive extracts of *H. elastica* are shown in **Table I**. Preliminary phytochemical analysis of successive (*n*-hexane, chloroform, ethyl acetate and ethanol) extractives revealed the presence of steroids and terpenoids in all the extracts, but alkaloid, coumarin and quinone were absent in all successive extracts. Glycosides and phenols were present in chloroform, ethyl acetate and alcoholic extracts. Positive colour reactions were obtained for flavone and tannin in ethyl acetate and alcoholic extracts. The plant was found to be a rich source of phenolic compounds which are generally responsible for antioxidant, antimicrobial, hepatoprotective, immunomodulatory activities of plant extracts. Presence of these secondary metabolites might contribute to its medicinal value. Based on preliminary phytochemical examination, detailed investigation was undertaken.

GCMS of *n*-hexane extract

List of compounds identified by GC-MS are listed in **Table II**. GC-MS revealed 28 compounds, of which,

Table I — Phytochemical present in successive extracts of *Helicanthus elastica*

Test	*n*-Hexane	Chloroform	Ethyl acetate	Alcohol
Alkaloid	-	-	-	-
Coumarin	-	-	-	-
Flavone	-	-	+	+
Glycoside/Sugar	-	+	+	+
Phenol	-	+	+	+
Quinone	-	-	-	-
Steroid	+	+	+	+
Tannin	-	-	+	+
Terpenoid	+	+	+	+

-, absent; +, present

Table II — Compounds identified by GC-MS of *n*-hexane extract of *Helicanthus elastica*

Peak	R_T (min)	Compound	Area (%)	SI (%)
1	15.431	1-Octadecene	0.06	86
2	19.934	Neophytadiene	0.22	90
3	22.489	Hexadecanoic acid ethyl ester	0.48	91
4	25.570	Octadecanoic acid ethyl ester	3.37	86
5	25.715	Stigmasterol	5.16	89
6	30.472	γ-Sitosterol	39.13	88
7	30.923	β-Stigmastan-3-ol	2.67	88
8	32.304	Pentacosane	1.93	96
9	35.256	Δ-Sitost-4-en-3-one	17.76	87

R_T, Retention time; SI, Superimposability

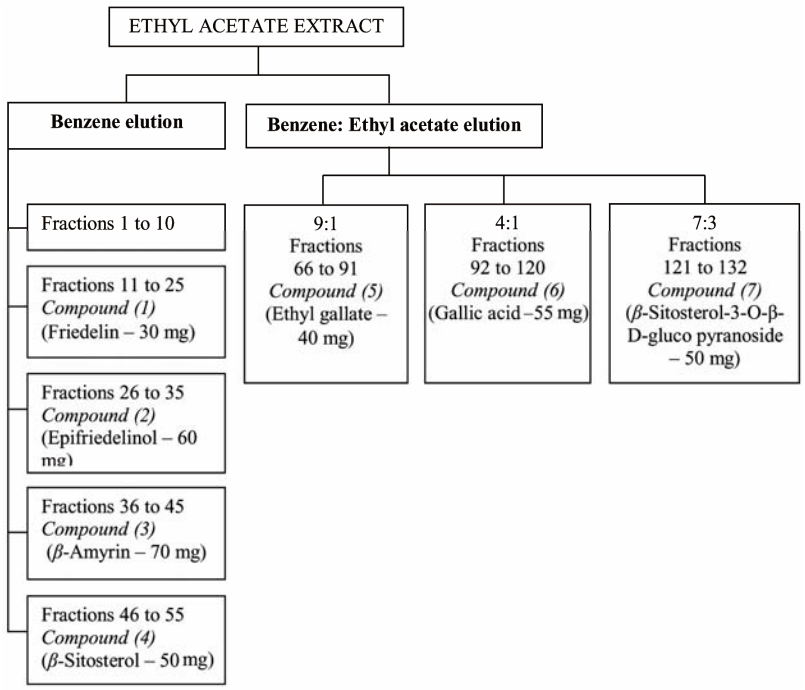
identity of 9 constituents were established by comparison with the mass spectra obtained to that available in the library with the help of SI factor. The study revealed γ-sitosterol (39.13%) as the major compound followed by Δ-sitost-4-en-3-one (17.76%), octadecanoic acid ethyl ester (3.37%), β-stigmastan-3-ol (2.67%) and pentacosane (1.93%). The other compounds such as hexadecanoic acid ethyl ester, 1-octadecene, neophy-tadiene and hexadecanoic acid ethyl ester were found to be in small quantities.

Column chromatography of ethyl acetate extract

Elution details of the compounds isolated are given in **Scheme I**.

1 (Friedelin): Elution of the column with benzene gave compound **1** which was identified as friedelin (m.p. 263°C, lit. m.p. 263°C, yield 30 mg). It answered Noller's test for triterpenoid.

The IR spectrum did not show any hydroxyl absorption. There was no olefinic absorption. Presence of ketone carbonyl and gem dimethyl group was shown by peaks at 1705 and 1380 cm^{-1} respectively. The ^1H NMR spectrum suggested the structure to be friedelin. There was no hydroxy methine signal corresponding to H-3, and that of olefinic protons. The spectrum showed eight methyl groups in the region δ 0.73-1.18. Seven tertiary methyls appeared at δ 0.72 (H-24), 0.87 (H-25), 0.95 (H-29), 1.00 (H-26), 1.01 (H-30), 1.05



Scheme I — Details of different fractions, their elution, and the compounds isolated from ethyl acetate extract of *H.elastica*

(H-27) and 1.18 (H-28). The secondary methyl H-23 appeared as a three proton doublet at δ 0.88 (J = 7.0 Hz). Two methylene protons adjacent to the carbonyl group appeared at δ 2.28 corresponding to H-2. H-4 appeared as one proton multiplet at δ 2.39. The ^{13}C NMR spectrum also identified the compound to be friedelin (see experimental). The identity was confirmed by comparison with the reported physical and spectroscopic data[6,7]. The identity as friedelin was further confirmed by comparison with an authentic sample (m.p., m.m.p., co-TLC and superimposable IR).

2 (Epifriedelinol): Elution of the column with benzene gave compound **2** which was identified as epifriedelinol (m.p. 288°C, lit. m.p. 286-88°C, yield 60 mg). It answered Noller's test for triterpenoid.

The IR spectrum showed hydroxyl group at 3435 cm^{-1} and gem dimethyl at 1389 cm^{-1}. No carbonyl peak was present. The ^{1}H NMR spectrum confirmed the structure to be epifriedelinol. H-3 appeared as one proton multiplet at δ 3.64. The compound showed eight methyl signals in the region δ 0.86-1.01. The secondary methyl H-23 appeared at δ 1.00 as doublet (J = 6.0 Hz). The ^{13}C NMR spectrum also confirmed the compound to be epifriedelinol (see Experimental Section). The identity was confirmed by comparison of the physical and spectroscopic data reported in literature[8]. The identity as epifriedelinol was further confirmed by comparison with an authentic sample (m.p., m.m.p., co-TLC and superimposable IR).

3 (β-Amyrin): Elution of the column with benzene gave compound **3** which was identified as β-amyrin (m.p. 198°C, lit. m.p. 197°C, yield 70 mg). It answered Noller's test for triterpenoid.

The IR spectrum showed the presence of hydroxyl group (3293 cm^{-1}), gem dimethyl group (1385 cm^{-1}), trisubstituted double bond (1647 and 830 cm^{-1}). The ^{1}H NMR spectrum showed eight tertiary methyl signals in the region δ 0.81-1.16 *viz.* 0.81 (H-24), 0.86 (H-23), 0.90 (H-29 and H-30), 0.96 (H-25), 0.99 (H-28), 1.02 (H-26), 1.16 (H-27). H-3 axial proton attached to C-3 carrying hydroxyl groups appeared as one proton triplet (J = 8.00 Hz) at δ 3.24. The olefinic proton H-12 appeared as one proton narrow multiplet at δ 5.20. The absence of-COOH at C-28 in triterpenoids is confirmed by IR and also by the absence of H-18 appearing at δ 2.80 corresponding to H-18 of oleanane triterpene.

The ^{13}C NMR spectral data were also in conformity with that of β-amyrin (see Experimental Section). The identity was confirmed by comparison of the physical and spectroscopic data reported in literature[9,10]. The identity as β-amyrin was further confirmed by comparison with an authentic sample (m.p., m.m.p., co-TLC and superimposable IR).

4 (*β*-Sitosterol): Elution of the column with benzene gave compound **4** which was identified as β-sitosterol (m.p. 136°C, lit. m.p. 137°C, yield 50 mg). It answered Liebermann-Burchard test for steroids.

The IR spectrum showed hydroxyl group (3480 cm^{-1}), tri substituted double bond (1637, 801 cm^{-1}) and isopropyl group (1381 cm^{-1}).

^{1}H NMR spectrum showed six methyls in the region δ 0.64-1.00. H-3 appeared as one proton broad multiplet at δ 3.55. The olefinic proton H-6 appeared as one proton broad singlet at δ 5.35.

The ^{13}C NMR spectrum also identified the compound to be β-sitosterol (see Experimental Section). The identity was confirmed by comparison of the physical and spectroscopic data reported in literature[11]. The identity as β-sitosterol was further confirmed by comparison with an authentic sample (m.p., m.m.p., co-TLC and superimposable IR).

5 (Ethyl gallate): Elution of the column with benzene-ethyl acetate (9:1) gave compound **5** identified as ethyl gallate (m.p. 146°C, lit. m.p. 148-52°C, yield 40 mg). It answered ferric chloride test for phenols.

The IR spectrum showed the presence of hydroxyl (3457 cm^{-1}), carbonyl (1707 cm^{-1}) and ester group (1254 cm^{-1}).

The ^{1}H NMR spectrum showed two equivalent aromatic protons H-2 and H-6 appeared at δ 7.0 as singlet. The presence of ethyl ester moiety was confirmed by the terminal methyl appearing as three proton triplet at δ 1.22 (J = 7.11 Hz). The methylene protons appeared as two proton quartet at δ 4.17 (J = 7.10 Hz).

The ^{13}C NMR spectrum showed the ester carbonyl at δ 167.76. The terminal methyl appeared at δ 14.25 as quartet. The methylene carbon appeared at δ 61.00. C-1 appeared at δ 121.17 and the C-4 carbon appeared at δ 138.08. The two equivalant carbons C-2 and C-6 appeared at δ 109.46 as doublet. Similarly, other two equivalent carbons at C-3 and C-5 appeared at δ 144.96.

The IR, ^{1}H and ^{13}C NMR and DEPT spectrum suggested the compound to be ethyl gallate (see Experimental Section). The identity was established by comparison of the physical and spectroscopic data reported in literature[12]. The identity as ethyl gallate was further confirmed by comparison with an authentic sample (m.p., m.m.p., co-TLC and superimposable IR).

6 (Gallic acid): Elution of the column with benzene-ethyl acetate (4:1) gave compound **6** identified as gallic acid (m.p. 244°C, lit. m.p. 250°C, yield 55 mg). It answered ferric chloride test for phenols.

The IR spectrum showed the presence of hydroxyl (3251 cm^{-1}), α,β-unsaturated carboxyl (1693 cm^{-1}) and presence of aromatic system (1612-863 cm^{-1}).

The ^1H NMR spectrum showed a singlet at δ 7.01 corresponding to H-2 and H-6.

The ^{13}C NMR spectrum also confirmed the compound to be gallic acid (see Experimental Section). The identity was confirmed by comparison of the physical and spectroscopic data reported in literature[13,14]. The identity as gallic acid was further confirmed by comparison with an authentic sample (m.p., m.m.p., co-TLC and superimposable IR).

7 (*β*-Sitosterol-3-O-β-D-glucopyranoside): Elution of the column with benzene-ethyl acetate (7:3) gave compound **7** identified as β-sitosterol-3-β-D-glucopyranoside (m.p. 281°C, lit. m.p. 282°C, yield 50 mg).

The IR spectrum showed hydroxyl group (3411 and 1024 cm^{-1}), CH_3 bending (1384 cm^{-1}) and tri substituted double bond (1631 and 836 cm^{-1}).

The identity was confirmed by comparison of the physical and spectroscopic data reported in literature[15]. The identity as β-sitosterol-3-β-D-glucopyranoside was further confirmed by comparison with an authentic sample (m.p., m.m.p., co-TLC and superimposable IR).

Column chromatography led to the isolation of friedelin, epifriedelinol, β-amyrin, β-sitosterol, ethyl gallate, gallic acid, and β-sitosterol-3-O-β-D-glucopyranoside. All these compounds are reported for the first time from this plant. The compounds isolated in the current investigation can be used as phytochemical reference standards[16] to establish the quality and efficacy of the unexplored medicinal mistletoe-*H. elastica.*

Materials and Methods

All solvents used were of analytical grade. For extraction of the plant material commercial grade solvents were purified and used. Chloroform and ethyl acetate were dried over anhydrous $CaCl_2$ and distilled. Commercial grade ethanol was dried over quick lime and distilled. Acme's silica gel (60-120 and 100-200 mesh) was used for column chromatography. Silica gel 60 F_{254} precoated aluminium plates (Merck, layer thickness 0.2 mm) were used for TLC. The spots were visualized by dipping in vanillin–sulphuric acid reagent and heating at 105°C till colour appearance.

Plant material

Fresh plants of the mistletoe growing on *Mangifera indica* were collected during flowering in the month of August, 2009 from Kasaragod District of Kerala, morphological features were compared with regional flora[17,18]. It was authenticated by Dr. S. Amerjothy, retired HOD of the Plant Biology and Biotechnology department, Presidency College, Chennai. Voucher specimen of the plant collected was deposited at the Pharmacognosy Department of Captain Srinivasa Murti Drug Research Institute for Ayurveda, Chennai. The voucher specimen number is 00637. Shade dried powder of the whole plant of *H. elastica* was used for phytochemical examination.

Preliminary phytochemical examination

Shade dried coarse powder (10g) was successively extracted with *n*-hexane, chloroform, ethyl acetate and alcohol was used for performing preliminary phytochemical tests as per standard procedure[19,20].

Extraction and isolation

Coarse powder (1 kg) was twice extracted with ethanol by cold percolation (48 hr). The ethanol extract was filtered, concentrated on a water bath and finally dried in vacuum. The total alcohol extract (180 g) was triturated with water, successively extracted with *n*-hexane (45 g), and then with ethyl acetate (80 g). The *n*-hexane extractive was analyzed by GC-MS and compounds were identified by comparison with GC-MS library data (NIST and WILEY). Ethyl acetate fraction (30 g) was subjected to column chromatography over silica gel (100-200 mesh) and the following seven compounds were isolated and identified by physical and spectroscopic data (IR, ^1H and ^{13}C NMR). The identities were further confirmed by comparison with authentic samples (m.p., m.m.p., co-TLC and super-imposable IR).

GC-MS analysis of *n*-hexane extract

Operating procedure

Instrument-GC-MS D5973 Agilent; Column-DB5 – MS; Coated Material-Dimethylsiloxane film thickness 25 µm; Column thickness-0.25 µ; Column length-30 m; Internal diameter-0.25 mm; Temperature programme-70°C for 2 min, 10°C/min up to 280°C; Injection temperature-180°C; Carrier gas – Helium; Flow rate-1 ml/min; Detector-Flame ionization detector; Library software-NIST and WILEY.

Column chromatography of ethyl acetate extract

All melting points were determined by open capillary method on a heating block instrument and uncorrected. IR spectra were taken in KBr disc on a Perkin-Elmer grating FT-IR instrument. ^{1}H and ^{13}C NMR were taken in $CDCl_3$ or $CDCl_3$ and CD_3OD mixture in a Bruker instrument (500 and 125 MHz, 300 and 75 MHz respectively). Chemical shifts are given in δ scale with TMS as the internal standard.

Experimental Section

1 Friedelin: Elution of the column with benzene (fractions 11 to 25) gave a compound crystallized as colourless needles (m.p. 263°C) from acetone. It answered Noller's test for triterpenoid and gave a single spot on TLC over silica gel (R_f = 0.74) with toluene-ethyl acetate (19:1) as the developing system.

IR (KBr): 2927, 2867; 1705 (ketone), 1455, 1380 (gem dimethyl), 1355, 1309, 1169, 1049, 999, 914, 789, 713 cm^{-1}; ^{1}H NMR (CD_3Cl_3, 500 MHz): δ 0.72 (3H, s, H-24), 0.87 (3H, s, H-25), 0.88 (3H, d, *J* = 7.0 Hz, H-23), 0.95 (3H, s, H-29), 1.00 (3H, s, H-26), 1.01 (3H, s, H-30), 1.05 (3H, s, H-27), 1.18 (3H, s, H-28), 2.29 (2H, m, H-2) and 2.39 (1H, m, H-4); ^{13}C NMR ($CDCl_3$ 125 MHz): δ 22.28 (C-1), 41.52 (C-2), 213.26 (C-3), 58.22 (C-4), 42.14 (C-5), 41.28 (C-6), 18.23 (C-7), 53.10 (C-8), 37.44 (C-9), 59.47 (C-10), 35.62 (C-11), 30.50 (C-12), 39.69 (C-13), 38.29 (C-14), 32.41 (C-15), 36.0 (C-16), 29.99 (C-17), 42.79 (C-18), 35.02 (C-19), 28.16 (C-20), 32.77 (C-21), 39.25 (C-22), 6.81 (C-23), 14.65 (C-24), 17.94 (C-25), 20.25 (C-26), 18.66 (C-27), 32.08 (C-28), 31.77 (C-29) and 35.34 (C-30).

2 Epifriedelinol: Elution of the column with benzene (fractions 26 to 35) gave a compound crystallized from *n*-hexane-chloroform mixture as colorless crystal (m.p. 288°C). It answered Noller's test for triterpenoid and gave a single spot on TLC over silica gel (R_f = 0.59) with toluene-ethyl acetate (19:1) as the developing system.

IR (KBr): 3435 (hydroxyl), 2926, 2869, 1634, 1459, 1389 (gem dimethyl), 1175, 1108, 1073, 1018, 799 cm^{-1}; ^{1}H NMR: (CD_3Cl_3, 500 MHz): δ 0.86 (3H, s, H-25) 0.93 (3H, s, H-24), 0.94 (6H, s, H-29 and H-30), 0.96 (3H, s, H-27), 0.99 (3H, s, H-28), 1.00 (3H, d, *J* = 6.0 Hz, H-23) 1.01 (3H, s, H-26) and 3.64 (1H, m, H-3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 17.54 (C-1), 35.33 (C-2), 72.75 (C-3), 49.16 (C-4), 37.09 (C-5), 41.72 (C-6), 15.78 (C-7), 53.19 (C-8), 37.82 (C-9), 61.34 (C-10), 35.62 (C-11), 30.02 (C-12), 39.66 (C-13), 38.36 (C-14), 32.32 (C-15), 35.55 (C-16), 29.70 (C-17), 42.81 (C-18), 35.18 (C-19), 28.17 (C-20), 32.80 (C-21), 39.27 (C-22), 11.61 (C-23), 16.39 (C-24), 18.24 (C-25), 20.11 (C-26), 18.64 (C-27), 32.08 (C-28), 31.78 (C-29) and 35.02 (C-30).

3 β-Amyrin: Elution of the column with benzene (fractions 36 to 45) gave a gummy material which on crystallisation from *n*-hexane gave colourless crystals (m.p. 198°C). It answered Noller's test for triterpenoid and gave a single spot (R_f = 0.39) on TLC over silica gel with toluene-ethyl acetate (9:1) as the developing system.

IR (KBr): 3293 (hydroxyl;), 2946, 2870; 1647 (tri-substituted double bond), 1463; 1385 (gem dimethyl group), 1361; 1035 (C-O stretching), 830 cm^{-1} (tri-substituted double bond); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.81 (3H, s, H-24), 0.86 (3H, s, H-23), 0.90 (6H, s, H-29 and H-30), 0.96 (3H, s, H-25), 0. 99 (3H, s, H-28), 1.02 (3H, s, H-26), 1.16 (3H, s, H-27), 3.24 (1H, t, *J* = 8. 0 Hz, H-3), and 5.20 (1H, m, H-12); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 38.61 (C-1), 25.99 (C-2), 79.05 (C-3), 38.78 (C-4), 55.20 (C-5), 18.39 (C-6), 32.68 (C-7), 39.82 (C-8), 47.66 (C-9), 36.97 (C-10), 23.54 (C-11), 121.74 (C-12), 145.20 (C-13), 41.74 (C-14), 28.10 (C-15), 26.17 (C-16), 32.50 (C-17), 47.26 (C-18), 46.85 (C-19), 31.08 (C-20), 34.75 (C-21), 37.16 (C-22), 27.25 (C-23), 15.50 (C-24), 15.58 (C-25), 16.82 (C-26), 426.96 (C-27), 28.40 (C-28), 33.33 (C-29) and 23.69 (C-30).

4 β-Sitosterol: Elution of the column with benzene (fractions 46 to 55) a compound crystallized from hexane-chloroform mixture as colourless needles (m.p. 136°C). It answered Leibermann-Burchard test for sterols and gave a single spot on TLC over silica gel (R_f 0.55) with toluene-ethyl acetate (9:1) as the developing system.

IR (KBr): 3480 (hydroxyl); 2943, 2391, 1637 (trisubstituted double bond); 1463, 1381 (isopropyl); 1241, 1132, 1061, 968, 801 cm^{-1} (trisubstituted double bond); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.64 to 1.00, (18H, 6xCH_3), 3.55 (1H, brm, H-3), 5.35 (1H, brs, H-6); ^{13}C NMR ($CDCl_3$, 125MHz): δ 37.20 (C-1), 31.53 (C-2), 71.77 (C-3), 42.17 (C-4), 140.70 (C-5), 121.67 (C-6), 31.86 (C-7), 31.53 (C-8), 50.08 (C-9), 36.10 (C-10), 21.04 (C-11), 39.73 (C-12), 42.8 (C-13), 56.72 (C-14), 24.25 (C-15), 28.20 (C-16), 56.02 (C-17), 11.94 (C-18), 19.35 (C-19), 36.46 (C-20), 18.73 (C-21), 33.90 (C-22), 26.05 (C-23), 45.79 (C-24), 29.12 (C-25), 19.77 (C-26), 18.99 (C-27), 23.03 (C-28), 11.81 (C-29).

5 Ethyl gallate: Elution of the column with benzene-ethyl acetate (9:1) afforded a pale brown solid (m.p. 146°C). It answered ferric chloride test for

phenol. It gave a single spot (R_f = 0.51) on TLC over silica gel with toluene-ethyl acetate-formic acid (1:0.5:0.1) as the developing system.

IR (KBr): 3457, 3292 (hydroxyl); 1707 (ester carbonyl), 1618 (aromatic), 1254 (ester) and 1021 cm^{-1}; ^1H NMR ($CDCl_3$ + CD_3OD, 300 MHz): δ 4.17 (2H, q, *J* = 7.10 Hz, CH_2), 1.22 (3H, t, *J* = 7.10 Hz, CH_3), 7.0 (2H, s, H-2 and H-6); ^{13}C NMR ($CDCl_3$ + CD_3OD, 75 MHz): δ 121.17 (C-1), 109.46 (C-2 & C-6), 144.93 (C-3 & C-5), 138.08 (C-4), 167.76 (C-7), 61.0 (C-8) and 14.25 (C-9)

6 Gallic acid: Elution of the column with benzene-ethyl acetate (4:1) afforded a pale brown solid (m.p. 244°C). It answered ferric chloride test for phenol and sodium bicarbonate test for carboxylic acids. It gave a single spot (R_f = 0.37) on TLC over silica gel with toluene-ethyl acetate-formic acid (1:0.5:0.1) as the developing system.

IR (KBr): 3251 (hydroxyl), 1693 (carboxyl), 1612, 1248, 1021 and 863 cm^{-1} (aromatic); ^1H NMR ($CDCl_3$ + CD_3OD, 300MHz): δ 7.01 (2H, s, H-2 and H-6); ^{13}C NMR ($CDCl_3$ + CD_3OD, 75MHz): δ 121.20 (C-1), 109.85 (C-2 and C-6), 144.96 (C-3 and C-5), 138.24 (C-4) and 169.69 (COOH).

7 β-Sitosterol-3-O-β-D-glucopyranoside: Elution of the column with benzene-ethyl acetate (7:3) which on crystallization in acetone gave colourless crystals (m.p. 281°C). It gave positive Liebermann Burchard test for steroids and also answered for sugar. It gave a single spot (R_f = 0.57) on TLC over silica gel with chloroform-methanol (8.5: 1.5) as the developing system.

IR (KBr): 3411 (OH), 2932, 1384 (C-H bending), 1165, 1071; 1024 (C-O bending of alcohol), 1631 and 836 cm^{-1} (trisubstituted double bond).

Conclusion

H. elastica is found to contain sterols, terpenoids, flavones, tannins and glycosides; alkaloids, quinones and coumarins were absent.

Compounds *viz.* 1-octadecene, neophytadiene, hexadecanoic acid ethyl ester, octadecanoic acid ethyl ester, stigmasterol, γ-sitosterol, β-stigmastan-3-ol, pentacosane and Δ-sitost-4-en-3-one have been identified by GC-MS analysis.

Friedelin, epifriedelinol, β-amyrin, β-sitosterol, ethyl gallate, gallic acid and β-sitosterol-3-β-D-glucopyranoside have been isolated from ethyl acetate extract by column chromatography over silica gel. All these constituents are reported for the first time from the plant. No phytochemical investigation of the plant has been previously reported. The compounds isolated in the current investigation can be used as chemical markers for routine analysis and standardization of *H. elastica*.

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