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Research Article

IXORA COCCINEA LINN: PHYTOCHEMICAL INVESTIGATION

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ABSTRACT

The present study was undertaken to carry out phytochemical screening of the ethanolic extract of the roots of *Ixora Coccinea* linn belonging to the family Rubiaceae. It is a fairly small, bushy shrub, with bright scarlet coloured flowers. The preliminary phytochemical screening of the ethanolic extract revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, tannins, resins, saponins, triterpenoids and steroids. Chemical investigation of the roots led to the isolation of six phytoconstituents namely 9, 12-Octadecadienoic acid, Di-n-octyl phthalate, β-Amyrin, Kaempferol-7-o-glucoside, Kaempferitrin and Quercitrin. All these constituents are reported for the first time from the roots of *I. Coccinea*.

Keywords: 12-Octadecadienoic acid, Kaempferol-7-o-glucoside, Kaempferitrin, Quercitrin.

INTRODUCTION

Ixora Coccinea is a glabrous shrub which grows to a height of about 0.6-0.9m, belonging to the family Rubiaceae.[1] The word “Ixora” is a Portuguese version of Iswari, name of Goddess Parvati to which the flowers of *I. Coccinea* are offered, while the word “Coccinea” is a Latin word meaning Scarlet coloured.[2] It bears flowers which are numerous having bright scarlet colour in dense, senssile corymbiform cymes. The plant *I. Coccinea* is native to India and is found mostly in Konkan region. It is cultivated throughout India as an ornamental plant.[3] The roots of the plant *I. Coccinea* are mostly used as an astringent, antiseptic, stomachic, sedative etc. Traditionally roots are also used in diarrhoea, dysentery, gonorrhoea, in loss of appetite, hiccups, fever, sores and chronic ulcers. Flowers are mostly used in dysmenorrhoea, leucorrhoea, haemoptysis, dysentery and catarrhal bronchitis. Decoction of flowers or bark is used as a lotion for eye troubles.[4] Preclinical studies have shown that the plant possess anti-inflammatory, anti-microbial, anti-oxidant, anti-ulcerogenic, anti-diarrheal, anti-nociceptive, anti-mutagenicity, hepatoprotective and hypolipidaemic activities.[5] From the literature survey it was revealed that the roots of *I. Coccinea* contains Δ 9,11 Octadecadienoic acid, Palmitic acid, stearic acid, oleic acid, linoleic acid and mannitol.[6] Two novel derivatized peptides viz. Ixorapeptide I and Ixorapeptide II along with other 28 known compounds from the aerial parts of the plant *I. coccinea,*[7] as well as compounds like Biochin A, Myricetin, Quercetin, Rutin, Diadzein and formononetin from the methanolic flower extract has been reported.[8] From the extensive literature survey it is learnt that no substantial work has been carried out on the roots of *I. Coccinea*. Hence an effort was made to investigate the phytoconstituents present from the ethanolic extract of the roots of *I. Coccinea*.

MATERIAL AND METHOD

All the melting points were recorded on Bio Technics India, Model No. BT2-38 melting point apparatus and were uncorrected. IR spectra of the compounds were recorded on Bruker α- T Spectrophotometer using KBr pellet method at National Facility for Clinical Trials ISISM, Kattankulathur, Tamil Nadu. ^{1}HNMR and Mass (LC-MS) spectra of compounds were taken on Bruker 500 MHz PMR spectrophotometer using $CDCl_3$ as the solvent and LC-MS Shimadzu LC 2020 respectively at the National Facility for Clinical Trial, Interdisciplinary School of Indian System

of Medicine, Chennai. Mass (ESI-MS) Spectra were recorded using ESI-MS Expression CMS Advion at SynZeal Research Laboratory, Ahmedabad. TLC was carried out using Aluchrosep Silica-gel 60/UV_{254} from S.D. Fine Chemicals Pvt. Ltd, Mumbai. TLC was carried out using Aluchrosep Silica- Gel 60/UV_{254} from S.D.Fine Chemicals Pvt Ltd, Mumbai. Column chromatography was carried out using Glass Column with a glass stopcock, 30 × 600mm from Merck Specialities Pvt Ltd., Mumbai, packed with Silica- gel (200- 400 mesh) from Molychem Pvt Ltd, Mumbai. All the chemicals and reagents used were obtained in high purity from S.D Fine chemicals Pvt. Ltd., Bombay and Molychem Pvt Ltd, Mumbai.

Authentication and collection

The roots of *I. Coccinea* were collected during October 2012 from Verna and Savordem, Goa, India. It was identified and authenticated by Prof.G.I.Hukkeri, Associate Professor in Botany, Dhempe College of Arts and Science, Miramar, Panaji, Goa, India.[9]

Preparation of ethanolic extract[10]

The roots were collected, washed and dried in shade. The dried roots were then powdered (1kg) and exhaustively extracted by maceration with ethanol (95%) for three days. After three days ethanolic layer was decanted off. The process was repeated three times. The solvent from the total extract was distilled off using rotary vacuum evaporator (Superfit) and then evaporated to dryness (45g).

Preliminary phytochemical screening[11, 12]

The ethanolic extract was subjected to preliminary phytochemical screening, for evaluation of major phytoconstituents such as alkaloids, glycoside, flavonoids, steroids, triterpenoids, tannins, saponins, resins etc. The results are tabulated in Table 1.

Isolation of phytoconstituents[10]

10g of the ethanolic extract of the roots of the plant *I. Coccinea* was adsorbed on 7g of silica and loaded on the top surface of the glass column which was previously filled with 180g of silica (240-400 mesh) prepared in Petroleum Ether. The column was eluted first with Petroleum ether 100%, followed by Petroleum ether: chloroform mixtures (95:5.90:10, 80:20, 70:30, 60:40 and 50:50), then chloroform 100% followed by graded mixtures of chloroform: ethyl acetate (95:5, 90:10, 80:20,70:30, 60:40 and 50:50) then with ethyl acetate 100%, followed by ethyl acetate: methanol graded mixtures (99:1,92:8,97:3,96:4 and 95:5). The elutions were monitored by TLC (Silica-gel G; visualisation by UV 254nm, 366nm and Vanillin-Sulphuric acid spraying reagent heated at 110^{0}C). Each time 10ml were collected and identical elutes (TLC monitored) were combined and concentrated to 5ml and kept aside.

Elution carried out with Petroleum ether: chloroform (50:50) resulted in a single spot on TLC (Petroleum ether: chloroform 50:50). After removing the solvent a pale yellow liquid resulted, which was designated as compound PS-1 (60 mg). Elution carried out with chloroform: ethyl acetate (90:10) resulted in a single spot on TLC (chloroform: ethyl acetate 90:10). After removing the solvent pale yellow oil obtained, which was designated as compound PS-2 (75mg).

Elution carried out with chloroform: ethyl acetate (60:40) resulted in a single spot on TLC (chloroform: ethyl acetate 60:40). After removing the solvent a pale yellow solid resulted, which was designated as compound PS-3 (75mg). Elution carried out with ethyl acetate (100%) resulted in a single spot on TLC (ethyl acetate 100%). After removing the solvent brown solid resulted, which was designated as compound PS-4 (65mg).

Elution carried out with ethyl acetate: methanol (98:2) resulted in a single spot on TLC (ethyl acetate: methanol 98:2). After removing the solvent a yellow solid resulted, which was designated as compound PS-5 (70mg). Elution carried out with ethyl acetate: methanol (95:5) resulted in a single spot on TLC (ethyl acetate: methanol 95:5). After removing the solvent a yellow solid resulted, which was designated as compound PS-6 (60mg).

The concentrates of the other elutes yielded resinous mass which was not processed further.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the ethanolic extract revealed the presence of alkaloids, carbohydrates, flavonoids, glycosides, tannins, resins, saponins, triterpenoids and steroids. Chemical investigation of the roots led to the isolation of six phytoconstituents namely 9, 12-Octadecadienoic acid, Di-n-octyl phthalate, β-Amyrin, Kaempferol-7-o-glucoside, Kaempferitrin and Quercitrin.

Compound 1 (9, 12- Octadecadienoic acid) b.p-230^{o}C; IR (KBr): 3379.47 cm^{-1} (br. OH), 2930.40 cm^{-1} (C-H stretching In CH_3), 2861.78 cm^{-1} (C-H stretching In CH_2), 1715.96 cm^{-1} (C=O), 1452.61 cm^{-1} (C-H deformation in CH_3), 1280.18 cm-1 (C-O stretching.); ^{1}HNMR ($CDCl_3$): δ0.871- δ 0.943(m, 3H, terminal methyl), δ1.325 (t, 7×CH_2, for methylene

protons), δ1.640 (m, 2H, C-3), δ2.083 (t, 2H, C-2), δ2.343 (t, 4H, C-8, 14), δ2.914 (t, 2H, C-11), δ5.431 (m, 4H, vinylic protons). The LC-MS spectra showed molecular ion peak at 280.15 [M^+], which was consistent to the molecular formula of $C_{18}H_{32}O_2$.[13]

Compound 2 (Di-n-octyl Phthalate), b.p-378^oC; IR (KBr): 2925.27 cm^{-1} (C-H str. in CH_3), 2858.82 cm^{-1} (C-H str. in CH_2),1728.71 cm^{-1} (C=O), 1600.40 cm^{-1} (C=C), 1475.85 cm^{-1} (C-H deformation in CH_3),1277.72 cm^{-1} (C-O str.); ^{1}HNMR ($CDCl_3$): δ 0.817 (m, 10H, C-7',7",8',8"), δ 1.181 (s, 12H, C-4',4",5',5",6',6") , δ 1.625 (m, 8H, C-2',2",3',3") , δ 4.005- δ 4.242 (m,4H,C-1',1"), δ 7.468 (d,2H, C-4,5), δ 7.630 (m, 2H, C-3,6). The LC-MS spectra showed molecular ion peak at 391.30 [M+1]$^+$ which is consistent with molecular formula $C_{24}H_{38}O_4$. The mass spectra showed fragment ion peaks at m/z 149.20 and 167.15 which is considered characteristic of alkyl phthalates.[14]

Compound 3 (β-Amyrin), m.p-196^oC; IR (KBr): 3381.24 cm^{-1} (br.OH), 2927.09 cm^{-1} (C-H stretching in CH_3), 2860.42 cm^{-1} (C-H stretching in CH_2), 1601.65 cm^{-1} (C=C), 1456.74 cm^{-1} (C-H deformation in CH_3), 1277.87 cm^{-1} (C-O stretching.); ^{1}HNMR($CDCl_3$): δ 0.830 (d, 3H, C-24), δ 0.880 (s, 6H, C-28,30), δ 0.936 (s, 3H, C-29), δ 0.960 (s, 3H, C-23), δ 0.994 (d, 3H, C-25), δ 1.163 (s, 3H, C-26), δ 1.188 (s,3H,C-27), δ 4.205- δ 4.319 (m,1H, C-3), δ 4.910- δ4.974 (m, 1H, 1×OH, C-3), δ 5.008 (d, 1H, C-12, vinylic protons), δ 1.202- δ 1.448 (m, 20H, 10×CH_2, C-1, 2, 6, 7, 11, 15, 16, 19, 21,22), δ 1.673- δ 1.717 (m, 3H, methine protons, C-5,9,18). The LC-MS spectrum gave a molecular ion peak at 427.35 [M+1]$^+$ and characteristic fragments at 218.35 and 258.20 resulting from Retro-Diels-Alder fragmentation of Δ7 pentacyclic triterpens. The molecular ion peak at 427.35 was consistent to the molecular formula $C_{30}H_{50}O$.[15, 16]

Compound 4 (Kaempferol-7-o-glucoside), m.p-269^oC; IR (KBr): 3775.98 cm^{-1}(glucoside), 3387.08 (br. OH), 2862.32 (C-H stretching in CH_2), 1727.70 cm^{-1}(C=O) , 1612.39cm^{-1}, 1457.42cm^{-1} (aromatic), 1383.22, 1280.04, 1127.22, 1072.30, 745.10; ^{1}HNMR ($CDCl_3$): δ 1.162- δ 1.425 (m, 2H, C-6"), δ 3.033 (d,1H, 1×OH, C-3), δ 3.406- δ 4.932 (m,1H, 8H, remaining sugar protons), δ 5.427 (d, 2H, 2×OH, C-5, 4'), δ 5.461 (d,1H, C-1", glucose anomeric protons), δ 6.035- δ 6.119 (m, 1H, C-6), δ 6.133 (t, 1H, C-8), δ 6.867 (t, 2H. C-3', 5'), δ 7.694- δ7.706 (m, 2H, C-2', 6'). The ESI-MS spectra gave a molecular ion peak at 450.6 [M+2]$^+$ corresponding to the molecular formula $C_{21}H_{20}O_{11}$.[17, 18, 19]

Compound 5 (Kaempferitrin), m.p-195^oC ; IR (KBr): 3379.47 cm^{-1}(br. OH), 2927.67 cm^{-1} (C-H stretching in CH_3), 1727.06 cm^{-1} (C=O), 1569.25 cm^{-1} (C=C), 1457.71 cm^{-1} (C-H deformation in CH_3), 1247.82 cm^{-1} (C-O stretching.); ^{1}HNMR ($CDCl_3$): δ 4.394 (d, 2H, 2×OH, C-5,4'), δ 6.861 (d, 2H, C-6,8), δ 7.704 (d, 2H, C-2',6'), δ 7.251 (d, 2H, C-3',5'), δ 4.485 (br.s, 1H, C-1"), δ 4.317 (d, 1H, C-2"), δ 3.929 (m, 1H, C-3"), δ 3.136 (d, 1H, C-4"), δ 3.576 (d, 1H, C-5"), δ 0.847- δ 0.976 (t, 3H, C-6"), δ 6.048 (s, 1H C-1"'), δ 4.221(d, 1H, C-2"'), δ 4.051(d, 1H, C-3"'), δ 3.300(t,1H, C4"'), δ 3.637 (d, 1H, C-5"'), δ 1.172 (d, 3H, C-6"'), δ 3.051 (t, 6H, 6× OH, C-2", 2"', 3", 3"', 4", 4"'). The ESI-MS spectrum gave a molecular ion peak at 579.3 [M+1]$^+$ corresponding to the molecular formula $C_{27}H_{30}O_{14}$. The spectrum also showed characteristic fragments at 433.2 (loss of rhamnose group) and 287.5 (aglycone group).[20, 21, 22]

Compound 6 (Quercitrin), m.p-175^oC; IR (KBr): 3382.86 cm^{-1} (br. OH), 2927.46 cm^{-1}(C-H stretching in CH_3), 1726.49 cm^{-1} (C=O), 1640.39 cm^{-1} (C=C), 1458.22 cm^{-1} (C-H deformation in CH_3), 1280.63 cm^{-1} (C-O stretching.); ^{1}HNMR ($CDCl_3$): Aglycone: δ 7.710 (m, 1H, C-6'), δ 7.519- δ 7.535 (m, 1H, C-2'), δ 5.837 (t, 1H, C-5'), δ 5.803- δ5.823 (m, 1H, C-8), δ 5.446 (t, 1H, C-6), δ 5.142 (d, 4H, 4×OH, C-7,5,4',5') Rhamnose: δ 4.914- δ 5.006 (m, 1H, C-1"), δ 3.583- δ 4.308 (m, 4H, C-2", 3", 4", 5"), δ 3.435 (m, 3H, 3×OH, C-2", 3", 4"), δ 0.843- δ0.925 (d, 3H, C-6"). The ESI-MS spectra gave a molecular ion at 449.2 [M+H]$^+$ consistent to the molecular formula $C_{21}H_{20}O_{11}$. Quercitrin then loses its terminal rhamnose unit and produces a product ion at 301.6, followed once again by the subsequent retro cyclization pathway to produce the product ion at m/z 181.2.[23, 24,25]

Table 1: Results of Preliminary phytochemical screening of the ethanolic extract of the *I. Coccinea* roots

S. No.	Preliminary Phytochemical Test	Result
1	Alkaloid	+ ve
2	Glycoside	+ve
3	Carbohydrates	+ ve
4	Flavonoids	+ ve
5	Proteins	- ve
6	Tannins	+ ve
7	Resins	+ ve
8	Saponins	+ ve
9	Triterpenoids	+ ve
10	Steroids	+ ve
11	Starch	- ve

+ = Present, - = Absent

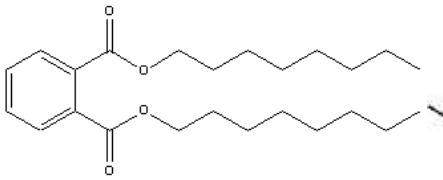

Fig. 1

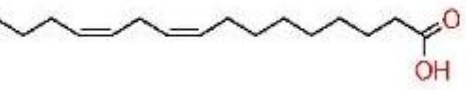

Fig. 2

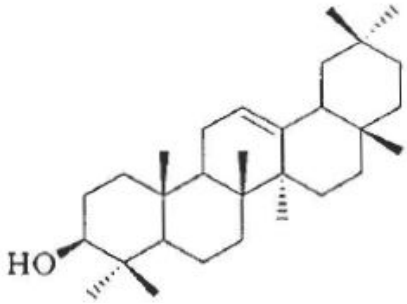

Fig. 3

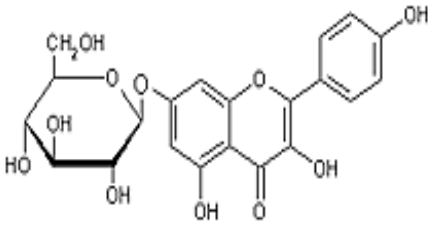

Fig. 4

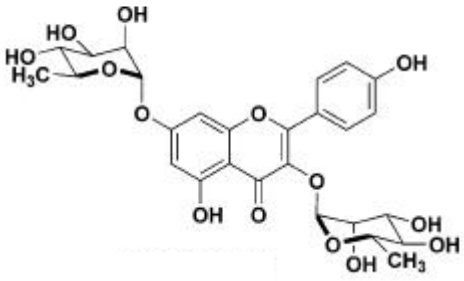

Fig. 5

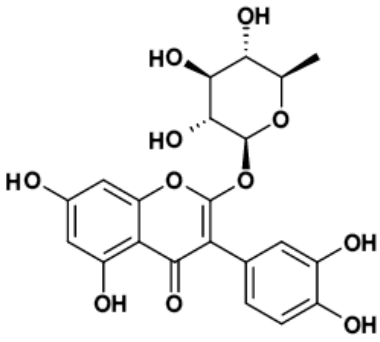

Fig. 6

CONCLUSION

The chemical investigation led to the isolation of six compounds from the ethanolic extract of the roots of *I. Coccinea*, which includes 9, 12-Octadecadienoic acid, Di-n-octyl phthalate, β-Amyrin, Kaempferol-7-o-glucoside, Kaempferitrin and Quercitrin. The isolated and characterised constituents can be categorised under the class of lipids, phthalates, triterpenoids, and flavonoidal glycosides. The above compounds were isolated for the first time from the roots of *I. Coccinea*.

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