

Phytochemical Analysis in the Leaves of *Chamaecrista nigricans* (Leguminosae)

Tangavelou AC^{1*}, Viswanathan MB², Balakrishna K³ and Patra A⁴

¹Sri Paramakalyani Centre for Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi, Tamil Nadu, India

²Department of Botany, Bharathidasan University, Tiruchirappalli 620024, Tamil Nadu, India

³Entomology Research Institute, Loyola College, Nungampakkam, Chennai 600034, Tamil Nadu, India

⁴Department of Chemistry, University College of Science, Kolkata, West Bengal, India

Abstract

Objective

In the present study, the plant *Chamaecrista nigricans* (Siruavuri in Tamil) was selected to isolate, elucidate and identify the chemical constituents present in it.

Methods

Leaves were collected, shade-dried, coarsely powdered using a pulverizer, successively extracted with various solvents of increasing polarity such as hexane, chloroform and methanol using Soxhlet apparatus. Methanol leaf extract was used for isolation and identification of chemical constituents. Column chromatography (CC) and thin layer chromatography (TLC) were used for separation and purification of chemical constituents while the isolated pure compounds were identified using UV-VIS, IR, ¹H and ¹³C NMR spectra. GC-MS analysis was carried out to identify the chemical constituents.

Results

Three anthraquinones such as emodin, chrysophanol and physcion were isolated and identified. GC-MS analysis helped to identify diisooctyl ester 1,2-benzenedicarboxylic acid, methyl ester, (Z, Z, Z)-9,12,15-octadecatrienoic acid, nitric acid nonyl ester, 4-C-methyl-myo-inositol, n-hexadecanoic acid, 2-methyl-butanoic acid, and, octadecanoic acid.

Conclusion

Medicinally valuable bioactive natural compounds in this plant proved its importance in drug industry for drug development against various diseases.

Keywords: *Chamaecrista nigricans*; Chemical constituents; Identification; Drug development

Introduction

The genus *Chamaecrista* (L.) Moench (Leguminosae) comprises of about 330 species [1] in the world most commonly found from Africa to Asia and also in South America. In India, 11 species are reported, of which 2 species are endemic. *Chamaecrista nigricans* (Vahl) Greene is an annual undershrub, locally known as Siruavuri in Tamil and commonly found in Thoothukudi, Tirunelveli and Virudhunagar districts of Tamil Nadu State in India. Locally, the leaves are used for the treatment of skin diseases. Traditionally, leaves are used as an appetite, fever, sore throat and various gastrointestinal disorders including diarrhea, peptic ulcer and in family planning [2-7], as an antipyretic and substituted for quinine in Senegal and Guinea and to heal wounds in Bamako region, Mali, West Africa [8]. Chemical constituents such as emodol, emodolanthrone and leucoanthocyanin have been reported from leaves [2,9,10]. Biological activities such as analgesic, anti-inflammatory, anti-diarrheal, antimicrobial, anti-plasmodial, anti-ulcer, contraceptive and estrogenic properties have also been reported [4-7,11].

Experimental

Plant material

Leaves were collected from the plains in Tirunelveli District, Tamil Nadu, India. Authentic herbarium specimen (MBV & ACT 17210) was deposited in the Herbarium of the Sri Paramakalyani Centre for Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi, Tamil Nadu, India.

Plant materials and extraction

Leaves of *Chamaecrista nigricans* (1 kg) were shade-dried, coarsely powdered using a pulverizer, successive extracted with various solvents of increasing polarity such as hexane, chloroform and methanol using Soxhlet apparatus. Methanol leaf extract was selected for isolation and identification of phytoconstituents.

Methods of separation

Chromatographic techniques such as column chromatography (CC) and thin layer chromatography (TLC) were mainly used for separation and purification of phytoconstituents. Silica gel (60-120 mesh) columns of 90 × 5 cm were prepared. The waxy material was removed by elution using hexane. Adding benzene, chloroform, ethyl acetate, methanol and their mixtures gradually increased the polarity of the eluting solvents. The elute fractions of 25-100 mL were collected and the solvents were distilled off on the Water Bath. The

*Corresponding author: Tangavelou AC, Sri Paramakalyani Centre for Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi, Tamil Nadu, India, Tel: +91 9585147029; E-mail: ac.tangavelou@tdu.edu.in; actangavelou@hotmail.com

Received August 30, 2017; Accepted March 23, 2018; Published March 29, 2018

Citation: Tangavelou AC, Viswanathan MB, Balakrishna K, Patra A (2018) Phytochemical Analysis in the Leaves of *Chamaecrista nigricans* (Leguminosae). Pharm Anal Acta 9: 582. doi: 10.4172/2153-2435.1000582

Copyright: © 2018 Tangavelou AC, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

concentrates were spotted on TLC plates of silica gel G of 0.5 mm thickness coating. The plates (20 × 5 cm) were developed with suitable solvents in a cylindrical TLC glass jar. The developed plates were air-dried, sprayed 50% sulphuric acid and heated in an Oven at 110°C for 5 min. Similar fractions of TLC patterns were combined, concentrated and rechromatographed repeatedly over silica gel to isolate pure compounds.

Methods of identification

The isolated pure compounds were identified for the class to which they belong based on the properties and color reactions. The properties include melting point for solid, boiling point for liquid, $[\alpha]_D$ for optically active compounds and R_f value on TLC. However, equally informative data for plant constituents are spectral data. The instruments used were Shimadzu UV-VIS spectrophotometer in methanol for UV-VIS (ultra violet-visible) spectra, the KBr disc on Perkin-Elmer grating spectrophotometer for IR (infrared), ¹H and ¹³C NMR spectra on Bruker FT-NMR instrument at 400 MHz and 100 MHz respectively, MS/GC-MS (Mass Spectroscopy/Gas Chromatography-Mass Spectroscopy) using Shimadzu Instruments. Confirmation was done by direct comparison with authentic compounds [12].

Gas chromatography-Mass spectrometry (GC-MS): For GC-MS analyses, the methanol extract was run on Perkin Elmer GC-MS system (GC Clarus 500) with Column Elite-1 (100% Dimethyl poly siloxane), 30 x 0.25 mm x 1 μm df. Oven temperature was programmed as follows: isothermal temperature at 50°C for 2 min, then increased to 200°C at the rate of 10°C/min, then increased up to 280°C at the rate of 5°C/min held for 9 min. Ionization of the sample components was performed in the EI mode (70 eV). The carrier gas was helium (1 mL/min) and the sample injected was 2 μL. The detector was Mass detector turbo mass gold-Perkin Elmer. The total running time for GC was 36 min and software used was Turbomass 5.2. The individual constituents were identified by comparing their mass spectra with the spectra of known compounds stored in the NIST spectral database.

Results

The methanol leaf extract (200 g) was column chromatographed over silica gel (Acme's silica gel 60-120 mesh) in hexane and eluted with solvents of increasing polarity. Three anthraquinones (CN1, CN2 and CN3) were isolated and identified (Figures 1-3).

Characterization of CN1 compound (Emodin)

Elution with hexane:benzene (7:3) yielded orange-colored needles (80 mg), crystallized from methanol (m.p. 256-257°C). It was soluble in aqueous Na₂CO₃, conc. H₂SO₄, NaOH and NH₃ and gave dark red solution. It gave positive ferric reaction for phenol. The compound gave orange color under daylight and UV light at 254 nm. A single yellow spot was observed on TLC with silica gel (R_f=0.59) and benzene:chloroform:ethylacetate (20:20:3) as the developing system, and it turned pink on exposure to NH₃ vapor. It gave pink color with alcoholic magnesium acetate, a characteristic feature of anthraquinones.

UV λ_{max} nm: 220, 252, 264, 289, 436;

IR ν_{max}^{KBr} cm⁻¹: 3388, 1667, 1622, 1577, 1563, 1478, 1369, 1333, 1301, 1271, 1217, 1166, 1101, 1033, 907, 874, 759;

¹H NMR (δ, CDCl₃, 400 MHz): 2.44 (3H, s, CH₃), 6.66 (1H, d, J=2.5 Hz, H-7), 7.07 (1H, s, H-2), 7.31 (1H, d, J=2.5 Hz, H-5), 7.61 (1H, br s, H-4), 12.17 (1H, s-OH), 12.27 (1H, s-OH);

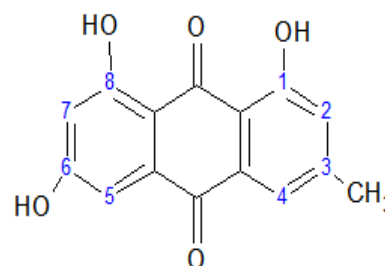


Figure 1: Emodin (1, 6, 8-trihydroxy, 3-methyl anthraquinone).

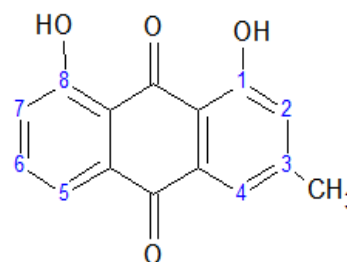


Figure 2: Chrysophanol (1, 8-dihydroxy, 3-methyl anthraquinone).

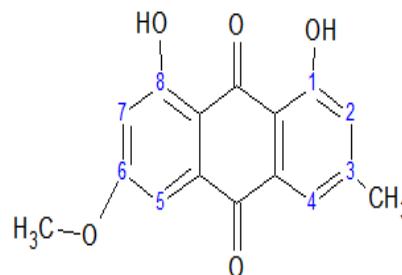


Figure 3: Physcion (1, 8-dihydroxy, 3-methyl-6-methoxy anthraquinone).

¹³C NMR (δ, CDCl₃, 100 MHz): 22.3 (-CH₃), 108.7 (C-5), 109.7 (C-12), 114.1 (s, C-13), 121.3 (C-4), 124.9 (C-2), 133.6 (C-14), 135.8 (C-11), 149.1 (C-3), 162.2 (C-1), 166.5 (C-6), 182.1 (C-10), 190.4 (C-9); and

EI-MS-m/z (% rel. int.): C₁₅H₁₀O₅ 270 (M⁺ 100%), 242 (12), 213 (14), 185 (8), 155 (8), 141 (10).

Characterization of CN2 compound (Chrysophanol)

Elution with benzene:chloroform (1:1) gave yellow-colored needles (62 mg), crystallized from methanol (m.p. 195-197°C). It was soluble in a solution of conc. H₂SO₄, aqueous NaOH and NH₃ and gave dark red color. It gave positive ferric reaction for phenol. It was insoluble in 5% aqueous Na₂CO₃. The compound was yellow color under daylight and UV light at 254 nm. A single spot was observed on TLC with silica gel (R_f=0.61) and chloroform: ethylacetate (2:1) as solvent system, and the spot turned pink on exposure to NH₃ vapor. The compound showed red color with methanolic NaOH and alcoholic magnesium acetate, a characteristic feature of anthraquinones.

IR ν_{max}^{KBr} cm⁻¹: 3047, 2848, 1677, 1627, 1475, 1452, 1270, 1208, 1159, 1086, 1024, 902, 869, 839, 752;

¹H NMR (δ, CDCl₃, 400 MHz): 2.45 (3H, s, CH₃-3), 7.09 (1H, brs,

H-2), 7.29 (1H, brd, J=8.0 Hz, H-7), 7.64 (1H, brs, H-4), 7.69 (1H, brs, H-6), 7.81 (1H, brd, J=8.0 Hz, H-5), 12.0 (1H, s, -OH), 12.11 (1H, s, -OH);

¹³C NMR (δ, CDCl₃, 100 MHz): 22.3 (-CH₃), 108.7 (C-5), 109.7 (C-12), 114.1 (s, C-13), 121.3 (C-4), 124.9 (C-2), 133.6 (C-14), 135.8 (C-11), 149.1 (C-3), 162.2 (C-1), 166.5 (C-6), 182.1 (C-10), 190.4 (C-9); and

EI-MS-m/z (% rel. int.): C₁₅H₁₀O₅ 270 (M⁺ 100%), 239 (8), 226 (10), 197 (9), 152 (6), 127 (4).

Characterization of CN3 compound (Physcion)

Elution with chloroform: methanol (1:1) gave yellow-colored needles (28 mg), crystallized from ethylacetate (m.p. 206-207°C). It was soluble in the solution of conc. H₂SO₄, NaOH and NH₄OH and gave dark red color. It gave positive ferric reaction for phenol. It was insoluble in 5% aqueous Na₂CO₃. The compound gave yellow color under daylight and UV light at 254 nm. A single spot was observed on TLC over silica gel (R_f=0.77) and developed with chloroform: methanol (5:2) as the solvent system, which turned pink on exposure to NH₃ vapor. The compound showed red color with methanolic NaOH and alcoholic magnesium acetate a characteristic feature of anthraquinones.

UV λ_{max} nm: 249, 265, 287, 406 (rh), 432;

IR ν_{max}^{KBr} cm⁻¹: 3405, 2921, 2833, 1628, 1478, 1365, 1325, 1273, 1225, 1160, 1033, 978, 900, 874, 849, 759, 714; ¹H NMR (δ, CDCl₃, 400 MHz): 2.45 (3H, s, CH₃), 3.94 (3H, s, -OCH₃), 6.69 (1H, d, J=3.0 Hz, H-7), 7.08 (1H, brs, H-2), 7.36 (1H, d, J=3.0 Hz, H-5), 7.62 (1H, d, J=3.0 Hz), 12.12 (1H, s, OH), 12.31 (1H, s, OH); and

EI-MS-m/z (%): 284 (M⁺, 100), 260 (18), 240 (12), 226 (11), 189 (6), 167 (7), 139 (10), 111 (4).

GC-MS analysis

GC-MS analysis of methanol extract showed seven peaks indicating the presence of seven phytochemical constituents (Table 1, Figures 4-10). They are nitric acid nonyl ester; 4-C-methyl-myo-inositol; 2-methyl-butanoic acid; n-hexadecanoic acid; methyl ester, (Z, Z, Z)-9, 12, 15-octadecatrienoic acid; octadecanoic acid and diisooctyl ester 1,2-benzenedicarboxylic acid respectively. Of which, 3 compounds each belonged to aliphatic esters and saturated fatty acids and one compound belonged to inositol. Comparatively, 4-C-methyl-myo-inositol was present in major quantity (57.01%) followed by diisooctyl ester 1,2-benzenedicarboxylic acid (41.22%) respectively.

Discussion

All the three compounds of emodin, chrysophanol and physcion developed red color with methanolic NaOH and magnesium acetate, characteristic of anthraquinones [13]. The development of pink color under 5% KOH in methanol on TLC silica gel indicates the presence

of hydroxyanthraquinones [14,15]. All the compounds gave positive ferric reaction for phenol.

Identification of CN1 compound (Emodin)

UV ν_{max}^{KBr} cm⁻¹ at 436 nm indicates the presence of chelated hydroxyl groups in the compound. Characteristic IR peaks at 3388,

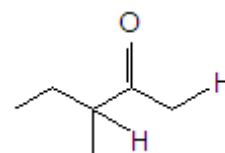


Figure 4: 2-Methyl-butanoic acid.

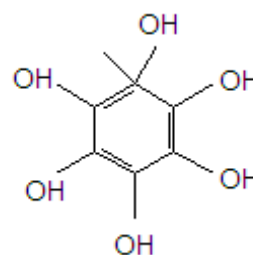


Figure 5: 4-C-Methyl-myo-inositol.

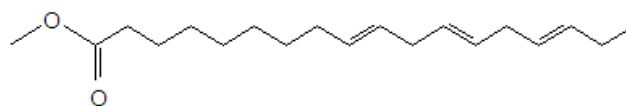


Figure 6: Methyl ester, (Z, Z, Z)-9, 12, 15-octadecatrienoic acid.

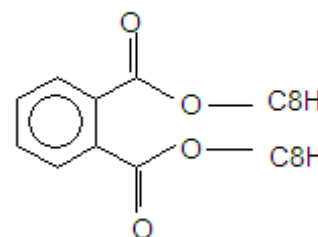
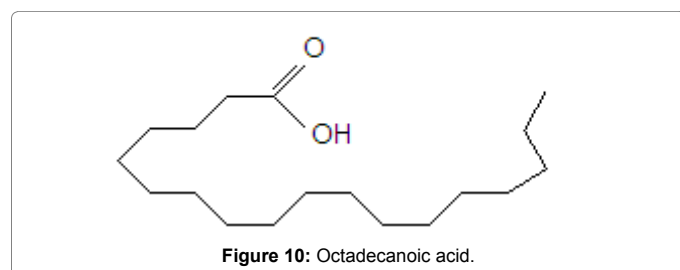
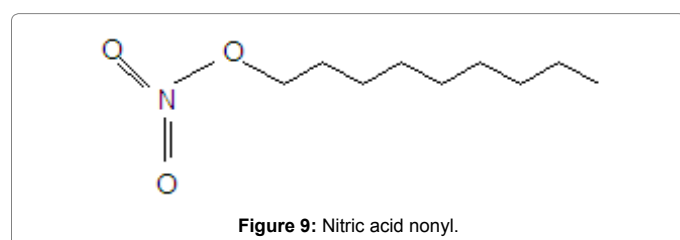
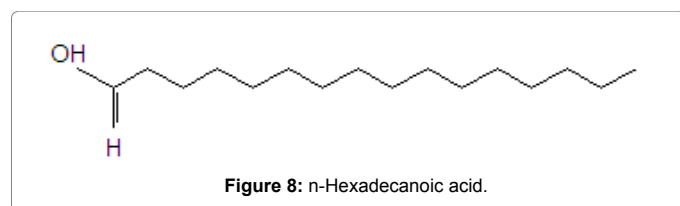


Figure 7: Diisooctyl ester 1,2-benzenedicarboxylic acid.

Name of the compound	Nature of compounds	Retention time	Molecular formula	Molecular weight	Peak area %
Diisooctyl ester 1, 2-benzenedicarboxylic acid	Aliphatic Ester	24.67	C ₂₄ H ₃₈ O ₄	390	41.33
Methyl ester, (Z, Z, Z)-9, 12, 15-octadecatrienoic acid	Aliphatic Ester	18.81	C ₁₉ H ₃₂ O ₂	292	0.46
Nitric acid nonyl ester	Aliphatic Ester	7.90	C ₉ H ₁₉ NO ₃	189	0.05
4-C-Methyl-myo-inositol	Inositol	13.63	C ₇ H ₁₄ O ₆	194	57.01
n-Hexadecanoic acid	Saturated Fatty acid	16.14	C ₁₆ H ₃₂ O ₂	256	1.01
2-Methyl-butanoic acid	Saturated Fatty acid	15.50	C ₅ H ₁₀ O ₂	102	0.10
Octadecanoic acid	Saturated Fatty acid	19.12	C ₁₈ H ₃₆ O ₂	284	0.05

Table 1: GC-MS Analysis of leaves of *Chamaecrista nigricans*.



1667, 1622, 1577, 1563, 1478, 1416, 907, 874, 759 and 720 cm^{-1} were observed for hydroxyl, non-chelated, chelated carbonyl groups and aromatic ring moieties respectively [16]. The ^1H NMR spectrum revealed the presence of an aromatic methyl group at $\delta 2.44$, typical of a β -methyl to C-1 OH bonded to the C-3 atom. It also showed 2 metacoupled protons appearing as broad singlet at $\delta 7.07$ and 7.61 assigned to H-2 and H-4. Two other metacoupled aromatic protons ($J=2.5$ Hz) appearing at $\delta 7.31$ and 6.66. They were assigned to H-5 and H-7 respectively. Two broad one proton singlets appearing at $\delta 12.17$ and 12.27 were assignable to the chelated hydroxyls at C-1 and C-8. These data suggested CN1 to be emodin. The ^{13}C NMR spectrum also confirmed the structure and it revealed the presence of methyl group at $\delta 22.4$. The mass spectrum also showed M^+ at m/z 270 corresponding to the molecular formula $\text{C}_{15}\text{H}_{10}\text{O}_5$ for emodin (1, 6, 8-trihydroxy, 3-methylanthraquinone). All these spectral data reported were comparable with those of emodin [17,18]. This compound was first reported in 1925 as frangula-emodin from the fungi *Dermocybe sanquinens* [19], later from *Penicillium* sp. [20], *Aspergillus* sp. [21], lichens [22] and higher plants including *Cassia* spp. [23,24], *Rheum* and *Rumex* spp. [16], *Polygonum* spp. and *Rhamnus* [25,26] and *Ventilago* spp. [27]. Thus the anthraquinone (CN1) was identified as emodin.

Identification of CN2 compound (Chrysophanol)

UV $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} at 436 nm indicates the presence of chelated hydroxyl groups in the compound. Characteristic IR peaks at 3047, 1677, 1627, 1475, 1452, 902, 869 and 752 cm^{-1} were observed for hydroxyl, non-chelated, chelated carbonyl groups and aromatic ring moieties respectively [16]. The ^1H NMR spectrum reveals the presence of an aromatic methyl group at $\delta 2.45$, typical of a β -methyl bonded to the C-3 atom. Two broad singlets at $\delta 7.09$ and 7.64 are attributed to H-2 and H-4 respectively. Two one proton broad doublets at $\delta 7.81$ (d, $J=8.0$ Hz) and 7.29 (d, $J=8.0$ Hz) were assigned to H-5 and H-7

respectively and the two singlet proton signals at δ 12.0 (s, 1H) and 12.11 (s, 1H) were assignable to chelated hydroxyl groups. The mass spectrum of the compound showed M^+ at m/z 254 corresponding to the molecular formula $\text{C}_{15}\text{H}_{10}\text{O}_4$ for chrysophanol (1, 8-dihydroxy, 3-methyl anthraquinone). All these spectral data reported were comparable with those of chrysophanol [17,18]. This compound was reported from the fungi *Phoma foveata* [28], lichens, *Asahinae chrysantha* [22] and higher plant families belonging to *Cassia* spp. [23,24], *Rheum* spp. [15], *Rumex* and *Polygonum* spp. [29], *Rhamnus* and *Ventilago* spp. [27]. Thus the anthraquinone (CN2) was identified as chrysophanol.

Identification of CN3 compound (Physcion)

UV $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} at 432 nm indicates the presence of chelated hydroxyl groups in the compound. Characteristic IR peaks at 3405, 1628, 1478, 977, 899, 874, 758 and 714 cm^{-1} were observed for hydroxyl, chelated carbonyl groups and aromatic ring moieties respectively [16]. The ^1H NMR spectrum revealed the presence of an aromatic methyl group at $\delta 2.44$, typical of a β -methyl bonded to the C-3 atom and a methoxy group at $\delta 3.94$ attached to C-6 atom. Two broad singlets at $\delta 7.07$ and 7.61 which are attributed to H2 and H4 respectively and two one proton meta-coupled doublets at $\delta 6.66$ (d, $J=3.0$ Hz) and 7.31 (d, $J=3.0$ Hz) were assignable to H-5 and H-7 respectively. The two broad single proton signal at $\delta 12.12$ (s, 1H) and 12.31 (s, 1H) represented chelated hydroxyl groups. The mass spectrum of the compound showed M^+ at m/z 284 corresponding to the molecular formula $\text{C}_{16}\text{H}_{12}\text{O}_5$ corresponding to physcion (1, 8-dihydroxy, 3-methyl-6-methoxy anthraquinone). All these spectral data reported were comparable with those of physcion [17,18]. This compound was widely distributed in fungi including *Aspergillus* spp., *Penicillium* spp., Lichens eg. *Parmelia* spp., higher plants including *Cassia* spp. [23,24], *Rheum* spp. [15], *Rumex* and *Polygonum* spp. [29] and *Rhamnus* and *Ventilago* spp. [27]. Thus the anthraquinone (CN3) was identified as physcion.

All these three anthraquinones possess a wide range of pharmacological activities. Emodin possesses a wide spectrum of biological activities such as anticancer, anti-inflammatory, antioxidant, antimicrobial, hepatoprotective [30-34]. Chrysophanol has antibacterial, lipid-lowering effects [35,36] while physcion has been reported for antimicrobial, anti-inflammatory, anti-cancer and hepatoprotective activities [37-41].

GC-MS analysis revealed the presence of three aliphatic compounds, three saturated fatty acids and single inositol compound. All these compounds are medicinally valuable and are reported to have pharmacological effects in experimental animals. The compound n-hexadecanoic acid is a fatty acid which has been reported to possess potential mosquito larvicide and anti-inflammatory activity [42,43] and 2-methyl-butanoic acid for antibacterial activity [44]. Octadecanoic acid (stearic acid) has been to have anti-inflammatory activity [45] and also accelerates the recovering of hepatic dysfunction of liver damage in rats [46]. The compound 4-C-methyl-myo-inositol was found to be present in major quantity and it has been reported as a promising treatment for the prevention of ovarian hyperstimulation syndrome in experimental rats.

Conclusion

In the present study, it is concluded that the presence of these medicinally valuable bioactive natural compounds including three anthraquinones in *C. nigricans* proved its importance in drug industry for drug development against various diseases.

Acknowledgement

The first author thanks the Council of Scientific and Industrial Research (CSIR), New Delhi, for the award of Senior Research Fellowship [CSIR Award No.9/652(13)] to carry out this research work.

References

- Lewis GP (2005) Tribe Cassieae. Legumes of the world. Royal Botanic Gardens, Kew. pp: 111-125.
- Dalziel JM (1937) The useful plants of West Tropical Africa. The Crown Agents for the Overseas Colonies. pp: 612.
- Council of Scientific & Industrial Research (1985). The Wealth of India: A dictionary of Indian raw materials & industrial products. Publications & Information Directorate, Council of Scientific & Industrial Research, New Delhi.
- Akah PA, Orisakwe OE, Gamaniel KS, Shitta A (1998) Evaluation of Nigerian traditional medicines: II effects of some Nigerian folk remedies on peptic ulcer. *J Ethnopharmacol* 62: 123-127.
- Chidume FC, Gamaniel K, Amos S, Akah P, Obodozie O, et al. (2001) Pharmacological activity of the methanolic extract of *Cassia nigricans* leaves. *Indian J Pharmacol* 33: 350-356.
- Nwafor PA, Okwuasaba FK (2001a) Contraceptive and esterogenic effect of a methanolic extract of *Cassia nigricans* leaves in Experimental animals. *Pharm Biol* 39: 424-428.
- Nwafor PA, Okwuasaba FK (2001b) Effect of methanolic extract of *Cassia nigricans* leaves on rat gastrointestinal tract. *Fitoterapia* 72: 206-214.
- Diallo D, Sogn C, Samaké FB, Paulsen BS, Michaelsen TE, et al. (2002) Wound healing plants in Mali, the Bamako Region: An ethnobotanical survey and complement fixation of water extracts from selected plants. *Pharm Biol* 40: 117-128.
- Sundararaj D, Balasubramanyam G (1959) Guide to the economic plants of South India. Amudha Nilyam Private Limited, Madras.
- Irwin FR (1961) Woody plants of Ghana with special reference to their uses. Oxford University Press, London.
- Silva O, Duarte A, Cabrita J, Pimentel M, Diniz A, et al. (1996) Antimicrobial activity of Guinea-Bissau traditional remedies. *J Ethnopharmacol* 50: 55-59.
- Harborne JB (1998) Phytochemical methods: A guide to modern techniques of plant analysis. Chapman & Hall, London.
- Robinson T (1963) The Organic constituents of higher plants: Their chemistry and interrelationships.
- Rai PP, Shok M (1981) Burgess, Minneapolis, Minn. Thin-layer chromatography of hydroxyanthraquinones in plant extracts. *Chromatographia* 14: 599-600.
- Ma X, Chen Y, Hui R (1989) Analysis of anthraquinones in *Rheum franzenbachii* Munt (Rhubarb) by thin-layer chromatography. *Chromatographia* 27: 465-466.
- Omur-Demirezer L, Kuruzum-Uz A, Bergere I, Schiewe HJ, Zecek A (2001) The structure of antioxidant and cytotoxic agents from natural source: Anthraquinones and tannins from roots of *Rumex patientia*. *Phytochemistry* 58: 1213-1217.
- Lee CK, Lee PH, Kuo IH (2001) Chemical constituents from the aril of *Cassia fistula* L. *J Chinese Chem Soc* 48: 1053-1058.
- Meselhy MR (2003) Constituents from Molghat, the roots of *Glossostemon bruguieri* (Desf.). *Molecules* 8: 614-621.
- Kogl F, Postowsky JJ (1925) Untersuchungen über Pilzfarbstoffe. II. Über die Farbstoffe des blutroten Hautkopfes (*Dermocybe sanguinea* Wulf.). *Justus Liebig's Ann Chem* 444: 1-7.
- Shibata S, Udagawa S (1963) Metabolic products of fungi. XIX. Isolation of rugulosin from *Penicillium brunneum* Udagawa. *Chem Pharm Bull* 11: 402-403.
- Anke H, Kolthum I, Zahner H, Laatsch H (1980) Metabolic products of microorganisms. 185. The anthraquinones of the *Aspergillus glaucus* Group. 1. Occurrence, isolation, identification and antimicrobial activity. *Arch Microbiol* 126: 223-230.
- Mishchenko NP, Stepanenko S, Krivoshechkova OE, Maksimov OB (1980) Anthraquinones of the lichens *Asahinea chrysantha*. *Chemistry of Natural Compounds*, 16: 117-121.
- Ganapaty S, Thomas PS, Ramana KV, Vidhyadhar K, Chakradhar V (2002) A review of phytochemical studies of *Cassia* species. *J Nat Remedies* 2: 102-120.
- Manojlovic I, Bogdanovic-Dusanovic G, Gritsanapan W, Manojlovic N (2006) Isolation and identification of anthraquinones of *Calopluca cerina* and *Cassia tora*. *Chem Pap* 60: 466-468.
- Dwivedi SP, Pandey VB, Shah AH, Rao YB (1988) Chemical constituents of *Rhamnus procumbens* and pharmacological actions of emodin. *Phytotherapy Res* 2: 51-53.
- Goel RK, Das Gupta G, Ram SN, Pandey VB (1991) Antiulcerogenic and anti-inflammatory effects of emodin, isolated from *Rhamnus triquerta* Wall. *Indian J Exp Biol* 29: 230-232.
- Izhaki I (2002) Emodin: A secondary metabolite with multiple ecological functions in higher plants. *New Phytol* 155: 205-217.
- Bick IRC, Rhee C (1966) Anthraquinone pigments from *Phoma foveata* Foister. *Biochem J* 98: 112-116.
- Yao S, Li Y, Kong L (2006) Preparative isolation and purification of chemical constituents from the root of *Polygonum multiflorum* by high-speed counter-current chromatography. *J Chromatogr A* 1111: 64-71.
- Lin CC, Chang CH, Yang JJ, Namba T, Hattori M (1996) Hepatoprotective effects of emodin from *Ventilago leiocarpa*. *J Ethnopharmacol* 52: 107-111.
- Dave H, Ledwani L (2012) A review on anthraquinones isolated from *Cassia* species and their applications. *Indian J Nat Prod Resour* 3: 291-319.
- Hsu SC, Chung JG (2012) Anticancer potential of emodin. *BioMedicine* 2: 108-116.
- Dong X, Fu J, Yin X, Cao S, Li X, et al. (2016) Emodin: A review of its pharmacology, toxicity and pharmacokinetics. *Phytother Res* 30: 1207-1218.
- Sharma R, Tiku AB, Giri A (2017) Pharmacological properties of emodin: Anthraquinone derivatives. *J Nat Prod Resour* 3: 97-101.
- Coopooosamy RM, Magwa ML (2006) Antibacterial activity of chrysophanol isolated from *Aloe excelsa* (Berger). *African J Biotech* 5: 1508-1510.
- Chen CQ, Wang YQF, Yu-Shui Xie YS, Yin ZF, Xu ZJ, et al. (2015) Application of chrysophanol in zebrafish to reduce dietary introduced lipid and its possible mechanism. *Int J Clin Exp Med* 8: 10558-10567.
- Tamokou JDD, Tala MF, Wabo HK, Kuate JR, Tane P (2009) Antimicrobial activities of methanol extract and compounds from stem bark of *Vismia rubescens*. *J Ethnopharmacol* 124: 571-575.
- Zhao YL, Wang JB, Zhou GD, Shan LM, Xiao XH (2009) Investigations of free anthraquinones from rhubarb against α -naphthylisothiocyanate-induced cholestatic liver injury in rats. *Basic Clin Pharmacol Toxicol* 104: 463-469.
- Almeida AP, Dethoup T, Singburadom N, Lima R, Vasconcelos MH, et al. (2010) The *in vitro* anticancer activity of the crude extract of the sponge-associated fungus *Eurotium cristatum* and its secondary metabolites. *J Nat Pharma* 1: 25-29.
- Ghosh S, Sarma MD, Patra A, Hazra B (2010) Anti-inflammatory and anticancer compounds isolated from *Ventilago madraspatana* Gaertn., *Rubia cordifolia* Linn. and *Lantana camara* Linn. *J Pharm Pharmacol* 62: 1158-1166.
- Wijesekera I, Zhang C, Van TQ, Vo TS, Li YX, et al. (2014) Physcion from marine-derived fungus *Microsporium* sp. induces apoptosis in human cervical carcinoma HeLa cells. *Microbiol Res* 169: 255-261.
- Rahaman AA, Gopalakrishnan G, Ghouse BS, Arumugam S, Himalayan B (2000) Effect of *Feronia limonia* on mosquito larvae. *Fitoterapia* 71: 553-555.
- Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, et al. (2012) Anti-inflammatory property of n-hexadecanoic acid: Structural evidence and kinetic assessment. *Chem Biol Drug Des* 80: 434-439.
- Hayashida-Soiza G, Uchida A, Mori N, Kuwahara Y, Ishida Y (2008) Purification and characterization of antibacterial substances produced by a marine bacterium *Pseudoalteromonas haloplanktis* strain. *J Applied Microbiol* 105: 1672-1677.
- Shaw B, Lambert S, Wong MH, Ralston JC, Stryjecki C, et al. (2013) Individual saturated and monounsaturated fatty acids trigger distinct transcriptional networks in differentiated 3T3-L1 preadipocytes. *J Nutrigenetics Nutrigenomics* 6: 1-15.
- Goradel NH, Ali EM, Darabi M, Roshangar L, Asadi M, et al. (2016) Improvement of liver cell therapy in rats by dietary stearic acid. *Iranian Biomedical J* 20: 217-222.