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

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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 pulvarizor, 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, ^{1}H and ^{13}C 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 pulvarizor, 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

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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 Rf 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 ^{13}C 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 Na_2CO_3, conc. H_2SO_4, NaOH and NH_3 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 NH_3 vapor. It gave pink color with alcoholic magnesium acetate, a characteristic feature of anthraquinones.

UV λmax nm: 220, 252, 264, 289, 436;

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

^{1}H NMR (δ, $CDCl_3$, 400 MHz): 2.44 (3H, s, CH_3), 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);

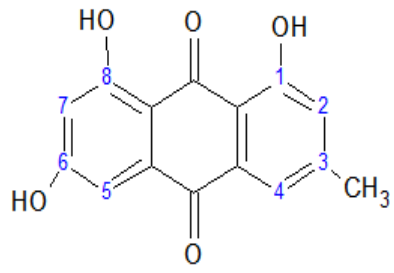

Figure 1: Emodin (1, 6, 8-trihydroxy, 3-methyl anthraquinone).

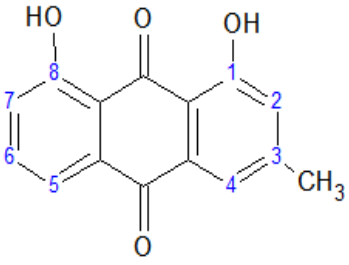

Figure 2: Chrysophanol (1, 8-dihydroxy, 3-methyl anthraquinone).

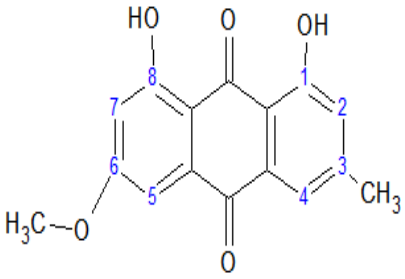

Figure 3: Physcion (1, 8-dihydroxy, 3-methyl-6-methoxy anthraquinone).

^{13}C NMR (δ, $CDCl_3$, 100 MHz): 22.3 (-CH_3), 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.): $C_{15}H_{10}O_5$ 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. H_2SO_4, aqueous NaOH and NH_3 and gave dark red color. It gave positive ferric reaction for phenol. It was insoluble in 5% aqueous Na_2CO_3. The compound was yellow color under daylight and UV light at 254 nm. A single spot was observed on TLC with silica gel (Rf=0.61) and chloroform: ethylacetate (2:1) as solvent system, and the spot turned pink on exposure to NH_3 vapor. The compound showed red color with methanolic NaOH and alcoholic magnesium acetate, a characteristic feature of anthraquinones.

IR $\nu_{max}^{KBr} cm^{-1}$**:** 3047, 2848, 1677, 1627, 1475, 1452, 1270, 1208, 1159, 1086, 1024, 902, 869, 839, 752;

^{1}H NMR (δ, $CDCl_3$, 400 MHz): 2.45 (3H, s, CH_3-3), 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);

^{13}C NMR (δ, $CDCl_3$, 100 MHz): 22.3 (-CH_3), 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.): $C_{15}H_{10}O_5$ 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. H_2SO_4, NaOH and NH_4OH and gave dark red color. It gave positive ferric reaction for phenol. It was insoluble in 5% aqueous Na_2CO_3. The compound gave yellow color under daylight and UV light at 254 nm. A single spot was observed on TLC over silica gel (Rf=0.77) and developed with chloroform: methanol (5:2) as the solvent system, which turned pink on exposure to NH_3 vapor. The compound showed red color with methanolic NaOH and alcoholic magnesium acetate a characteristic feature of anthraquinones.

UV λmax nm: 249, 265, 287, 406 (rh), 432;

IR $\nu_{max}^{KBr} cm^{-1}$: 3405, 2921, 2833, 1628, 1478, 1365, 1325, 1273, 1225, 1160, 1033, 978, 900, 874, 849, 759, 714; **^{1}H NMR (δ, $CDCl_3$, 400 MHz):** 2.45 (3H, s, CH_3), 3.94 (3H, s,-OCH_3), 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 physician 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 $\nu_{max}^{KBr} cm^{-1}$ at 436 nm indicates the presence of chelated hydroxyl groups in the compound. Characteristic IR peaks at 3388,

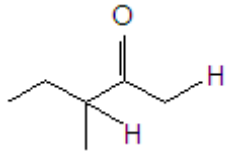

Figure 4: 2-Methyl-butanoic acid.

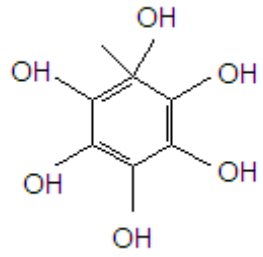

Figure 5: 4-C-Methyl-myo-inositol.

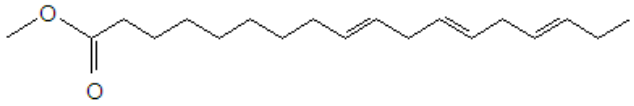

Figure 6: Methyl ester, (Z, Z, Z)-9, 12, 15-octadecatrienoic acid.

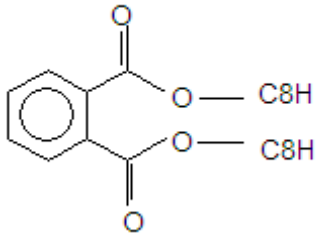

Figure 7: Diisooctyl ester 1, 2-benzenedicarboxylic acid.

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	$C_9H_{19}NO_3$	189	0.05
4-C-Methyl-myo-inositol	Inositol	13.63	$C_7H_{14}O_6$	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	$C_5H_{10}O_2$	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*.

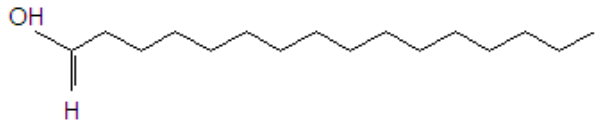

Figure 8: n-Hexadecanoic acid.

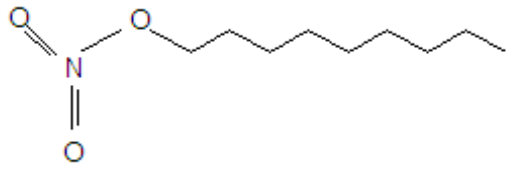

Figure 9: Nitric acid nonyl.

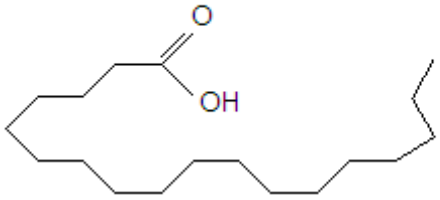

Figure 10: Octadecanoic acid.

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 ^{1}H NMR spectrum revealed the presence of an aromatic methyl group at δ2.44, 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 δ7.07 and 7.61 assigned to H-2 and H-4. Two other metacoupled aromatic protons (J=2.5 Hz) appearing at δ7.31 and 6.66. They were assigned to H-5 and H-7 respectively. Two broad one proton singlets appearing at δ12.17 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 δ22.4. The mass spectrum also showed M^+ at m/z 270 corresponding to the molecular formula $C_{15}H_{10}O_5$ 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 $\nu_{max}^{KBr} cm^{-1}$ 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 ^{1}H NMR spectrum reveals the presence of an aromatic methyl group at δ2.45, typical of a β-methyl bonded to the C-3 atom. Two broad singlets at δ7.09 and 7.64 are attributed to H-2 and H-4 respectively. Two one proton broad doublets at δ7.81 (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 $C_{15}H_{10}O_4$ 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 $\nu_{max}^{KBr} cm^{-1}$ 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 ^{1}H NMR spectrum revealed the presence of an aromatic methyl group at δ2.44, typical of a β-methyl bonded to the C-3 atom and a methoxy group at δ3.94 attached to C-6 atom. Two broad singlets at δ7.07 and 7.61 which are attributed to H2 and H4 respectively and two one proton meta-coupled doublets at δ6.66 (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 δ12.12 (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 $C_{16}H_{12}O_5$ 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.

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