Research Article

Isolation, Purification and Characterization of 2, 2-dimethylchromene 7-methoxy-6-*O*-*β*-glucopyranoside (chromene derivatives) from *Crotalaria longipes* Wight & Arn

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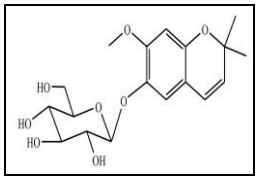
Abstract

The aerial part of *Crotalaria longipes* of family Fabaceae was subjected to isolation and identification of chemical constituents. The extract was purified and isolated by column chromatography and thin layer chromatography (TLC). The isolated compound was then subjected to UV spectrum, FT-IR for identification of functional groups and ^{1}H-NMR and ^{13}C-NMR for identification of protons and carbon atoms. ESI-MS was done to identify the molecular weight of the isolated compound. From the spectra obtained from FT-IR, ^{1}H-NMR, ^{13}C-NMR and ESI-MS, the isolated compound was found to be 2, 2-dimethylchromene 7-methoxy-6-*O*-*β*-glucopyranoside (chromene derivatives).



2, 2-dimethylchromene 7-methoxy-6-*O*-*β*-glucopyranoside

Keywords: *Crotalaria longipes*, chromene derivatives, TLC, spectroscopy

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Introduction

The genus *Crotalaria* (family Fabaceae) is considered to be the largest genus, with around 600 species distributed throughout the tropics and subtropics region of the world [1]. *Crotalaria* species have been reported to contain alkaloids, saponins and flavonoids as notable chemical markers with basic N-oxide of genera Leguminaceae having antileukemic, antitumour, antispasmodic, antineoplastic, cardiodepressant, hypotensive properties [2-4]. The leaves are the excellent remedy for ptyalism, diarrhea, scabies and impetigo. The seeds were powdered and boiled in the milk and were used for enhancing body strength, life span and also for curing skin diseases, leprosy, flatulence and fever [5]. The plants are still important for the discovery of new drugs as provider of the drugs based on secondary compounds from plants. Many scientific research has been carried out on these plants and their secondary metabolites of medicinal importance i.e. alkaloids, flavonoids and terpenoids, etc. have been reported [6]. According to literature survey various phytoconstituents have been isolated from different plants [7-9].

The genus *Crotalaria* has the largest number of threatened species listed in the Red Data Book. This is the genus known for the presence of pyrrolizidine alkaloids. Twenty four alkaloids have been isolated from 18 different species of *Crotalaria*. *Crotalaria longipes* is one among the 15 species listed on the red data books. It is a woody shrub growing upto 4m tall with bright yellow flowers endemic to Nilgiris and Kolli hills. The main objective of the present study is to isolate compound from *Crotalaria longipes* aerial part by extraction, column chromatography followed by characterization using UV, IR, Mass spectrum, ^{1}H-NMR and ^{13}C-NMR spectra.

Experimental

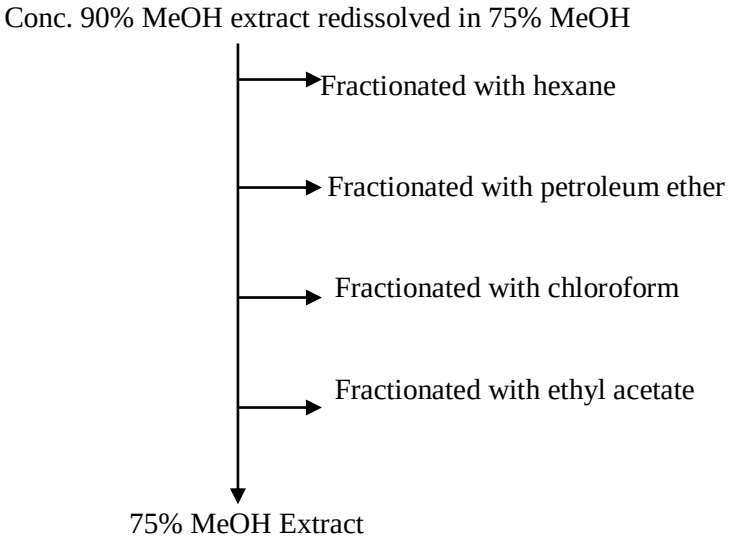
Materials and Reagents

The aerial part of *Crotalaria longipes* were collected from Kothagiri, Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu. Hexane, petroleum ether, chloroform, ethyl acetate, acetone, methanol and ethanol were of analytical grade procured from Merck. Column chromatography was performed on column (length 50 & diameter 150 mm),

silica gel (60-120 mesh) and Merck TLC readymade sheets 20 x 20 cm. The spectrophotometer systems used were Shimatzu UV spectrophotometer, Shimadzu spectrum 1 FT-IR spectrometer and ESI-MS analysis (TofSpec 2E MALDI time - of flight (TOF) Instrument (Micromass, Manchester, UK). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Brucker spectrometer using $CDCl_3$ as solvent and TMS as internal standard. The observed chemical shifts (8) were recorded in ppm and the coupling constants (J) were recorded in Hz.

Extraction

100g of aerial part powder of *Crotalaria longipes* were extracted with 90% methanol using Soxhlet apparatus and concentrated for further using simple distillation method. The concentrated plant extracts liquid fractionated with the solvents hexane, petroleum ether, chloroform, ethyl acetate. Altogether, 4 fractions were obtained and used for separation of pure isolates using column chromatography method.



Column chromatography

60-120 mesh size silica gel was dissolved in the low polarity solvent hexane and tightly packed in 50 X 150 mm glass column up to 100 mm height without air bubbles. Then the experimental extracts were loaded in individual glass columns and fractionated with solvents hexane, petroleum ether, chloroform, ethyl acetate, acetone and methanol at various proportion of solvent mixture.

Screening of purity for column chromatography fractions using TLC

15 ml of fractions were collected using each solvents and the collected fractions were screened for purity using thin layer chromatography (Merck TLC Readymade sheets 20 X 20 cm) with appropriate solvent systems (Petroleum ether : Hexane : Chloroform : Ethyl acetate : Acetone : methanol : ethanol 7 : 1 : 1 : 0.5 : 0 : 0.5 : 0.5).

Preparative TLC

The closely mixture fractions was re-separated using PTLC. The mixture fractions were spotted on TLC for separation individual components and scraped using sterile needles and dissolved in methanol. Then it is centrifuged at 10,000 rpm. The supernatant was taken for further characterization like TLC, UV-VIS spectrophotometer, FTIR, ESI-MS, ^{1}H-NMR, ^{13}C-NMR and structure elucidation.

Result and Discussion

The chloroform fraction was purified using chloroform and methanol as eluent in the combination of 9:3 by silica gel column chromatography (60-120 mesh). The colourless fractions 21 to 63 showed similar TLC pattern with some minor mixture of other compounds. Subsequently, flash column was used to separate the pure compound with silica gel column (200 mesh). The mobile solvent is chloroform: methanol in the ratio of 9.7 : 0.3. The fractions 11 to 43 showed similar spots on TLC under iodine vapour and it was taken for further characterization *viz.* TLC, UV scanning, FTIR, ESIMS, ^{1}H NMR and ^{13}C NMR.

TLC

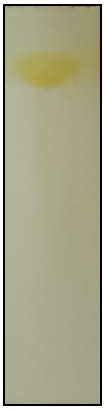


Figure 1 TLC of isolated compound

Colourless substance. Rf value was 0.77

UV (MeOH)

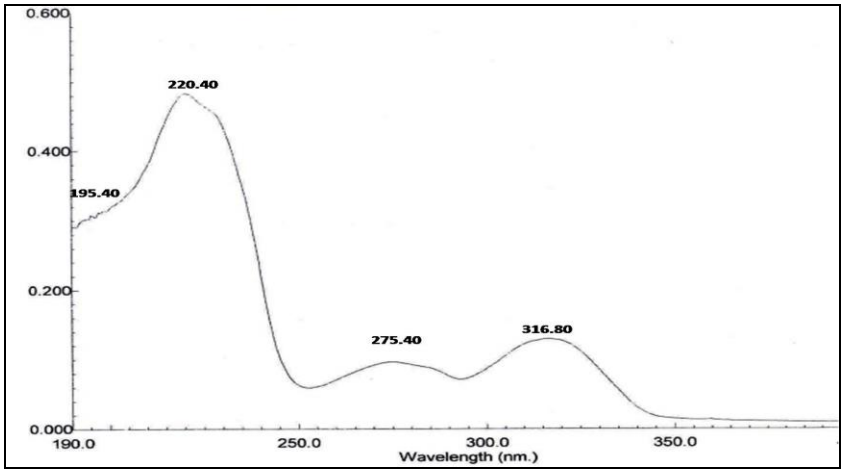


Figure 2 UV spectrum of isolated compound

The λ max for the isolated substance was recorded as 195.40 (4.15), 220.40 (4.35), 275.40 (3.65), 316.80 (3.77) nm.

FT-IR (KBr)

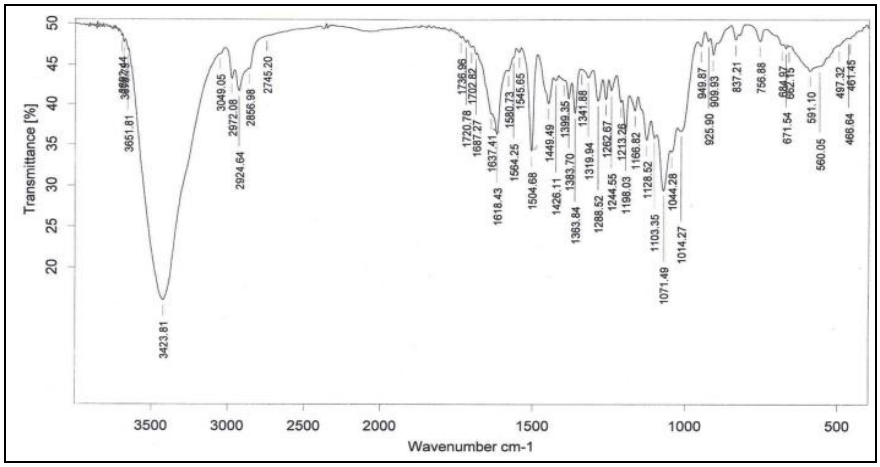


Figure 3 FT-IR spectrum of isolated compound

The maxima values observed for the isolated substance are the following: 3424, 2925, 1618, 1505, 1071 (cm-1).

EI-MS *m/z* (%)

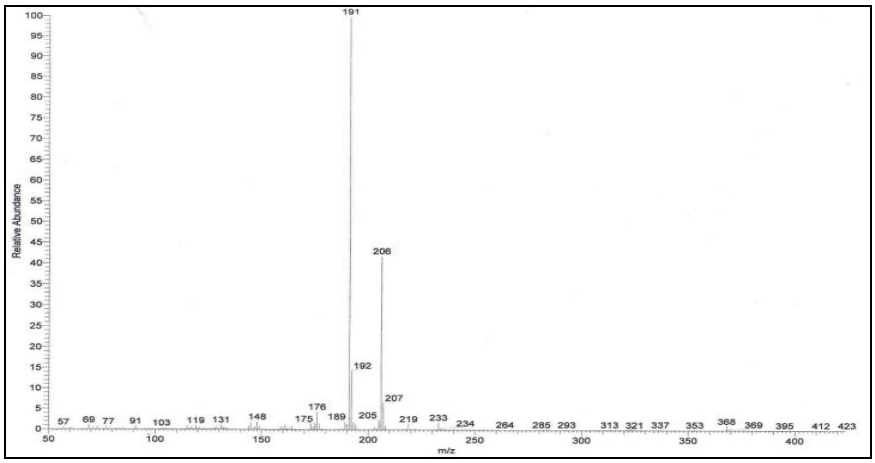


Figure 4 EI-MS spectrum of isolated compound

368 [M] + (1), 206[M-162] + (42), 191 (100) - Positive HR-ESI MS: *m/z* 369.1542 [M+H]+ (calcd. 369.1549 [M+H]+)

NMR

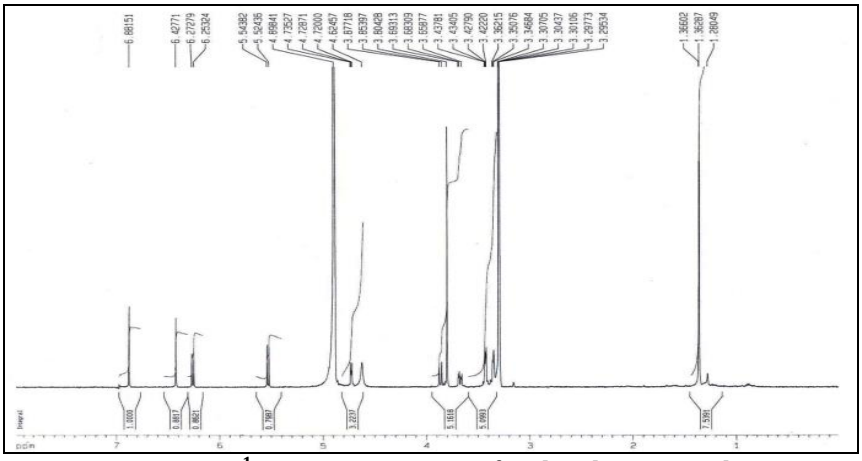


Figure 5 [1] H-NMR spectrum of isolated compound

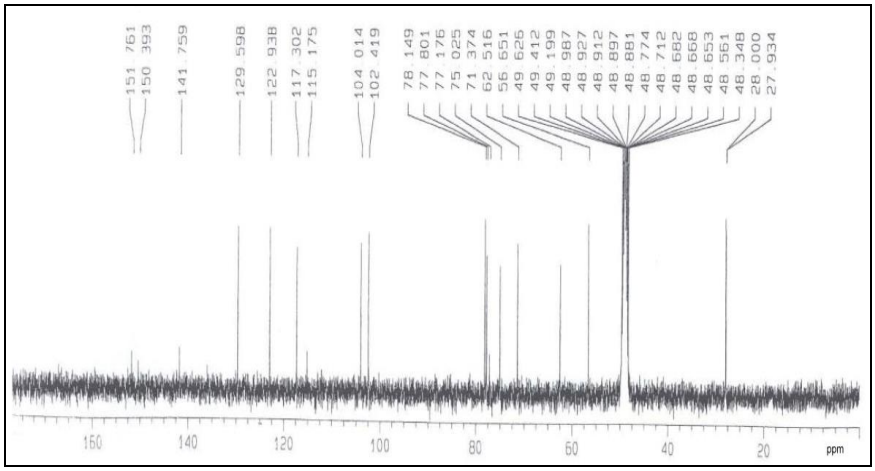


Figure 6 ^{13}C-NMR spectrum of isolated compound

^{1}H-NMR (CDCl3) δH values are 5.53 (1H, d, *J* = 9.7 Hz, H-3), 6.26 (1H, d, *J* = 9.8 Hz, H-4), 6.43 (1H, s, H-5), 6.88 (1H, s, H-8), 1.36 (3H, s, CH3), 1.37 (3H, s, CH3), 4.73 (1H, d, *J* = 7.8 Hz, H-1'), 3.30-3.86 (6H, m, H-2'~6'), 3.80 (3H, s, -OCH3).

^{13}C-NMR (100 MHz, CD3OD) δC values are 77.2(s, C-2), 129.6(d, C-3), 123.0(d, C-4), 117.3(s, C-4a), 115.2(d, C-5), 141.8(s, C-6), 151.8(s, C-7), 104.0(d, C-8), 150.4(s, C-8a), 28.0(q, C-9), 27.9(q, C-10), 102.4(d, C-1'), 75.0 (d, C-2'), 78.1 (d, C-3'), 71.4(d, C-4'), 77.8 (d, C-5'), 62.5(t, C-6'), 56.7(q, -OCH3 at C-7).

Based on spectral data base and through the literature the observed compound is identified as 2, 2-dimethylchromene-7-*O*-*β*-glucopyranoside.

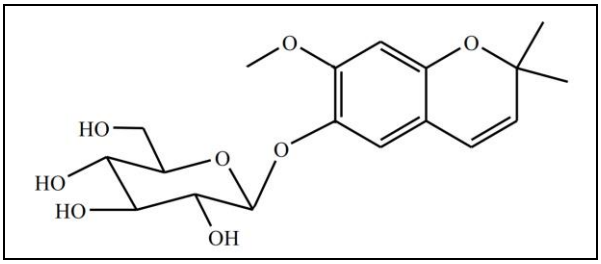


Figure 7 Chemical Structure of isolated compound -2,2-dimethylchromene 7-methoxy-6-*O*-*β*-glucopyranoside

The chromene ring is an important pharmacophore in modern drug delivery. The literature has given more attention to the chromene nucleus as a source of new anticancer and antiinflammatory agent [10]. The knowledge gained by various researches has suggested that substituted chromene which interact easily with the receptors and possess different pharmacological activities with lower toxicity. It is known that certain natural and synthetic chromene derivatives possess important biological activities such as antimicrobial, antioxidant, estrogenic, herbicidal and analgesic activity [11-13]. It also plays an important role in the production of fluorescent dyes for synthetic fibers and electroluminescent [14].

Conclusion

The developed method is useful for isolation, purification and characterization of -2,2-dimethylchromene 7-methoxy-6-*O*-*β*-glucopyranoside (chromene derivatives) found in *Crotalaria longipes*. The method does not require any elaborate treatment and tedious extraction procedure for isolation and purification. It is simple, precise and reproducible approach for further characterisation.

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