RESEARCH PAPER

Pharmacognostic Evaluation, Isolation and Multi-Technique Characterization of Coumarin from Ageratina adenophora (Spreng.) of Uttarakhand: A Chromatographic Marker for Standardization

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ABSTRACT

Background: Ageratina adenophora is an invasive Asteraceae species containing chemically diverse secondary metabolites. Establishing a reliable phytochemical marker requires agreement between botanical authentication, chromatographic behaviour and complementary spectroscopic evidence.

Objective: To isolate a coumarin-type compound from the leaves and aerial parts of A. adenophora collected in Uttarakhand and evaluate its identity and purity using HPTLC, HPLC, mass spectrometry, 1H-NMR and FTIR spectroscopy.

Methods: Authenticated plant material was examined by transverse-section and powder microscopy and extracted with 70% ethanol. The concentrated extract was partitioned with chloroform, monitored by HPTLC and fractionated by silica-gel column chromatography. The isolate was recrystallized and evaluated by HPLC, positive-mode LC-ESI-MS, 1H-NMR and FTIR spectroscopy.

Results: The crude extract yield was 12.40% w/w; the chloroform-enriched fraction represented 5.85% w/w of the crude extract; and the isolated compound represented 1.68% w/w of the starting extract. The isolate formed white to off-white crystalline needles with a melting point of 68 °C. HPTLC showed a single isolate zone at Rf 0.744, closely matching the coumarin standard at Rf 0.745. HPLC showed a major isolate peak at 7.323 min with 97.58% area-normalized purity, closely matching the standard at 7.328 min. Positive-mode mass spectrometry showed a dominant $[M+H]^+$ ion at m/z 147.03. FTIR bands at 1693.20 and 1665.93 cm^{-1} supported a conjugated lactone carbonyl, while bands at 1618.02 and 1572.67 cm^{-1} supported an aromatic conjugated system. The 1H-NMR spectrum contained aromatic signals at δ 7.31–7.43, result support the analysis.

Conclusion: The agreement of HPTLC and HPLC retention behaviour with an authentic standard, together with the molecular ion, NMR and diagnostic FTIR absorptions, supports assignment of the isolate as a coumarin-type compound and its use as a chromatographic marker for A. adenophora.

Keywords: *Ageratina adenophora*; coumarin; isolation; HPTLC; HPLC; mass spectrometry; NMR; FTIR; phytochemical marker.

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1. INTRODUCTION

The dependability of herbal medicines rests on correct botanical authentication and on pharmacognostic and phytochemical standardization, because the level of secondary metabolites in a plant shifts with genotype, geographical origin, climate, harvest season and post-harvest handling [1–4]. *Ageratina adenophora* (Spreng.) R.M. King & H. Rob. (syn. *Eupatorium adenophorum* Spreng.; Asteraceae), commonly called Crofton weed, is an invasive species that has become naturalized across many regions [5–7]. Its aerial parts have been reported to contain flavonoids, phenylpropanoids, quinic-acid and thymol derivatives, monoterpenes, sesquiterpenes and coumarins [8–15], and extracts have displayed antimicrobial, antioxidant, anti-inflammatory, antiviral and cytotoxic effects [16–22]. The plant also elaborates hepatotoxic sesquiterpenes, notably 9-oxo-10,11-dehydroageraphorone [23–27]; marker-based chemical work on this species must therefore be read alongside its safety profile.

Coumarins are benzopyrone natural products with antioxidant, anti-inflammatory, antimicrobial and anticoagulant properties, and they are widely used as chemotaxonomic and quality-control markers in botanical research [34–36]. A coumarin constituent has

been mentioned in earlier surveys of *A. adenophora* [8], yet its dedicated isolation, supported by HPTLC, HPLC, MS, NMR and FTIR evidence, has not been documented for plants growing in Uttarakhand. This study set out to isolate coumarin from authenticated *A. adenophora* collected in Uttarakhand and to describe its identity, purity and spectral behaviour.

2. MATERIALS AND METHODS

2.1 Plant material and authentication

Healthy, fully grown leaves and aerial parts of *A. adenophora* were gathered near Dol Ashram in the Kumaon hills of Uttarakhand, India, in May 2024. The material was authenticated at the Botanical Survey of India, Dehradun, and a voucher specimen was lodged under Acc. number 1434.

2.2 Microscopic evaluation

Fresh mature leaves were washed, and thin free-hand sections through the midrib and lamina were cut with a razor blade. The sections were cleared in chloral hydrate solution, stained with safranin, washed and mounted in glycerine. For powder microscopy, shade-dried leaf powder was cleared by gentle warming in chloral hydrate solution, washed and mounted in glycerine. The slides were observed under a LaboMed compound microscope with a digital camera, and the diagnostic tissues and cell inclusions were recognized and photographed following standard pharmacognostic practice [28,29,39].

2.3 Chemicals and reagents

The coumarin reference standard (purity 96.60%) was purchased from TCI. HPLC-grade methanol and acetonitrile, and analytical-grade ethanol, chloroform, n-hexane, ethyl acetate and formic acid were used, together with silica gel (60–120 mesh) for column chromatography and pre-coated silica gel 60 F_{254} plates (Supelco) for TLC/HPTLC. Chloral hydrate, safranin and glycerine were used for microscopy, and HPLC-grade water was used throughout.

2.4 Extraction and fractionation

One kilogram of shade-dried, coarsely powdered plant material was macerated in 70% ethanol at room temperature. The filtrate was concentrated under reduced pressure and then partitioned successively with chloroform to give a coumarin-enriched fraction. The partitioning was followed by TLC on silica gel 60 F_{254} with n-hexane : ethyl acetate : formic acid (7 : 3 : 0.2, v/v/v) as the mobile phase, and the plates were inspected at 254 and 366 nm.

2.5 Isolation of coumarin

The chloroform-enriched fraction was loaded on a silica gel (60–120 mesh) column and eluted with n-hexane : ethyl acetate in stepwise increasing polarity. Fractions that gave a single spot matching the coumarin standard were combined, evaporated and recrystallized from ethanol. The isolation yield was calculated as the mass of isolate divided by the mass of starting extract, multiplied by 100.

2.6 HPTLC analysis

HPTLC was carried out on Supelco silica gel 60 F_{254} HPTLC plates (200 × 100 mm). Methanolic solutions of the raw material, the coumarin standard and five enriched samples were sprayed as 8.0 mm bands with a CAMAG Linomat 5 applicator, using methanol as sample solvent, application volumes were 2.0–6.0 µL per track. Development was performed in a CAMAG twin-trough chamber (20 × 10 cm) containing n-hexane : ethyl acetate : formic acid (7 : 3 : 0.2, v/v/v). The plate was developed to a solvent front and dried for 5 min at room temperature.

The developed plate was scanned at 254 nm and then derivatized by spraying with anisaldehyde–sulphuric acid reagent and heated for 5 min at 105 °C in a hot-air oven. The derivatized plate was observed in visible light. The R_f values of the samples were compared with that of the standard [37,38].

2.7 HPLC analysis and quantification

HPLC was performed on a Shimadzu i-Series LC-2050C system fitted with a Restek ROC column (250 × 4.6 mm, 5 µm). Elution was isocratic with methanol : water (40 : 60) at 1 mL/min and detection at 254 nm. The isolate was identified by comparing its retention time with that of the coumarin standard, and its purity was calculated by area normalization. Linearity, LOD, LOQ, precision, accuracy and system suitability were evaluated according to ICH Q2(R2) [33].

2.8 Mass spectrometry (MS)

The isolate was examined by LC-ESI-MS/MS on a Shimadzu LCMS-8045 triple quadrupole instrument in positive electrospray mode (ESI+). The molecular ion and fragmentation pattern were compared with the known behaviour of coumarin ($C_9H_6O_2$; calculated $[M+H]^+$ m/z 147).

2.9 NMR spectroscopy

The ^{1}H-NMR spectrum was acquired in DMSO-d_6 at 400 MHz. Chemical shifts (δ, ppm) were referenced to the residual solvent signal [30,31].

2.10 FTIR spectroscopy

Infrared spectra were measured between 4000 and 400 cm^{-1} on a Perkin Elmer Spectrum Two, FTIR spectrophotometer, to detect the lactone carbonyl, aromatic C=C and C–O–C absorptions expected for coumarin [29,32].

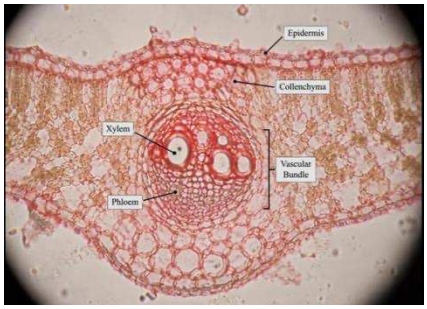
2.11 Statistical analysis

All experiments were performed in triplicate (n = 3), and the data are expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA), and differences were considered statistically significant at $p < 0.05$.

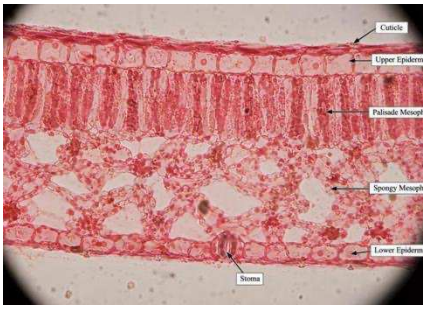
3. RESULTS

3.1 Authentication and microscopic evaluation

The plant material was confirmed as *A. adenophora* (Acc. no. 1434). In transverse section (Figure 1) the leaf had a single-layered upper and lower epidermis with a cuticle, a dorsiventral mesophyll made up of palisade and spongy parenchyma, and stomata on the lower surface (Figure 1b). The midrib contained subepidermal collenchyma and a collateral vascular bundle, with xylem towards the adaxial and phloem towards the abaxial side (Figure 1a). Powder microscopy (Figure 2) showed uniseriate multicellular covering trichomes (2c); multicellular glandular trichomes with a short stalk and an enlarged secretory head filled with brownish-yellow granular matter (2d); spirally thickened xylem vessels (2e); chloroplast-bearing palisade cells (2f); epidermal cells with sinuous anticlinal walls and a stoma (2g); and polygonal parenchyma cells (2h).

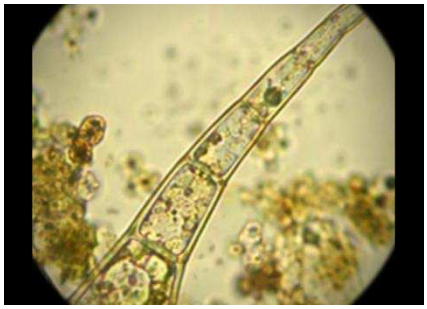


(a) Vascular bundle (xylem and phloem)

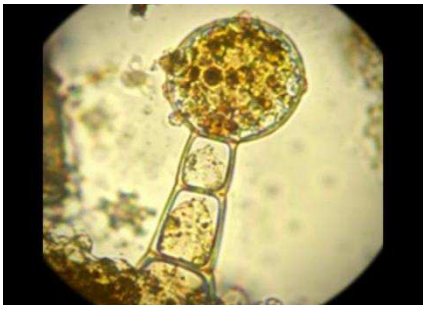


(b) Leaf transverse section (lamina)

Figure 1. Transverse sections of the *A. adenophora* leaf: **(a)** midrib showing epidermis, collenchyma, xylem, phloem and vascular bundle; **(b)** lamina showing cuticle, upper epidermis, palisade and spongy mesophyll, lower epidermis and stoma.



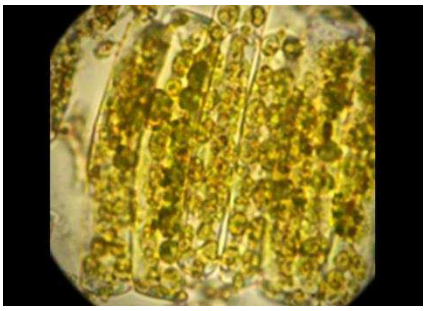
(c) Covering trichome



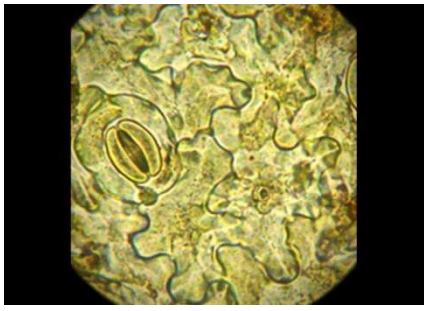
(d) Glandular trichome with secretory head



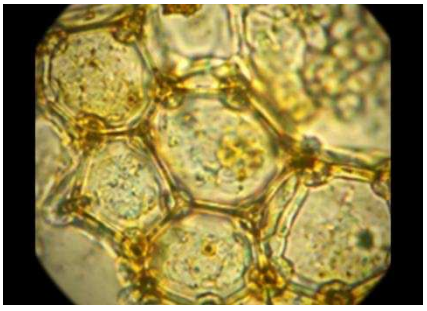
(e) Spirally thickened xylem vessels



(f) Palisade cells with chloroplasts



(g) Epidermal cells with stoma



(h) Parenchyma cells

Figure 2. Powder microscopy of the *A. adenophora* leaf: **(c)** covering trichome; **(d)** glandular trichome with multicellular stalk and secretory head; **(e)** spirally thickened xylem vessels; **(f)** palisade cells with chloroplasts; **(g)** epidermal cells with sinuous walls and a stoma; **(h)** polygonal parenchyma cells.

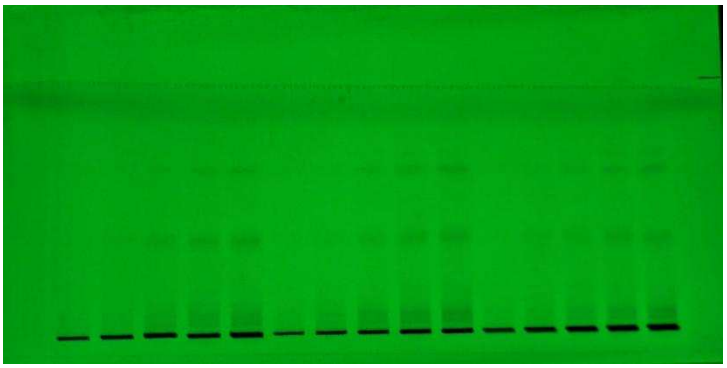
3.2 Extraction and isolation yield

Table 1. Extraction and isolation yields and physical characteristics of the isolate.

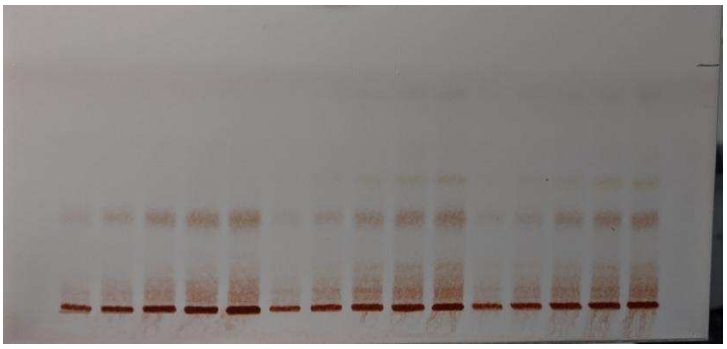
Parameter	**Result**
Extractive yield (%)	12.40% w/w
Coumarin-enriched fraction yield (%)	5.85% w/w (of crude extract; chloroform fraction)
Isolated coumarin yield (% w/w of starting extract)	1.68% w/w
Physical appearance (colour, form)	White to off-white crystalline needles
Melting point (°C)	68

3.3 HPTLC profile

The plate was documented under UV light at 254 nm and in visible light after derivatization (Figure 3). Densitometric scanning at 254 nm (Figure 4) showed the coumarin standard at Rf 0.745. The raw material and enriched samples contained a corresponding coumarin-region zone at Rf 0.726–0.747 at the applied volumes. The isolated compound produced a single zone at Rf 0.744, closely matching the standard.



(a) UV 254 nm



(b) Visible light after derivatization

Figure 3. HPTLC plate containing 15 tracks of raw material, coumarin standard and enriched fractions, including the isolated compound applied at 4 and 6 µL. Mobile phase: n-hexane : ethyl acetate : formic acid (7 : 3 : 0.2, v/v/v). Plates were documented (a) under UV light at 254 nm and (b) in visible light after derivatization.

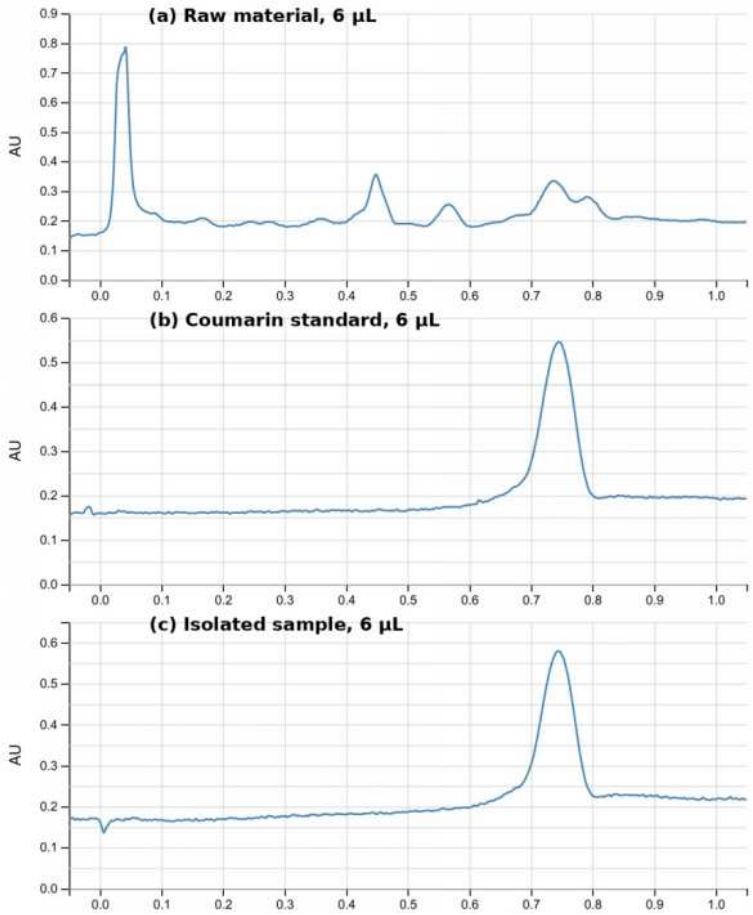
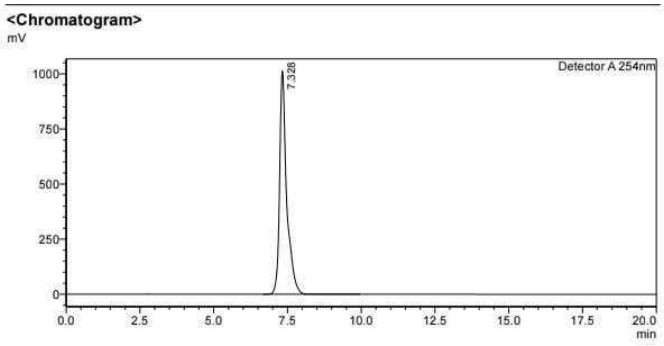


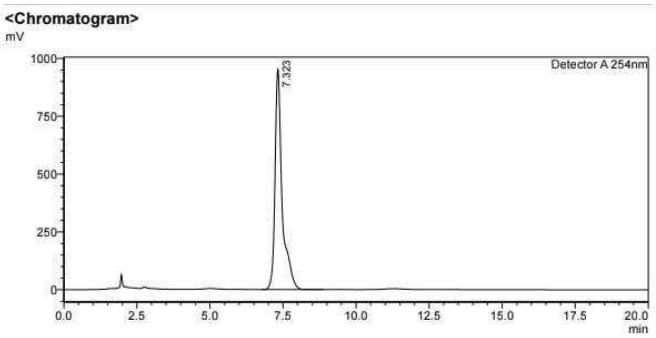
Figure 4. HPTLC densitograms (254 nm) **(a)** raw material, **(b)** coumarin standard and **(c)** the isolated sample

3.4 HPLC identification and purity

HPLC analysis of the isolate produced one major peak at 7.323 min with an area-normalized purity of 97.58%. The coumarin reference standard eluted at 7.328 min and had a reported purity of 98.04%. The method was linear over 1–50 µg/mL (r^2 = 0.9992), with an LOD of 0.08 µg/mL and an LOQ of 0.25 µg/mL.



(a) Coumarin standard (t_R 7.328 min)



(b) Isolated compound (t_R 7.323 min)

Figure 5. HPLC chromatograms (254 nm) of **(a)** the coumarin standard and **(b)** the isolated compound.

3.5 Mass spectrometry characterization

Positive-mode ESI-MS/MS of the isolated compound showed a single dominant ion at m/z 147.03, assigned to the protonated molecule $[M+H]^+$ of $C_9H_6O_2$ (theoretical m/z 147.04; MW 146.14), which is consistent with coumarin (benzopyran-2-one).

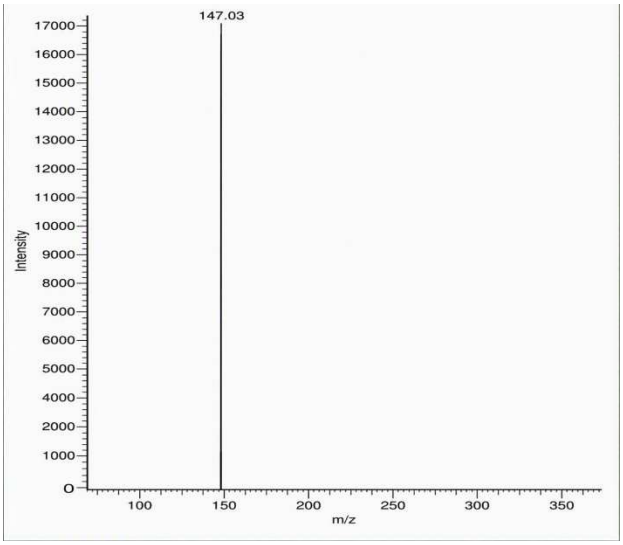


Figure 6. ESI+ full-scan mass spectrum of the isolate showing the molecular ion at m/z 147

3.6 NMR characterization

The ^{1}H-NMR spectrum (400 MHz, DMSO-d_6) of the isolated fraction showed aromatic signals at δ 7.31–7.43 (2H, m), a solvent signal at δ 2.55 and aliphatic signals at δ 0.83–1.09. These data support the presence of an aromatic compound; however, the expected lactone-region resonances were not adequately resolved and the residual aliphatic signals indicate remaining impurities. The available spectrum was therefore not considered independently conclusive for structural identification (Figure 7).

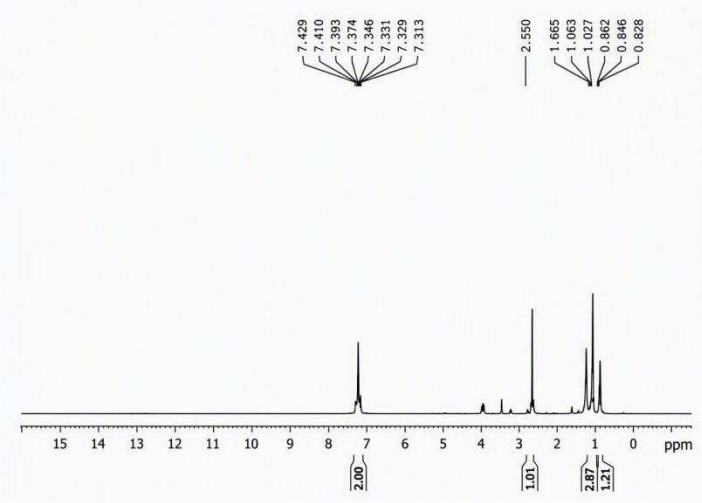


Figure 7. ^{1}H-NMR spectrum of the isolate (DMSO-d_6, 400 MHz)

3.7 FTIR characterization

The FTIR spectrum of the isolated compound showed a strong carbonyl absorption at 1693.20 and 1665.93 cm^{-1}, assigned to the conjugated C=O stretching of the α-pyrone (lactone) ring. Bands at 1618.02 and 1572.67 cm^{-1} corresponded to C=C stretching of the conjugated aromatic system, and those at 1499.59 cm^{-1} and near 1480 cm^{-1} to aromatic skeletal vibrations. Absorptions at 1300.13 and 1277.36 cm^{-1} were attributed to C–O stretching, with further C–O/C–O–C bands at 1115.65 and 1014.51 cm^{-1}. Bands at 922.06, 833.76, 744.71 and 713.82 cm^{-1} were assigned to aromatic C–H out-of-plane bending. Overall, the spectrum was consistent with the functional groups of a coumarin-type benzopyran-2-one skeleton. (Figure 8)

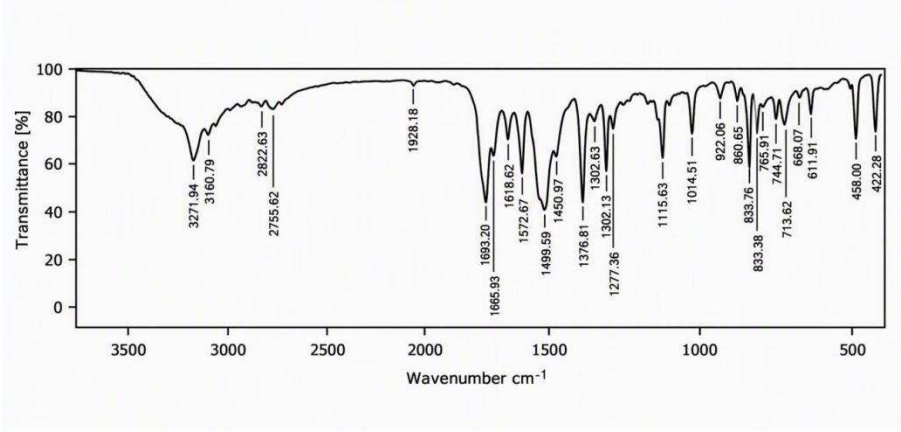


Figure 8. FTIR spectrum of the isolate with annotated absorption bands.

4. DISCUSSION

The combined results support assignment of the isolated material as a coumarin-type compound, although the evidential strength and limitations of each analytical technique must be considered.

(i) Botanical authentication. Microscopy established a dorsiventral leaf with a collateral vascular bundle, uniseriate covering trichomes, glandular trichomes with a secretory head, spirally thickened xylem vessels and sinuous-walled epidermal cells with stomata. These observations, together with authentication by the Botanical Survey of India and voucher accession number 1434, support the identity of the plant material used for extraction.

(ii) Extraction and chromatographic evidence. The 70% ethanolic extraction yielded 12.40% w/w crude extract, while the chloroform-enriched fraction represented 5.85% w/w of the crude extract and the isolated compound represented 1.68% w/w of the starting extract. On HPTLC, the isolate produced a single zone at Rf 0.744, closely matching the reference standard at Rf 0.745. HPLC provided a second chromatographic

comparison: the isolate eluted at 7.323 min and the reference standard at 7.328 min. The isolate showed 97.58% area-normalized purity. Agreement across two chromatographic systems supports the identity and high chromatographic purity of the isolate, although retention agreement alone is not sufficient for complete structural confirmation.

(iii) Mass spectrometric evidence. Positive-mode analysis showed a dominant ion at m/z 147.03, consistent with the protonated molecule $[M+H]^+$ expected for $C_9H_6O_2$ (theoretical m/z 147.04). This molecular-ion agreement supports a coumarin-type molecular composition. Because the presented spectrum is a unit-resolution full-scan spectrum, no exact-mass, elemental-composition or ppm-error claim is made.

(iv) FTIR evidence. The absorptions at 1693.20 and 1665.93 cm^{-1} are consistent with a conjugated lactone carbonyl. Bands at 1618.02 and 1572.67 cm^{-1} support a conjugated aromatic system, while absorptions at 1300.13, 1277.36, 1115.65 and 1014.51 cm^{-1} are compatible with C–O and C–O–C stretching. Together, these functional-group features support a benzopyran-2-one framework. The broad band near 3272 cm^{-1} may reflect adsorbed moisture rather than a structural hydroxyl group.

(v) NMR evidence and limitation. The 1H-NMR spectrum contained aromatic resonances at δ 7.31–7.43, supporting an aromatic nucleus. Consequently, the available NMR spectrum independently confirm the complete coumarin structure.

(vi) Overall interpretation. The melting point of 68 °C, close chromatographic agreement with the authentic coumarin standard, the $[M+H]^+$ ion at m/z 147.03 and the FTIR pattern collectively support identification as a coumarin-type compound. The conclusion is therefore based on convergent chromatographic and spectroscopic evidence.

(vii) Relevance and safety considerations. The isolate can serve as a working chromatographic marker for standardization of A. adenophora extracts and for subsequent formulation research. This study establishes chemical-marker evidence only; it does not establish therapeutic efficacy or safety. Because hepatotoxic sesquiterpenes have been reported from the species [23–27], toxicological assessment and a formal benefit–risk evaluation remain necessary before any internal-use product development.

5. CONCLUSION

A coumarin-type compound was isolated from authenticated A. adenophora collected in Uttarakhand at a yield of 1.68% w/w of the starting extract. The isolate produced a single HPTLC zone at Rf 0.744, closely matching the coumarin standard at Rf 0.745, and a major HPLC peak at 7.323 min with 97.58% area-normalized purity, closely matching the standard retention time of 7.328 min. The dominant $[M+H]^+$ ion at m/z 147.03 and the FTIR bands attributable to a conjugated lactone carbonyl, aromatic C=C and C–O/C–O–C groups provide complementary support for a coumarin-type benzopyran-2-one structure. The available 1H-NMR spectrum supported an aromatic compound independently conclusive. Accordingly, the isolate is suitable as a working chromatographic marker for A. adenophora. These findings do not establish the safety or efficacy of the plant or isolate for therapeutic use.

DECLARATIONS

Author contributions: Shubhangi Mehra: conceptualization, investigation, isolation and spectroscopic analysis, data curation, manuscript preparation. C. S. Tailor: supervision, methodological guidance, critical review. Gaurav Paliwal: supervision, methodological guidance, HPTLC and chromatographic support. Sugam Saraswat: supervision, methodological guidance and conceptualization. All authors approved the final manuscript and take responsibility for the integrity of the work.

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Conflict of interest: The authors declare no conflict of interest.

Ethical approval: Not applicable. The work comprised phytochemical isolation and spectroscopic characterization of plant material and involved no human participants or experimental animals.

Data availability: The data generated during the study are available from the corresponding author on reasonable request.

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REFERENCES

1. Evans WC. Trease and Evans' Pharmacognosy. 16th ed. Edinburgh: Saunders Elsevier; 2009.
2. Verpoorte R, Choi YH, Kim HK. Ethnopharmacology and systems biology: a perfect holistic match. J Ethnopharmacol. 2005;100(1-2):53-56.
3. Gobbo-Neto L, Lopes NP. Plantas medicinais: fatores de influencia no conteudo de metabolitos secundarios. Quim Nova. 2007;30(2):374-381.
4. Figueiredo AC, Barroso JG, Pedro LG, Scheffer JJC. Factors affecting secondary metabolite production

in plants: volatile components and essential oils. Flavour Fragrance J. 2008;23:213-226.

5. Qiang S. The history and status of the study on Crofton weed (Eupatorium adenophorum Spreng.), a worst worldwide weed. J Wuhan Bot Res. 1998;16:366-372.
6. Sun XY, Lu ZH, Sang WG. Review on studies of Eupatorium adenophorum — an important invasive species in China. J For Res. 2004;15:319-322.
7. Wang JJ. The Crofton weed, Ageratina adenophora. In: Wan FH, Zheng XB, Guo JY, editors. Biology and Management of Invasive Alien Species in Agriculture and Forestry. Beijing: Science Press; 2005. p. 650-661.
8. Li RT, Ding ZH, Ding JK. Chemical constituents from Eupatorium adenophorum. Plant Divers. 1997;19(2):1-3.
9. Zhang M, Liu WX, Zheng MF, et al. Bioactive quinic acid derivatives from Ageratina adenophora. Molecules. 2013;18(11):14096-14104.
10. Zhang ML, Wu M, Zhang JJ, Irwin D, Gu YC, Shi QW. Chemical constituents of plants from the genus Eupatorium. Chem Biodivers. 2008;5(1):40-55.
11. Geng H. Chemical constituents and their bioactivities of plants from the genus Eupatorium (2015–Present). Biology. 2024;13(5):288.
12. Zhang M, Ouyang JK, Xu QL, et al. Thymol derivatives with antibacterial and cytotoxic activity from the aerial parts of Ageratina adenophora. RSC Adv. 2021;11:5755-5761.
13. Ahluwalia V, Sisodia R, Walia S, Sati OP, Kumar J, Kundu A. Chemical analysis of essential oils of Eupatorium adenophorum and their antimicrobial, antioxidant and phytotoxic properties. J Pest Sci. 2014;87:341-349.
14. Kundu A, Walia S, Dutta TK, Singh J, Kaur C. Chemical composition of essential oil from aerial parts of Eupatorium adenophorum Spreng. J Essent Oil Res. 2013;25:1-7.
15. Pandey AK, Mohan M, Singh P, et al. Chemical composition, antibacterial and antioxidant activity of essential oil of Eupatorium adenophorum Spreng. from Eastern Uttar Pradesh, India. Food Biosci. 2014;7:80-87.
16. Negi A, Semwal A. Antimicrobial potential of Eupatorium adenophorum Spreng. Pharmacogn J. 2010;2(18):61-64.
17. Chakravarty AK, Mazumder T, Chatterjee SN. Anti-inflammatory potential of ethanolic leaf extract of Eupatorium adenophorum Spreng. Evid Based Complement Alternat Med. 2011;2011:471074.
18. Jin Y, Zhang Y, Wan C, et al. Immunomodulatory activity and protective effects of polysaccharide from Eupatorium adenophorum leaf extract on highly pathogenic H5N1 influenza infection. Evid Based Complement Alternat Med. 2013;2013:194976.
19. Jin Y, Hou L, Zhang M, et al. Antiviral activity of Eupatorium adenophorum leaf extract against tobacco mosaic virus. Crop Prot. 2014;60:28-33.
20. Liu X, Ouyang C, Li Y, et al. Evaluation of the antimicrobial activity of 9-oxoagerophorone against soil borne pathogens. J Plant Dis Prot. 2016;123:163-170.
21. Chen H, Zhou B, Yang J, et al. Essential oil derived from Eupatorium adenophorum Spreng. mediates anticancer effect by inhibiting STAT3 and AKT activation. Front Pharmacol. 2018;9:483.
22. Sun W, Liu SS, Zhao CC. Biological properties of active compounds from Ageratina adenophora. SAGE Open Med. 2023;11:20503121231167964.
23. An Y, Zhang Y, Wang H, et al. An overview: the toxicity of Ageratina adenophora on animals and its possible interventions. Int J Mol Sci. 2021;22(21):11581.
24. Oelrichs PB, Calanasan CA, MacLeod JK, et al. Isolation of a compound from Eupatorium adenophorum causing hepatotoxicity in mice. Nat Toxins. 1995;3(5):350-354.
25. Kaushal V, Dawra RK, Sharma OP, et al. Hepatotoxicity in rat induced by partially purified toxins from Eupatorium adenophorum. Toxicon. 2001;39:615-619.
26. Bhardwaj R, Singh A, Sharma OP, et al. Hepatotoxicity and cholestasis in rats induced by the sesquiterpene 9-oxo-10,11-dehydroageraphorone isolated from Eupatorium adenophorum. J Biochem Mol Toxicol. 2001;15:279-286.
27. Kaushal V, Dawra RK, Sharma OP, et al. Biochemical alterations in the blood plasma of rats associated with hepatotoxicity induced by Eupatorium adenophorum. Vet Res Commun. 2001;25:601-608.
28. World Health Organization. Quality Control Methods for Herbal Materials. Geneva: World Health Organization; 2011.
29. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. Pune: Nirali Prakashan.
30. Pavia DL, Lampman GM, Kriz GS, Vyvyan JR. Introduction to Spectroscopy. 5th ed. Boston: Cengage Learning; 2015.
31. Claridge TDW. High-Resolution NMR Techniques in Organic Chemistry. 3rd ed. Oxford: Elsevier; 2016.

32. Silverstein RM, Webster FX, Kiemle DJ, Bryce DL. Spectrometric Identification of Organic Compounds. 8th ed. Hoboken: Wiley; 2014.

33. International Council for Harmonisation. ICH Harmonised Guideline Q2(R2): Validation of Analytical Procedures. Geneva: ICH; 2023.

34. Lake BG. Coumarin metabolism, toxicity and carcinogenicity: relevance for human risk assessment. Food Chem Toxicol. 1999;37(4):423-453.

35. Venugopala KN, Rashmi V, Odhav B. Review on natural coumarin lead compounds for their pharmacological activity. BioMed Res Int. 2013;2013:963248.

36. Stefanachi A, Leonetti F, Pisani L, Catto M, Carotti A. Coumarin: a natural, privileged and versatile scaffold for bioactive compounds. Molecules. 2018;23(2):250.

37. Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd ed. Berlin: Springer; 1996.

38. Reich E, Schibli A. High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plants. New York: Thieme; 2007.

39. Khandelwal KR. Practical Pharmacognosy: Techniques and Experiments. Pune: Nirali Prakashan.