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ORIGINAL ARTICLE

# Isolation, Characterization, and Chromatographic Estimation of Esculin: A Potential Fluorescent Marker from *Launaea pinnatifida* Cass

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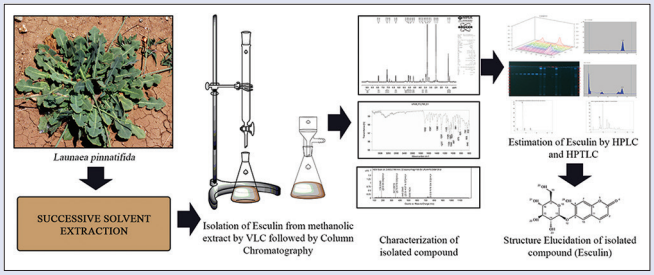
## ABSTRACT

**Background:** *Launaea pinnatifida* (Willd.) Sch. Bip ex Kunze (*Asteraceae*) is an Indian Ocean intertidal sand dune herb that is useful as traditional medicine. The plant is one of the species of traditional Indian Ayurvedic herb "Gojihva." **Objectives:** The present study was performed to isolate, characterize, and estimate a potential marker compound from *L. pinnatifida*. **Materials and Methods:** The leaves of the plant were subjected to successive solvent extraction with the help of solvents of varying polarity. Based on bioactivity studies performed using these extracts, the most bioactive methanolic extract was further subjected to vacuum liquid chromatography using chloroform and methanol in varying proportions. A phytoconstituent was isolated from fraction chloroform: methanol (90:10), elucidated to be Esculin based on its analytical (FTIR, liquid chromatography–mass spectrometry, $^1$H nuclear magnetic resonance, and ultraviolet) data. For the quantitative analysis of esculin in *L. pinnatifida* leaves, simple and validated high-performance liquid chromatography (HPLC) and high-performance thin-layer chromatography (HPTLC) methods were developed. **Results:** The purity of isolated as well as purchased standard esculin was found to be 97.66% and 98.2%, respectively, by HPLC, whereas the yield of esculin in the methanolic extract of leaves was found to be 1.40% w/w by HPTLC. **Conclusion:** Esculin has been reported for the first time in this plant and can be considered a potent phytochemical marker. It can also be useful for the standardization and quality control of *L. pinnatifida* formulations.

**Key words:** Gojihva, high-performance liquid chromatography, high-performance thin-layer chromatography, quality control, standardization

## SUMMARY

- Esculin was isolated from *Launaea pinnatifida* for the first time and characterized using several analytical instruments such as FTIR, liquid chromatography–mass spectrometry, $^1$H nuclear magnetic resonance, and ultraviolet spectroscopy. Simple and validated high-performance liquid chromatography and high-performance thin-layer chromatography procedures were established for the quantitative analysis of esculin in *L. pinnatifida* leaves. Esculin could be used as a marker for standardization of this plant, allowing it to be distinguished from other species of the controversial plant *Gojihva*.




**Abbreviations used:** NMR: Nuclear magnetic resonance; LC-MS/MS: Liquid chromatography-mass spectrometry; DMSO: Dimethyl sulfoxide; FT-IR: Fourier transform– Infrared; $^1$H NMR: Proton nuclear magnetic resonance; HPLC: High-performance liquid chromatography; HPTLC: High-performance thin-layer chromatography; LCMS: Liquid chromatography–mass spectroscopy; *L. pinnatifida: Launaea pinnatifida;* LPLPE: *Launaea pinnatifida* leaves petroleum ether extract; LPLC: *Launaea pinnatifida* leaves chloroform extract; LPLM: *Launaea pinnatifida* leaves methanol extract; LPLW: *Launaea pinnatifida* leaves water extract; LPRPE: *Launaea pinnatifida* root petroleum ether extract; LPRC: *Launaea pinnatifida* root chloroform extract; LPRM: *Launaea pinnatifida* root methanol extract; LPRW: *Launaea pinnatifida* root water extract; SSE: Successive solvent extraction; TLC: Thin-layer chromatography; UV: Ultraviolet; VLC: Vacuum liquid chromatography.

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## INTRODUCTION

*Launaea pinnatifida* (*L. pinnatifida*) is also recognized as *Launaea sarmentosa* (Willd.) sch. Bip. Ex Kuntze belongs to the family *Asteraceae* (Compositae). *L. pinnatifida* is a perennial, prostrate, stoloniferous herb rooting at each rosette and exudates white milky latex from its root, leaf, and stem when broken.[1]

According to the traditional medicinal literature, *L. pinnatifida* possesses diuretic and hepatoprotective action.[1,2] It has been traditionally used as an herbal remedy for many elements. The plant is a galactagogue. Juice of the plant is tonic, diuretic, aperients; applied in rheumatic affections, and given as a soporific to children. As far as pharmacological studies have been concerned, this plant possesses antioxidant, antidiarrheal, and hepatoprotective.[3] A different fraction of *L. pinnatifida* leaves extract also possesses significant antioxidant activity, among that ethyl acetate fraction had promising antioxidant activity. Taraxasterol has



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been isolated from leaves and taraxeryl acetate from the roots of the *L. pinnatifida*.[4] Apart from this, antifungal saponins were isolated from the methanolic extract of the seed of *L. pinnatifida*.[4] However, the plant remains largely unexplored.

*L. pinnatifida* is one of the species of the controversial drug "Gojihva." According to the literature survey, several species are considered as Gojihva, which includes *L. pinnatifida*, *Anchusa strigosa, Coccinia glauca, Macrotomia benthamii, Onosma bracteatum*, and *Elephantopus scaber*.[5,6] However, Gojihva is a controversial drug as its true botanical has still not been ascertained. Hence, there is an urgent need to obtain phytochemical profiling of various species known as Gojihva and to isolate marker compounds therefrom for specific differentiation. The present work has been done in continuation with the bioactivity-guided studies on *L. pinnatifida* for the aforementioned purpose.[7]

# MATERIALS AND METHODS

## Chemical and reagents

HPLC grade acetonitrile (ACN), methanol (MeOH), and water were procured from scientific sales and services, Ahmadabad. All laboratory-grade solvents and reagents were purchased from Finar Ltd., Ahmedabad. Standard esculin was procured from Yucca Enterprise, Mumbai. Silica gel G 100–200 mesh was purchased from Finar Ltd., Ahmedabad. Silica gel G 60–120 mesh (for column chromatography) was purchased from Spectrochem Pvt. Ltd., Mumbai, Maharashtra, India.

## Plant material

In May 2017, fresh leaves of the plant *L. pinnatifida* were obtained from Nagoa Beach in Diu (a union territory in west India). Authentication of the plant was done by Dr. Hitesh Solanki, Professor, Department of Botany, School of Science, Gujarat University, Ahmedabad. The geographical coordinate of plant location was 20°C 42'30.5 ''N, 70°C 53'35.3"E. For future reference, the voucher specimen (Voucher number. HM1) was deposited in the Department of Botany, School of Science, Gujarat University, Ahmedabad, Gujarat, India. The dried leaves and roots of the plant were employed in this research.

## Preparation of plant extracts by successive solvent extraction method

The leaves of *L. pinnatifida* were shade dried under the normal environmental condition and homogenized to a coarse powder (20 mesh size) and stored in aluminum bags. 150 g leaves were successively extracted separately using a Soxhlet apparatus and various organic solvents (petroleum ether, chloroform, methanol, and water) of analytical reagent quality. Each extract was then concentrated using a rotary evaporator at 40°C under vacuum and the dried residue was collected in an evaporating dish and kept overnight in desiccators for further studies.

## Vacuum liquid chromatography

As per our previously published work regarding bioactivity-guided studies on this plant, among all fractions, the methanol fraction of the leaves (*Launaea pinnatifida* leaves methanol extract [LPLM]) showed better hepatoprotective and antioxidant activity.[7] Therefore, the LPLM fraction was used for further phytochemical isolation. LPLM (10.12 g) fraction was subjected to vacuum liquid chromatography (VLC) for separating phytocompounds. The bioactive fraction was adsorbed on silica (60–120 mesh size) 1:1 ratio. Different solvents ratios were used for isolation of bioactive compound, for example, 100% ethyl acetate (LPLM/F1), 50:50 v/v ethyl acetate: methanol (LPLM/F2), and 100% methanol (LPLM/F3). The volume of each fraction collected was 900 ml. Each fraction was concentrated under reduced pressure at 40°C using a rotary evaporator.

## Isolation of phytocompounds from bioactive fraction using column chromatography

### *Column chromatography: (Launaea pinnatifida leaves methanol extract/F3)*

The most potent antioxidant and hepatoprotective extract (LPLM/F3) of leaves of *L. pinnatifida* were subjected to column chromatography to isolate the bioactive lead molecule using different polarity grade solvents as a mobile phase.[8] A glass column (60 inches in length and 4inch diameter with 500 ml reservoir) was selected and rinsed with acetone and further dried. A cotton layer was placed at the bottom and course sand was also placed to minimize the merging of the compound during elution. The column was packed with 300 g activated silica gel (100–120 mesh) and charged with the solvent (chloroform) and stationary phase (Silica 100–200 mesh). Extract bioactive fraction LPLM/F3 (8 g) dissolved in methanol and adsorbed onto dry silica gel (16 g). Then, the solvent was allowed to evaporate to dryness for 3 h. at 60°C and the dry powder with brownish color was poured into the column that was already packed with silica gel slurry and chloroform. The charged column was left for a few hours for complete saturation and removal of air bubbles to make the bed static. The column was first eluted with 100% chloroform at a flow rate of 1 ml per min. The polarity of the mobile phase was gradually increased with methanol by 10% increments. The fractions (100 ml) collected were dried in a rotary evaporator (Aditya Scientific, Model No. RE-5A with 5 L capacity) and weighed. All the fractions were subjected to thin-layer chromatography (TLC) for the identification of the compounds. A total of 110 fractions of column chromatography were collected each of 50–100 ml based on the TLC profile.

The fractions that have the same TLC fingerprinting profile (based on Rf) were merged and concentrate to dryness under reduced pressure using a rotary evaporator.[9] F90 fraction was found with the crystals while drying in the rotary evaporator which is white in color and amorphous in nature hence kept aside for further evaluation of the crystals (120 mg). The purity of the isolated compound was achieved by a repeated recrystallization technique using cold methanol.[10] TLC solvent system chloroform: methanol: formic acid (6.5:3:0.5) indicates the single spot at $R_f$ value 0.70. Hence, a further study was carried out for the elucidation of structure.

## Characterization of the isolated compound

The isolated bioactive pure compound was then characterized by the various spectral techniques, namely FTIR, $^1$H nuclear magnetic resonance (NMR), liquid chromatography–mass spectrometry (LC-MS), and UV as well as a comparison of these data with data in the literature.[11]

### *Ultraviolet–visible analysis*

The absorbance of various concentrations of isolated compounds at different wavelengths was measured by UV–Visible Spectrophotometer Shimadzu 1800, Japan, with serial no. A11455009148 and UVProbe software (version 2.70).

### *Liquid chromatography–mass spectrometry analysis*

The isolated compound in the LPLM fraction was evaluated using an Agilent UHPLC 1290 instrument coupled to Agilent LCQToF 6545 mass spectrometer. The mass spectrometric behavior of active fractions was studied for positive ion mode. The sample was prepared in methanol. The following instrument settings were used for analysis:

column Agilent $C_{18}$ Poroshell (100 mm × 4.6 mm × 2.7 µm); the column was kept at room temperature; ionizing voltage, 3500 V; nebulizer gas (N2), 35 psig, 11 L/min; fragmentor voltage, 130 V; nozzle voltage, 1000 V; drying gas (N2), 11 L/min; acquisition range, 100–1500 *m/z*; a mixture of ammonium acetate (A) and CAN (B) gradient 80:10%; and ammonium acetate for 30 min was selected as the mobile phase at a flow rate of 0.4 ml/min.

### *$^{13}$C nuclear magnetic resonance analysis*

$^{1}$H NMR spectra of the isolated compound were measured by Bruker ultrashield 500Mz instrument at the National Institute of Pharmaceutical Education and Research Gandhinagar, Gujarat, India. Dimethyl sulfoxide (DMSO) was used as solvent and TMS was used as an internal standard. Chemical shift values were recorded δ (ppm) and are depicted in Figure 1.

### *FTIR analysis*

FTIR study was performed and FTIR spectra were recorded on Bruker compact FTIR spectrometer with software OPUS version 7.5 at AUM Research Labs., Ahmedabad. Isolated compound C1 was pelleted with potassium bromide (KBr). FTIR spectral data were recorded and are depicted in Figure 1b. Functional group stretching and bending wavelength was matched with literature published data.

## Estimation of an isolated compound in *Launaea pinnatifida* leaves methanol extract fraction

### *High-performance liquid chromatography analysis*

The optimized chromatographic method was entirely validated utilizing the procedures outlined in ICH guidelines Q2 (R1) for analytical method validation.[12] The calibration curve for esculin was obtained by plotting the peak area against the injected amount of sample to the column. The limit of detection (LOD) and limit of quantification (LOQ) were determined as per the guideline given in ICH.[12]

Ten milligram of standard esculin dissolved in 10 ml of methanol. This solution was used as primary stock solution and further dilutions were prepared from it. The proposed HPLC method was used to test the linearity of the calibration curve in esculin standard solution over the concentration range of 0.008–0.25 mg/mL.

HPLC estimation of the isolated compound was performed using Agilent 1200 infinity instrument (Agilent Technology, USA), which equipped with a DAD detector, an autosampler, a column heater, and Welchrom® $C_{18}$ (4.67 mm ID, 250 mm, 5 µm particle size) column. The column oven temperature was set to 30°C. The mobile phase was consisting of two solvent (A) 0.14 g of anhydrous potassium dihydrogen orthophosphate ($KH_2PO$) dissolved in 900 ml HPLC grade water with the addition of 0.5 ml orthophosphoric acid, make up to 1000 ml with water, and filtered through 0.45 µ membrane filter and degassed in a sonicator for 3 min. (B) Acetonitrile (HPLC grade). Gradient elution was performed at flow rate of 1.5 ml per min with different ratio of solvent A and B using 350nm detection wavelength. Twenty microliter sample volume was injected into the system under the optimized chromatographic condition to evaluate the suitability of the system. 10.24 mg and 10.03 mg of purchased standard and isolated compound esculin (consider as standard with purity 97.668%), respectively, were dissolved in 10 ml methanol in the same manner 62.65 mg LPLM/F3 (consider as sample) extract was dissolved in 10 ml methanol and HPLC was performed for standard and sample. The analytical HPLC chromatogram of isolated marker compound and LPLM crude fraction is depicted in Figure 2a and b, respectively.

### *High-performance thin-layer chromatography analysis*

HPLTC analysis of LPLM and LPLM/F3 fractions was performed on percolated high-performance thin-layer chromatography (HPTLC) plate silica gel 60 $F_{254}$ (20.0 cm × 10.0 cm) manufactured by E. MERCK using a Camag Linomat V sample applicator and a Hamilton syringe with 100 µl volume capacity. A total of 12 bands were spotted on a silica plate with a 6 mm bandwidth, 15 mm margin at X position, and 8 mm margin from Y position (bottom).[13] A constant application rate of 150 nl/s was used using inert gas. TLC plate was developed in the mobile phase of chloroform: methanol: formic acid (6.5: 3: 0.5) for separation. Camag twin trough glass chamber (20 cm × 10 cm) was used for the linear ascending development, which is 30 min presaturated with 10 ml of the mobile phase. After the completion of plate development, the HPTLC

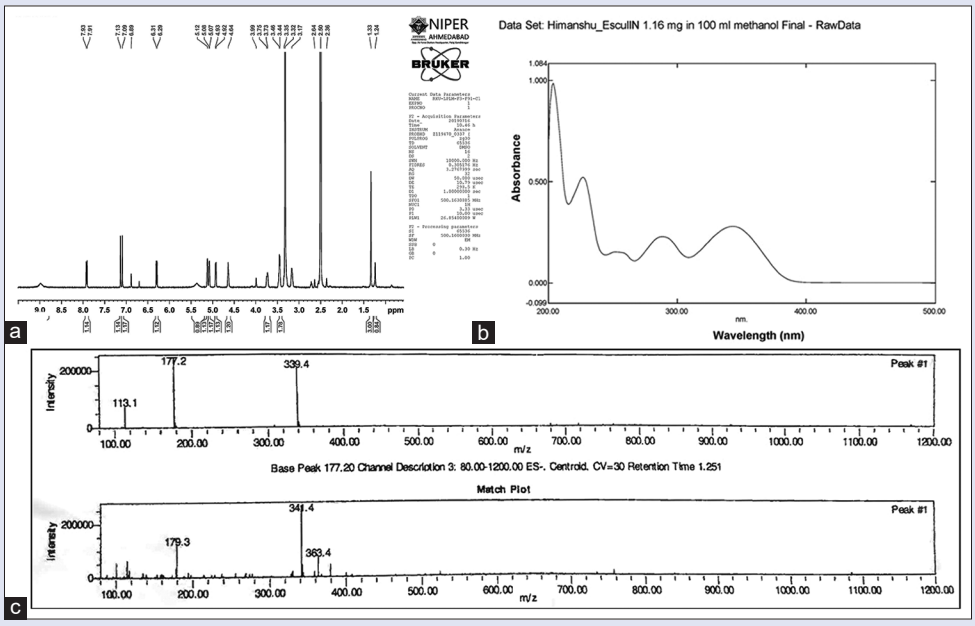


**Figure 1:** Characterization of an isolated compound by (a) $^{1}$H nuclear magnetic resonance spectra of an isolated compound, (b) ultraviolet visible spectra, (c) Liquid chromatography–mass spectrometry spectra

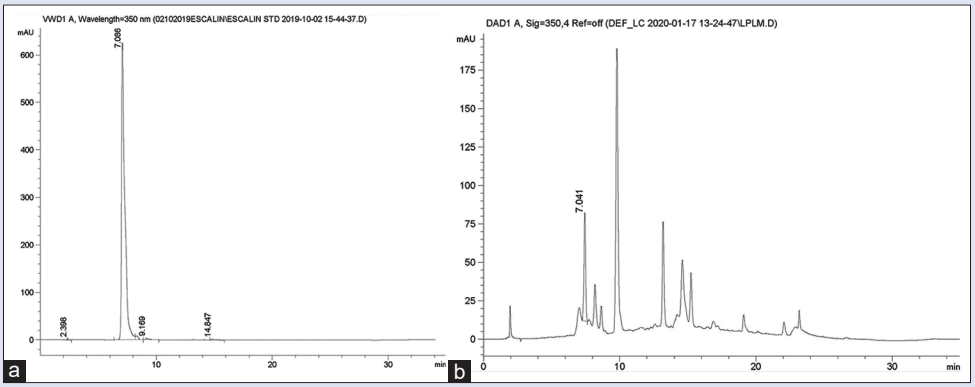


**Figure 2:** Analytical high-performance liquid chromatography chromatogram of (a) Isolated compound esculin from *Launaea pinnatifida* methanol fraction. (b) Crude methanol fraction (*Launaea pinnatifida* leaves methanol extract) from *Launaea pinnatifida*

plate was dried with a hairdryer at 50°C for 5 min. The slit dimension settings of length 4 mm and width 0.30 mm and a scanning rate of 20 $MMS^{-1}$ were employed. Densitometric scanning was performed with reflectance mode at $\lambda_{max}$ at 343 nm using on Camag TLC scanner 2.01.02 and incorporation with by win CATS software. Deuterium lamp (D2 lamp) was used as a source of radiation. The estimation of the isolated compound was done via height and peak area normalization and chromatograms are depicted in Figure 3.

## RESULTS AND DISCUSSION

LPLM leaves after successive solvent extraction showed good activity and hence subjected to further phytochemical investigation. This may have a better therapeutic value. After VLC, LPLM/F3 was also more therapeutically potent as compared to LPLM fractions hence considered for investigation in the direction of phytochemical isolation.

Fraction F90 has shown a single bend of the compound on the TLC plate and was kept aside for further purification. $R_f$ value at 0.15 was considered because of the more intense bends on the TLC chromatogram. TLC Fingerprinting profile clearly showed the single spot of the isolated compound C1 at $R_f$ 0.15. A compound is florescent at UV 366 nm; hence, it indicates the coumarin nature of the compound.[14] Results data were matched with published data regarding coumarin and its derivatives. A clear single spot indicates the purity of the compound. Spot turns to orange after spraying with a 1% methanolic KOH solution, which confirms that the isolated compound must be coumarin derivatives only.[15]

### Structure elucidation of the isolated compound C1

Crystals obtained from fraction F90 from column chromatography were white amorphous (120 mg) and further characterized using different spectral analyses that were performed. The melting point of white crystals of C1 was found to be 215°C–217°C. The melting point was searched in the Combined Chemical Dictionary database for the best match with plant/family name. Esculin was found to be the best match. Mother fraction LPLM/F3, fraction F90, and isolated Compound C1 were subjected to UV spectroscopy analysis. The compound showed λ max at 343 nm [Figure 1b] which was matching with esculin λ max.[16] UV-VIS spectrum shows absorption at 343 nm (ε =8.18 × 103), 289 (ε =6.68 × 103), and 252 nm (ε =4.48 × 103) for isolated compound esculin (concentration 0.0116 g/lit). A UV spectrum of esculin is depicted in Figure 1b.

LPLM/F3 extract was subjected to LC-MS analysis. The compound showed a mass of 341 (with positive ionization) which was matching with esculin [Figure 1c].[17] $^1$H NMR was carried out in DMSO. Additional structural information was conclusively revealed regarding the pure isolated compounds by the proton NMR. NMR signals were matched with literature and shown the similarity with the esculin structure.[18]

#### *$^1$H nuclear magnetic resonance spectra*

$^1$H NMR (500MHz, DMSO-d6) δ: 7.91 (d, j = 9.5 Hz, 1H, H-4), δ: 7.13 (s, 1H, H-5) δ: 6.84 (s, 1H, H-8), δ: 6.29 (d, j = 9.3 Hz, 1 h, H-3), δ: 5.15 (d, j = 3.7 Hz 1H), δ: 5.07 (d, j = 5.21 Hz 1H), δ: 4.92 (d, j = 7.147 Hz, 1H), δ: 4.64 (bs, 1H), δ: 3.75 (d, j = 11.537, 1H), δ: 3.46 (m, 1H), δ: 3.32 (m, 1H).

The FTIR spectral maxima indicated the presence of stretching bands CH2-asym (2909.1051 $cm^1$), aromatic ester (ketone) (1691.5434 $cm^1$) (1463.0441 $cm^1$), ether (1276.9662 $cm^1$), ester (1138.5159 $cm^1$), and an aliphatic ether (1127.9407 $cm^1$) in compound C1. All the signals were matched with the literature data of esculin.[19]

Esculin was obtained as off-white color amorphous to crystalline powder which is soluble in methanol. The melting point was found to be 203°C–205°C, matched with the Combined Chemical Dictionary database, which was matched with esculin. The percentage yield of esculin was found to be 120 mg (1.5% w/w to dry extract of LPLM).

### Estimation of isolated compound esculin by high-performance liquid chromatography and high-performance thin-layer chromatography

The percentage purity of the purchased standard was found to be 98.6%, whereas the percentage purity of the isolated compound C1 was found to be 97.66%, as shown in Figure 2a and b. This was good enough for other spectral analyses such as NMR, LC-MS, UV, and FTIR for the elucidation of structures? The isolated compound in the mother fraction of LPLM was found to be 0.76% by HPLC analysis. The retention time of standard esculin, isolated compound, and LPLM fraction was found to be 7.01, 7.08, and 7.04, respectively with no interference of endogenous peaks at their respective retention times. Quantification was achieved with UV detection at 343 nm based on peak area. The retention time of standard esculin, isolated compound, and LPLM/F3 fractions was found to be 7.01, 7.08, and 7.04, respectively. The improved approach was tested following ICH guidelines. Linearity was established for the range of 0.008–0.25 mg/mL with a correlation coefficient of 0.9994 [Table 1]. The validated method's mean percentage recovery or accuracy was found to be 99.77 ± 4.17 (mean ± standard deviation), reflecting good accuracy [Table 1]. The results of the LOD and LOQ tests were 0.008 and 0.025, respectively. Esculin was found to be 0.76% of the LPLM fraction.

Esculin was quantified by HPTLC for the first time in *L. pinnatifida*. Esculin was identified by HPTLC chromatogram when scanned at 343 nm.[20] The peak-area and height response was a polynomial function

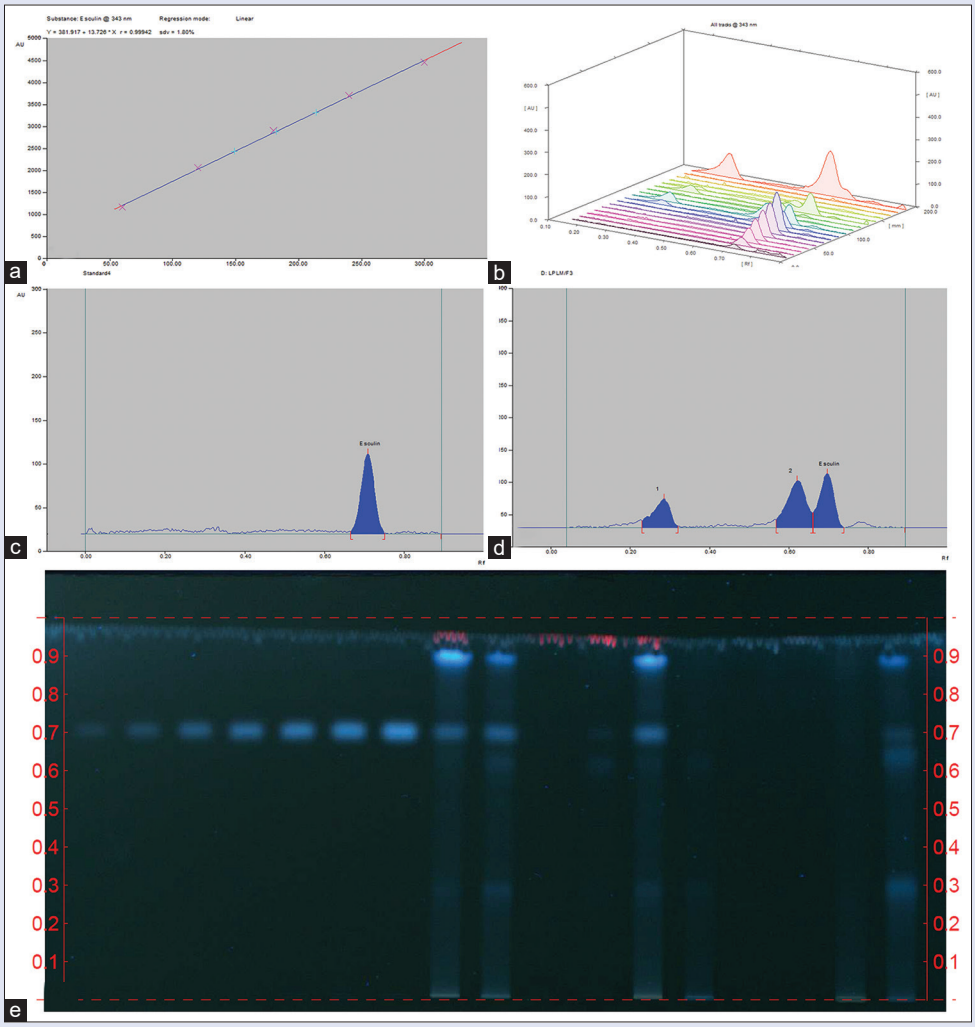


**Figure 3:** Analytical high-performance thin-layer chromatography chromatogram, (a) Calibration curve as per peak area, (b) three-dimensional chromatogram of various standard concentration, *Launaea pinnatifida* leaves methanol extract and *Launaea pinnatifida* leaves methanol extract/F3 fraction, (c) Isolated compound esculin as standard, (d) *Launaea pinnatifida* leaves methanol extract fraction as sample, (e) HPTLC plate spotted with standard (1–7 spot), *Launaea pinnatifida* leaves methanol extract (8 spot) and *Launaea pinnatifida* leaves methanol extract/F3 (9 spot)

**Table 1:** High-performance liquid chromatography method validation parameters

| Assay validation parameters | Results |
|---|---|
| Accuracy | 99.77±4.17 |
| Slope | 10325 |
| Intercept | 3.0323 |
| Linearity range | 0.008-0.25 mg/ml |
| Correlation coefficient ($r^2$) | 0.9994 |
| SE of intercept | 14.8101031 |
| SD of intercept | 36.26994248 |
| LOD | 0.008290963 |
| LOQ | 0.02512413 |

SE: Standard error; SD: Standard deviation; LOD: Limit of detection; LOQ: Limit of quantification

of the amounts of standards esculin in the ranges of 30–420 ng, according to the calibration plots [Figure 3a and b]. The correlation coefficient as per area and height was found to be 0.9975 and 0.9943 with RSD 0.138 and 0.112, respectively, in the developed method. The average percentage recovery of esculin as per area and height of peak was found to be 97.33% and 96.48%, respectively. As a result, the developed method was found to be accurate and satisfactory. Table 2 shows a summary of all validation parameters for the developed HPTLC method for quantification of esculin in *L. pinnatifida* methanolic extract.

Identification and quantification of esculin in methanol extract, LPLM, and LPLM/F3 were confirmed by HPTLC and developed chromatogram at 343 nm, as shown in Figure 3c and d. The percentage content of esculin in LPLM and LPLM/F3 was found to be 1.40, and 1.76% w/w, respectively, as shown in Table 3. $R_f$ value of esculin in LPLM and LPLM/F3 was found to be 0.70 as shown in Figure 3d. Based on calibration data and average recovery results, the approach was judged to be precise, reliable, and acceptable. As a result, the current HPTLC method permits quantification of esculin in *L. pinnatifida* plants in a simple, rapid, and accurate manner.

Esculin is a fluorescent hydroxycoumarin extracted primarily from barley (*Hordeum vulgare*),[21] horse chestnut (*Aesculus hippocastanum*),[22] daphnin (the dark green resin of *Daphne mezereum*),[23] Casava (*Manihot esculenta* Crantz),[24] and *Fraxinus sieboldiana* Blume[25] among other plants. Esculin also has antioxidant effects, as it protects triglycerides from auto-oxidation at high temperatures. The antioxidant property could also explain some of the product's anti-inflammatory effects,

**Table 2:** Calibration curve data and estimation of esculin in *Launaea pinnatifida* leaves methanol and *Launaea pinnatifida* leaves methanol/F3 fraction by high-performance thin-layer chromatography analysis

| Track | Sample ID | Concentration (ng/spot) | $R_f$ | Peak area | Peak height | Calculated esculin (µg) as per peak height | Calculated esculin (µg) as per peak area |
|---|---|---|---|---|---|---|---|
| 1 | Esculin | 30 | 0.72 | 19.68 | 603.71 | - | - |
| 2 | Esculin | 60 | 0.71 | 36.71 | 1157.83 | - | - |
| 3 | Esculin | 120 | 0.71 | 64.27 | 2053.52 | - | - |
| 4 | Esculin | 180 | 0.71 | 91.43 | 2899.15 | - | - |
| 5 | Esculin | 240 | 0.71 | 115.53 | 3700.44 | - | - |
| 6 | Esculin | 300 | 0.71 | 134.69 | 4452.22 | - | - |
| 7 | Esculin | 420 | 0.71 | 168.24 | 5532.97 | - | - |
| 8 | LPLM | 10,000 | 0.70 | 70.06 | 2426.46 | 0.140 | 0.154 |
| 9 | LPLM/F3 | 10,000 | 0.70 | 83.60 | 2880.21 | 0.176 | 0.190 |

LPLM: *Launaea pinnatifida* leaves methanol; VLC: Vacuum liquid chromatography; LPLM/F3: 100% methanol fraction of LPLM after VLC 1

**Table 3:** Method validation parameters for quantification of esculin

| Parameters | Values | |
|---|---|---|
| | Peak height | Peak area |
| Accuracy | 96.48±13.31 | 97.33±10.90 |
| Slope | 0.4281 | 14.16 |
| Intercept | 10.695 | 283.02 |
| Linearity range (ng/spot) | 30-420 | 30-420 |
| Correlation coefficient | 0.9943 | 0.9975 |
| SE of intercept | 2.94783918 | 64.7092906 |
| SD of intercept | 7.219258152 | 158.4730527 |
| LOD | 55.64950222 | 36.93227922 |
| LOQ | 168.6348552 | 111.9159977 |

LOD: Limit of detection; LOQ: Limit of quantification; SE: Standard error; SD: Standard deviation

making it a good choice for after-sun treatments. Due to its excellent sensitivity, selectivity, and fluorescence characteristics, esculin has recently been reported to be employed as a fluorescence sensor for uranyl detection.[26,27] Thus, *L. pinnatifida* could be an alternative source for the development of a new formulation for free radical-mediated degenerative diseases, mainly regarding the liver.

## CONCLUSION

Esculin has been isolated for the first time from *L. pinnatifida*. It may serve as a phytochemical reference marker for the standardization of the plant which is one of the species of the controversial drug "Gojihva".


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## Conflict of interest

There are no conflicts of interest.

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