

Physicochemical, Phytochemical, Qualitative HPTLC and Antioxidant Study of Medicinal Plant *Leucas aspera*

Rakhi Mishra^{1,2,*}, Binit Dwivedi² and Deepshikha Gupta¹

¹Amity Institute of Applied Sciences, Amity University, Department of Chemistry, Uttar Pradesh, India

²Dr. D.P. Rastogi Central Research Institute of Homoeopathy, Under Central Council for Research in Homoeopathy, Ministry of AYUSH, Government of India, Drug Standardization Unit, Department of Chemistry, Uttar Pradesh, India

(*Corresponding author's e-mail: rakhimishra020@gmail.com)

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Abstract

Introduction: *Leucas aspera* (family-Lamiaceae) has great therapeutic significance in the Indian system of medicine due to its rich antioxidant activity. The main objective of the present work was to evaluate physicochemical, phytochemical, qualitative high-performance thin layer chromatography (HPTLC), and antioxidant study of *L. aspera* in-house mother tincture (alcoholic extract) and 2 market samples of reputed brands. Materials and methods: Three samples of mother tincture were used for the study, in-house-mother tincture (A) of *L. aspera* and market samples (B and C). For qualitative HPTLC studies, precoated silica gel 60 F₂₅₄ plate was used, mobile phase used was toluene: ethyl acetate: formic acid (7:3:0.3, v/v/v), and UV detection was performed at 254 and 366 nm. For derivatization, anisaldehyde-sulphuric acid reagent was used. In the antioxidant study, total phenolic content (TPC), DPPH, and ABTS assay were studied. Results and discussion: Result shows that the mother tinctures prepared by authenticated plant sample showed maximum active constituents and prominent antioxidant activity as compared to the mother tinctures procured from the market. Conclusions: The present physicochemical, phytochemical, HPTLC, and antioxidant study justify *L. aspera* medicinal usage in Homeopathy. This is the reason for the cure and healing property of *L. aspera* whole plant extract.

Keywords: HPTLC, *Leucas aspera*, Antioxidant activity, Homeopathy, Mother tincture

Introduction

Leucas aspera (Wild) Linn, family Lamiaceae [1], is commonly called as “Thumbai” [2,3]. It is a common weed in India and is widely distributed throughout the country. *L. aspera* whole plant is used traditionally as an antipyretic [4], and insecticide [5,6] drug. Its extract has been shown to have anti-inflammatory [7-9], antinociceptive, antidiabetic [10], antimicrobial [11], antioxidant [12,13], and cytotoxic properties [14,15]. Medicinal value of each part of *L. aspera* is different, such as, its flowers are valued as a drug that helps to increase the activity of the central nervous system by acting as a stimulant and helps to treat cough by acting as an expectorant [16-18], helps to treat constipation acting as aperients [19], helps in perspiration act as diaphoretic, showed insecticidal properties, helps in increasing menstrual flow act as an emmenagogue and nasal disorders. The root is used for the treatment of pneumonia and pneumonitis [20], leaves used for rheumatism [21], juice used for psoriasis and other chronic skin eruptions, stems used for oral antihyperglycemic [22], whole plant [23] used as febrifuge and antidote to snake venom. Leaf extracts of *L. aspera* also show larvicidal activity against *Anopheles stephensi* larvae [24]. Silver nanoparticles prepared using leaf extract of *L. aspera* have shown cytotoxic activity against the HeLa cell line with IC₅₀ values of 36 µg/ml [25]. Hexane, ethyl acetate, acetone, and ethanol extracts of *L. aspera* were tested for anti-elastase assay revealed that hexane extract possessed the highest activity with IC₅₀ of 247.42 µg/ml, at a significant level (α) <0.05 indicating that it can be used as a cosmetic ingredient to deal with skin aging and related problems [26].

In homoeopathic system *L. aspera* mother tincture is used for the treatment of dysentery, jaundice, fever, the bite of venomous animals [27], and enlargement of the liver and spleen. The main bioactive constituents of *L. aspera* are ursolic and oleanolic acid [28]. Due to the presence of ursolic [29] and

oleanolic acid-like triterpenes [30] (**Figure 1**), they possess many pharmacological [31,32] properties. They act as an anti-inflammatory, hepatoprotective [33], antitumor [34], anticancer [35], antimicrobial anti-HIV, antifungal, gastroprotective, hypoglycemic, and antihyperlipidemic properties. Also, they are non-toxic in nature and used widely in cosmetic and health products. Ursolic and oleanolic acid are isomers and are difficult to separate in HPTLC. So, the objective of this work is to establish a suitable mobile phase in HPTLC for the separation and identification of these isomers from *L. aspera* extract. In the present research work, a single mobile phase was successfully developed for the determination of triterpenes isomers ursolic and oleanolic acid in chloroform extract of mother tincture of *L. aspera* by HPTLC. The present HPTLC method is beneficial in terms of the time taken and the amount of solvent used thus promoting further research in the quantification study of these isomers in *L. aspera* mother tincture. For measurement of antioxidant potential, total phenolic content (TPC), DPPH radical scavenging assay, and ABTS radical cation decolorization assay were carried out.

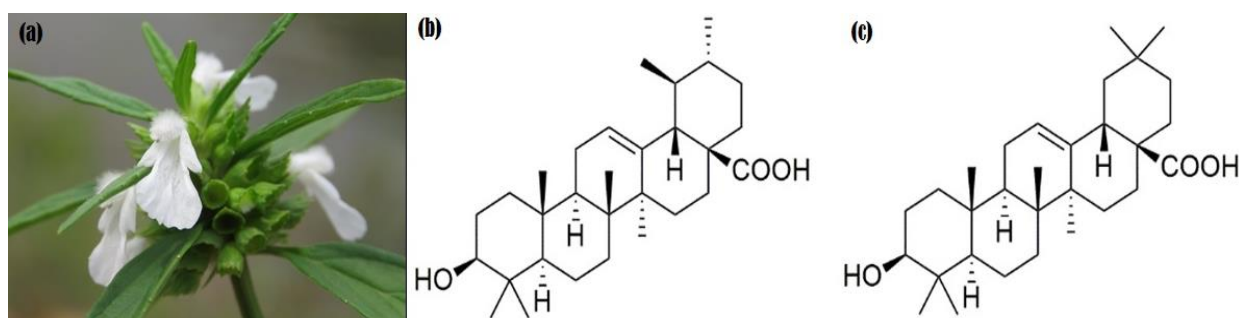


Figure 1 (a) *Leucas aspera* inflorescence, (b) Ursolic acid, and (c) Oleanolic acid.

Materials and methods

Raw materials

The plant material of *L. aspera* was collected and authenticated by staff at the Center of Medicinal Plants Research in Homoeopathy (CMPRH), Emerald, Tamil Nadu. The voucher specimen was deposited in the CSIR National Institute of Science Communication and Information Resources raw material herbarium, Delhi, India, for future reference number NISCAIR/RHMD/Consult/2020/3717-18-3. Authentic plant material was used to prepare the mother tincture. Ursolic acid ($C_{30}H_{48}O_3$, melting point $292\text{ }^{\circ}\text{C}$ with purity by HPLC $>90\%$) and oleanolic acid ($C_{30}H_{48}O_3$, melting point $300\text{ }^{\circ}\text{C}$ with purity by HPLC $>97\%$) were purchased from Sigma Aldrich, USA. Solvents used were ethanol, methanol, HPLC water, and chloroform of analytical grade purity (Merck Ltd., India).

Physicochemical studies for raw drug standardization

Moisture content was determined by the loss on drying method. Total ash, water-soluble ash, foreign matter, and acid-insoluble ash parameters were performed as per methods recommended in Homoeopathic Pharmacopeia of India [36].

Loss on drying

Loss on drying method used for determination of moisture content as per methods recommended in Homoeopathic Pharmacopeia of India [34]. Percentage loss on drying was calculated.

Foreign matter determination

For foreign matter determination, 100 g of plant raw material has been taken and outspread in a thin layer. The sample was examined by a $6\times$ lens or with the unaided eye, the foreign organic matter was picked manually. The ratio of total foreign matter weighed, and the weight of the drug taken gave the % of foreign matter [37].

Total ash value determination

In the drug, the impurity present in the form of organic matter was determined with the help of total ash value. For its determination, 2 g of the dried raw drug was weighed in powdered form in a pre-weighed silica crucible. The sample was incinerated in a silica crucible by gradually increasing the

temperature up to 450°C for 4 h or until it became carbon-free. The crucible was cooled and weighed until a constant weight was obtained [36]. Percent of total ash value was then calculated by taking the ratio of loss in weight to the weight of the sample taken.

Acid-insoluble ash value determination

After total ash value determination, 25 ml of 5 M hydrochloric acid was added to the dried ash and boiled in a water bath for 10 min. The solution was concentrated till its color changed to yellow. Acid insoluble matter was filtered using ashless Whatman paper 1 followed by washing with distilled water. The paper was again ignited in a crucible at a temperature not more than 450°C for 4 h, after which the crucible was kept in a desiccator, cooled, and weighed [36]. With reference to the originally taken air-dried powdered drug, % of acid insoluble ash value was calculated [37].

Water-soluble extractive value determination

For determination of water extractive value, 2 g of sample was accurately weighed, air dried powdered drug was put in a conical flask with 100 ml water added in it. The solution was allowed to stand for 24 h with intermittent shaking of the flask after every 4 h. The water-soluble extractive was filtered using Whatman filter paper. Twenty-five ml of this filtrate was completely dried on a pre-weighed petri dish at 105°C. The increase in weight of the petri dish was noted to calculate the water-soluble extractive value determination [34]. With reference to originally taken air dried powdered drug, % of water-soluble extractive value was calculated [37].

Alcohol-soluble extractive value determination

For determination of alcohol soluble extractive value accurately weighed 2 g air dried powdered drug was put in a conical flask and 100 ml absolute alcohol added to it. The whole solution was left for 24 h for complete extraction at room temperature. The solution was shaken vigorously for a few minutes after every 6 h. The extract was filtered with the help of Whatman filter paper taking precautions to avoid evaporation loss of alcohol from the extract. Weighed the empty flat-bottomed petri dish. The petri dish with 25 ml of filtrate was heated at 105°C in an electric oven then cooled in a desiccator and weighed [36]. With reference to originally taken air dried powdered % of alcohol-soluble extractive value calculated and recorded in **Table 2**.

Preparation of crude extract/in-house mother tinctures

One hundred g of coarsely dried powdered *L. aspera* whole plant was taken in which 100 ml distilled water and 635 ml strong alcohol (95%) was added to make 1,000 milliliters of the mother tincture using the percolation method [38] (as per Homoeopathic Pharmacopoeia of India). This tincture was transferred to a tightly packed amber glass container and stored for further study.

Qualitative phytochemical screening

Phytochemical tests were performed on crude extract for qualitative estimation of triterpenes with all respective testing procedures including Triterpenes tests, Lieberman Burchardt, Salkowski, and Tschugajen tests as described in the textbook by JB Harborne [39].

Standardization of mother tincture

Standardization of mother tincture was conducted to identify the organoleptic and physicochemical properties of mother tincture. Organoleptic properties measurement was done for color, odor, and clarity of solution. The samples were tested for various physicochemical properties like sediments, pH, total solids, Wt/ml, and total alcohol content.

TLC (Thin Layer Chromatography) study

TLC study has been used for isolating active compounds from mother tinctures or chloroform or alcohol extract. For the TLC study, the plate used is of silica gel 60 F₂₅₄ pre-coated plate as adsorbent for elution. Various trial methods were used, and the best result was observed in toluene: ethyl acetate: formic acid (7:3:0.3, v/v/v) as mobile phase. TLC spots were observed at 254 and 366 nm wavelengths.

Preparation of standard Oleanolic acid and Ursolic acid

Dissolved 10 mg of oleanolic acid in 10 mL ethanol in a volumetric flask and sonicated for 10 min to prepare a working standard of oleanolic acid with a concentration of 1 mg/ml. Similarly, ursolic acid standard was prepared.

Preparation of chloroform extract

Twenty-five ml of Mother Tincture (A) and 2 market samples (B and C) were taken in a 50ml beaker. To remove ethanol, the solution was evaporated on a water bath and extracted 3 times with 20ml chloroform. Upon combining the extracts, and concentrating up to 2 ml volume, TLC of chloroform extract of A, B, and C and reference standards ursolic acid and oleanolic acid on silica gel 60 F₂₅₄ pre-coated plate.

HPTLC fingerprinting profile study

For HPTLC fingerprinting study a densitometric HPTLC CAMAG LinomatV (Switzerland) system was used. In the HPTLC system, CAMAG Linomat V was used as a sample applicator, for the development of the mobile phase a saturating chamber CAMAG Twin Trough glass chamber was used. CAMAG TLC Scanner and software version CATS were used. HPLC grade solvents were used for all the extracts preparation. Samples were applied on silica gel 60 F₂₅₄ pre-coated plate (Merck) 20×10 cm² plate with an aid of a sampling machine, solvent front was run up to 70mm height. Volumes applied for the standard and sample were 6 and 10 µl respectively. The mobile phase used was toluene: ethyl acetate: formic acid (7:3:0.3, v/v/v), and TLC spots were visualized after illumination at 254 and 366 nm. Anisaldehyde-sulphuric acid reagent was used as a derivatizing agent for HPTLC profiling which was prepared by mixing 0.5 ml anisaldehyde and 10 mL glacial acetic acid, followed by the addition of 85 ml methanol and 5 ml concentrated sulphuric. Pure oleanolic and ursolic acid were used as reference standards.

Study of antioxidant potential

Determination of Total Phenolic Content (TPC)

The (TPC) of the extracts was determined by Folin-Ciocalteu's reagent procedure reported by Singleton *et al.* [40]. The total phenol content was estimated in *L. aspera* in-house mother tincture sample A and its market samples (B and C). Ascorbic acid was used as the reference standard. Different concentrations (0.2661-8.517 mM) of ascorbic acid were prepared and analyzed at 736 nm and a calibration curve was plotted as absorbance versus concentration. TPC was estimated by using ascorbic acid as standard approximately 50 µl of the mother tincture was mixed with 5 ml of 10 % Folin-Ciocalteu's (phenol reagent) and 4 ml of sodium carbonate. The mixture was allowed to stand for 1 h in dark. After 1 h the color changed from yellow to blue. The absorbance of the solutions was measured at λ_{max} 736 nm using a UV-VIS spectrophotometer (UV-Visible Spectrophotometer SPECORD 200 plus Analytik Jena, Germany). The TPC was calculated from the calibration curve and in the results, the TPC of the *L. aspera* sample A (in-house mother tincture) and market samples B and C were calculated as the ascorbic acid equivalents (AAE) using standard ascorbic acid (Y = 0.0753 × +0.0256, R² 0.9995) curve standardized in the lab for the calculation of ascorbic acid equivalent. TPC was expressed in mM concentration of ascorbic acid equivalent.

DPPH radical scavenging assay

The free radical scavenging activity of *L. aspera* sample in-house mother tincture (A) and market samples B and C were measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The standard solution of DPPH was prepared by dissolving 0.025 g in 25 ml methanol and different concentrations of standards/mother tincture sample (100 µL) were mixed with 4 ml methanol and 1 ml of DPPH standard. The mixture was allowed to stand for 1 h in dark, after which the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (UV-Visible Spectrophotometer SPECORD 200 plus Analytik Jena, Germany). The percentage inhibition was determined by comparing the result of the test and the control (methanol used as solvent blank) [41]. Percentage degradation was calculated by the formula:

$$DPPH\ radical\ Scavenging(\%) = [(B - A) \times 100] \div B \quad (1)$$

Where,

A= absorbance of sample

B= absorbance of control

The inhibiting effects of the mother tincture showed varied levels of DPPH radical scavenging activity, expressed as percentage degradation.

Determination of ABTS assay

Free radical scavenging activity of mother tincture and market samples were determined by ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)] radical cation decolorization assay. ABTS⁺ cation radical was produced by the reaction between 7mM ABTS in water and 2.45 mM potassium persulfate (1:1) stored in the dark at room temperature for 16 h before use. ABTS⁺ solution was then diluted with methanol to obtain an absorbance of 0.700 at 746 nm. After the addition of 10 µlof mother tincture/potencies/standard in 2 ml of diluted ABTS⁺ solution, the absorbance was measured at 5 min after the initial mixing. An appropriate solvent blank (methanol) was run in each assay [41]. Percent inhibition of absorbance at 746 nm was calculated using the formula:

$$ABTS \text{ ion scavenging effect (\%)} = [(AB - AA) \times 100] \div AB \quad (2)$$

Where,

AB is the absorbance of ABTS radical + methanol

AA is the absorbance of ABTS radical+ sample/standard.

Trolox was used as a standard substance used.

Results and discussion

Results of physicochemical and phytochemical studies

The physicochemical properties of the tinctures of in-house drug sample (A) and comparison with 2 procured market samples (B and C) for parameters like Sediments, pH, total solids, alcohol content, and weight per ml were analysed and tabulated in **Table 1(a)**. The results obtained for various physiochemical studies of raw drugs are tabulated in **Table 1(b)**. Phytochemical triterpenes tests performed on the crude extract of the whole plant of *L. aspera* showed positive results for Lieberman Burchardt, Salkowski, and Tschugajen tests as mentioned in **Table 2**. Organoleptic observations of the prepared in-house mother tincture indicated the formation of a clear brown solution with a characteristic tincture odor.

Table 1(a) Result of physicochemical properties of in-house (A) and commercial market samples (B and C) of *L. aspera* mother tinctures.

S.No.	Parameter	In-house sample A	Market sample B	Market sample C
1	Sediments	Nil	Nil	Nil
2	pH	5.86	6.00	6.36
3	Total solid	1.43 % w/v	1.26 % w/v	1.14% w/v
4	Wt/ml	0.913 g	0.910 g	0.916 g
5	Alcohol content	60.0 % v/v	59.0 % v/v	55.3 % v/v

Table 1(b) Results of test of physicochemical properties of raw drug material.

Name of test	Result (%)
Foreign matter	2.00
Loss on drying	13.80
Total ash value	9.89
Acid-insoluble ash value	0.35
Water-soluble extractive value	13.60
Alcohol-soluble extractive value	2.63

Table 2 Results of phytochemical tests for screening of triterpenes present in mother tincture of *L. aspera*.

Name of phytochemical triterpenoid tests	Procedure	Observation	Result
Lieberman Burchardt test	Chloroform solution of the extract with a few drops of acetic acid and 1 mL concentrated sulphuric acid.	Deep red at the junction of the two layers	Positive
Salkowski test	Chloroform solution of the extract when shaken concentrated sulphuric acid.	Lower layer turns yellow on standing	Positive
Tschugajen test	Chloroform solution of the extract with an excess of acetyl chloride and a pinch of zinc chloride warmed on water bath.	Red color	Positive

Result of HPTLC study

Based on extensive literature reviews, various combinations of solvent systems were studied with an aim to have an appropriate mobile phase composition for the best and most efficient HPTLC chromatographic separation of oleanolic and ursolic acid in *L. aspera* chloroform extract. In the mobile phase toluene: ethyl acetate: acetic acid (5.5:4.5:1, v/v/v), toluene: ethyl acetate: formic acid (7:5:1, v/v/v), and toluene: methanol (9:1, v/v) no appropriate resolution of the band was observed whereas in mobile phase toluene: ethyl acetate: formic acid (7:3:0.3, v/v/v) efficient band resolution of both oleanolic and ursolic acid standards were observed with improved R_f value of 0.54 and 0.55 respectively. Among all the mobile phase combinations studied, toluene: ethyl acetate: formic acid (7:3:0.3, v/v/v) was finalized to be the ideal one for the evaluation of both the compounds in *L. aspera*. Thus, it was finalized the best appropriate mobile phase composition for the entire HPTLC method development study. **Table 3** recorded various mobile phase combinations used for the preliminary screening study for the best possible separation of bands.

Table 3 Comparison of various mobile phase combinations used for preliminary screening study for best possible chromatographic separations of oleanolic and ursolic acid.

Used Mobile phase combinations for evaluation of oleanolic acid and ursolic acid	R_f value	Observations
Toluene: Ethyl acetate: Acetic acid (5.5:4.5:1, v/v/v)	0.50	Poor resolution of band
Toluene: Ethyl acetate: Formic acid (7:5:1, v/v/v)	0.56	Poor resolution of band
Toluene: Methanol (9:1, v/v)	0.23	No appropriate resolution of band
Toluene: Ethyl acetate: Formic acid (7:3:0.3, v/v/v)	0.55 for ursolic acid and 0.54 for oleanolic acid	Efficient band resolution with improved R_f .

Qualitative HPTLC study of the in-house mother tincture and market samples

HPTLC study of *L. aspera* chloroform extract of the in-house mother tincture (A), 2 market samples (B and C), and standard oleanolic and ursolic acid was carried out by using selected mobile phase toluene: ethyl acetate: formic acid in the ratio of volume (7:3:0.3, v/v/v). At U.V light 254 and 366 nm, no spots of ursolic acid and oleanolic acid were observed for any of the samples. Therefore, for better resolution, anisaldehyde-sulfuric acid reagent was used as derivatizing agent. After derivatizing the plate with anisaldehyde-sulfuric acid reagent, blue spot of ursolic acid was observed at R_f 0.55 (**Figure 2**) and a pink spot of oleanolic acid was observed at R_f 0.54 (**Figure 5**). After derivatization at 366nm, 2 distinguished colored spots were observed at R_f 0.55 (for standard ursolic acid, yellow) (**Figure 3**) and at

R_f 0.54 (for oleanolic acid, blue) (**Figure 6**) in in-house sample (A) and in market sample (B and C). 3D diagram of HPTLC densitogram displayed the presence of ursolic and oleanolic acid in mother tinctures of in-house sample A and market samples B and C displayed in **Figures 4** and **7** respectively.

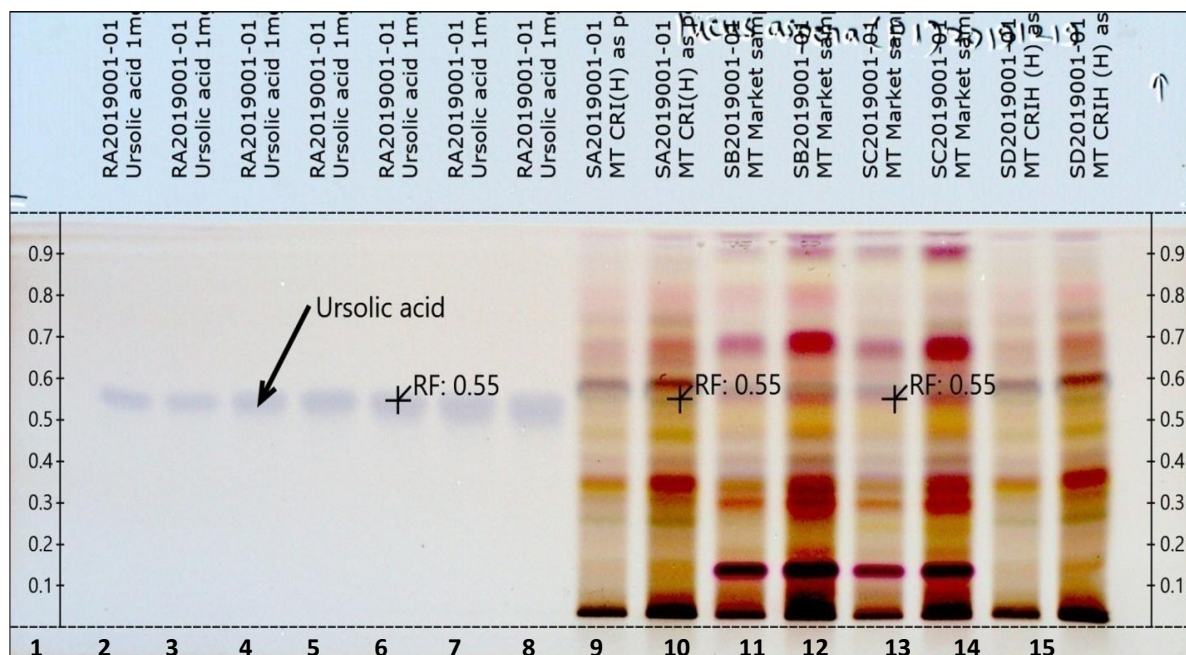


Figure 2 High-performance TLC fingerprints of *L. aspera* after derivatization with anisaldehyde sulphuric acid reagent viewed in white light. Standard ursolic acid Track (1-7), Track (8-9) in-house sample A CRI (H), Track (10-11) commercial market sample B, Track (12-13) market sample C and Track (14-15) in-house sample A CRI (H).

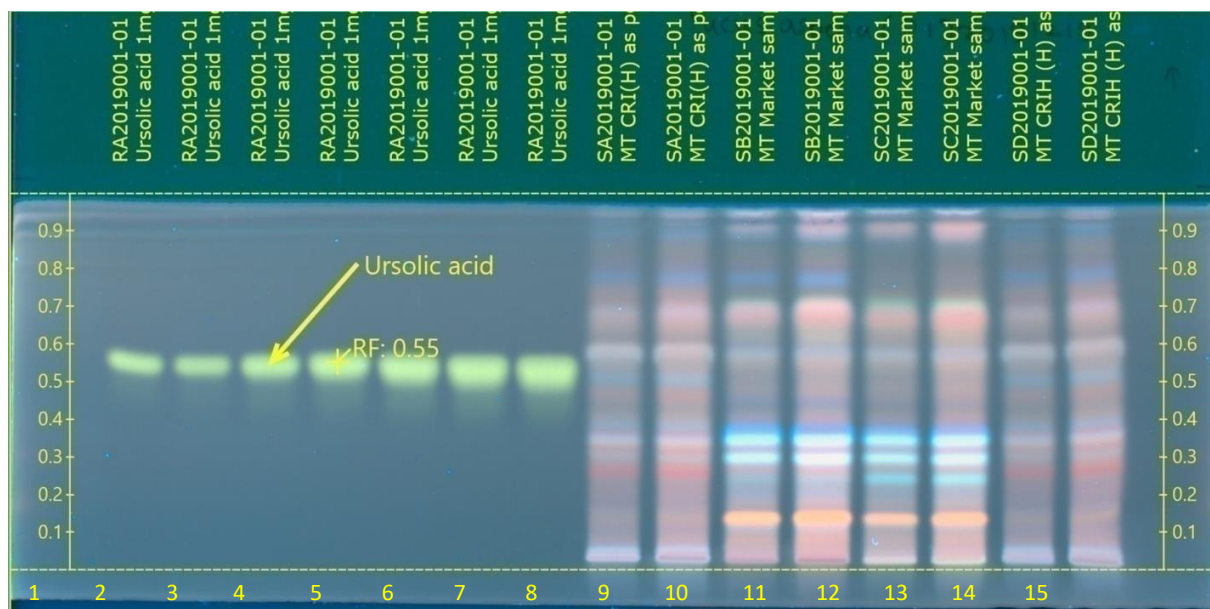


Figure 3 High-performance TLC fingerprints of *L. aspera* after derivatization with anisaldehyde sulphuric acid reagent at 366 nm. Standard ursolic acid Track (1-7), Track (8-9) in-house sample A CRI (H), Track (10-11) commercial market sample B, Track (12-13) market sample C and Track (14-15) in-house sample A CRI (H).

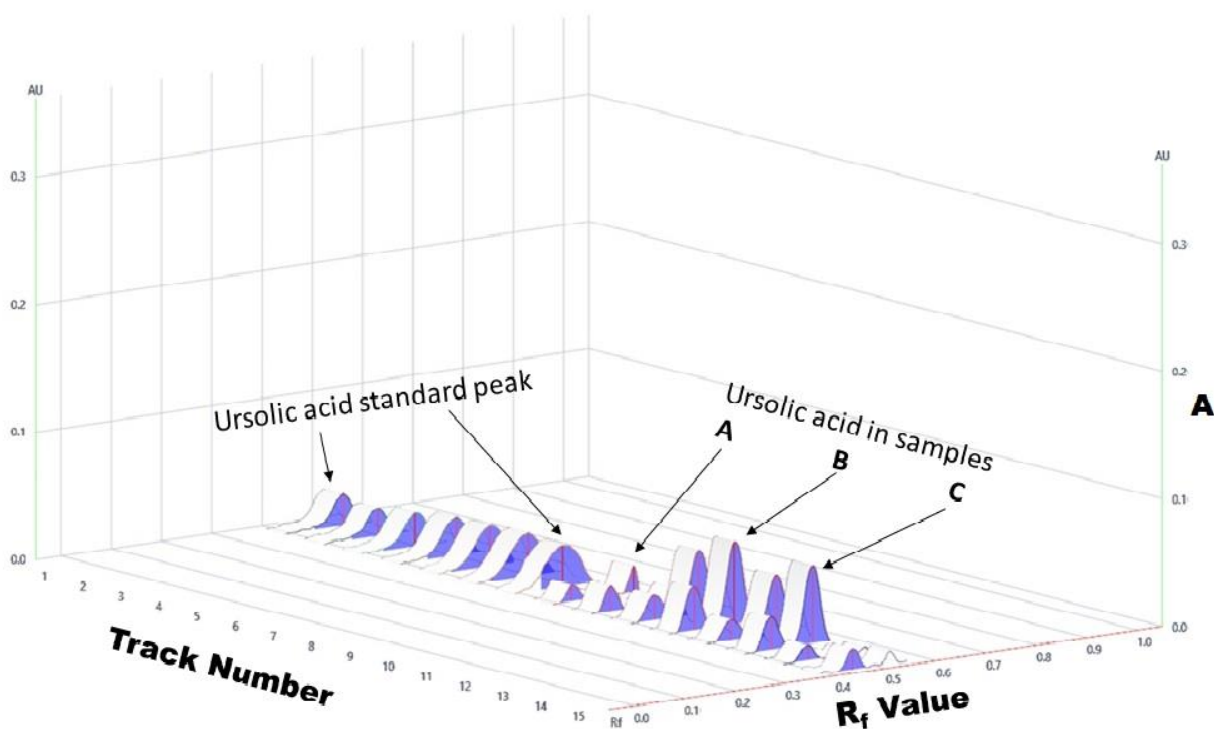


Figure 4 3D view of HPTLC densitogram for chloroform extract of *L. asperain*-house sample A and market samples B and C with respective standard (Track 1-7).

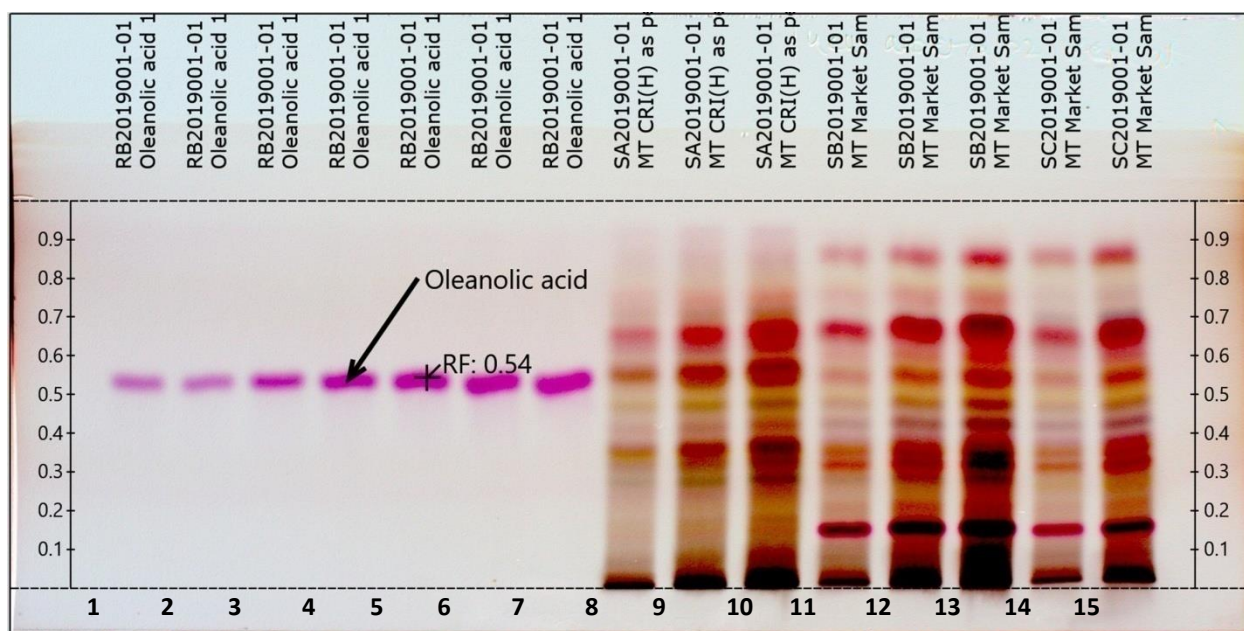


Figure 5 High-performance thinlayer chromatography fingerprints of *L. aspera* after derivatization with anisaldehyde sulphuric acid reagent in white light. Standard oleanolic acid Track (1-7), Track (8-10) in-house sample A, Track (11-13) commercial market sample B and Track (14-15) market sample C.

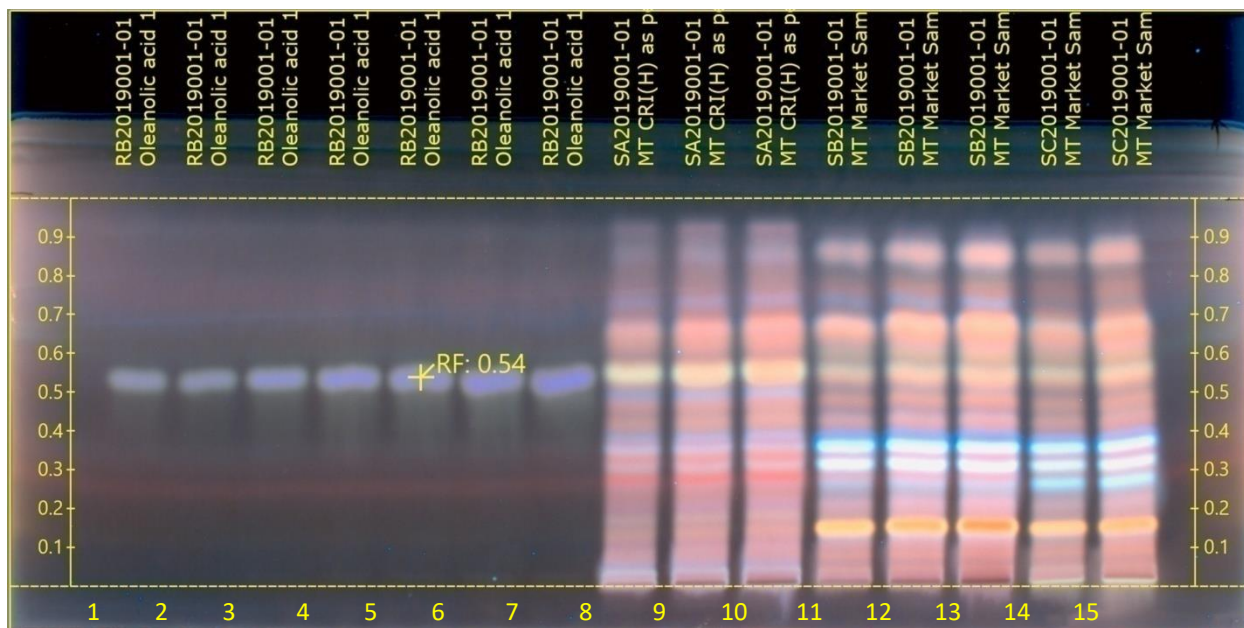


Figure 6 High-performance TLC fingerprints of *L. aspera* after derivatization with anisaldehyde sulphuric acid reagent at 366 nm. Standard oleanolic acid Track (1-7), Track (8 -10) in-house sample A, Track (11-13) commercial market sample B and Track (14-15) market sample C.

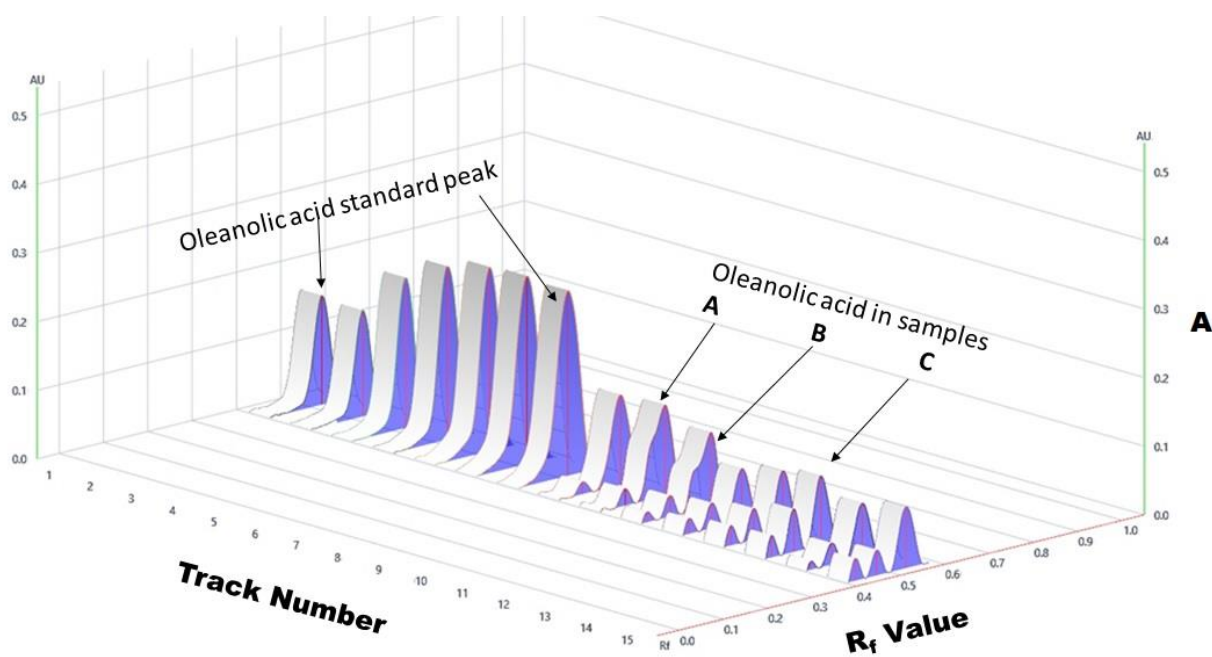


Figure 7 3D diagram of HPTLC densitogram display for chloroform extract of *L. aspera* in-house sample A and market samples B and C with respective standards.

Result of antioxidant activity

In the present study, the TPC of *L. aspera* in-house sample A and its market samples B and C were determined by Folin-Ciocalteu method and reported as AAE. Study reveals TPC found in *L. aspera* in-house sample A, market samples B, and C (50µL) were 9.26, 6.40, and 5.64 AAE (**Table 4**).

Table 4 Result of TPC in mother tincture of *L. aspera* in-house sample A and market samples B and C.

S.No.	Sample	Concentration in (mM) of AAE	Absorbance
1	<i>L. aspera</i> in-house sample A	9.26	0.7223
2	<i>L. aspera</i> market sample B	6.40	0.5075
3	<i>L. aspera</i> market sample C	5.64	0.4501
4	Control	0.09	0.0187

Table 5 Result of DPPH scavenging activity against *L. aspera* in-house sample A and market samples B and C.

S.No.	Sample	Concentration in (mM)	Absorbance	% Degradation
1	<i>L. aspera</i> in-house sample A	8.17	0.5315	78.50%
2	<i>L. aspera</i> market sample B	4.96	1.3572	45.13%
3	<i>L. aspera</i> market sample C	7.42	0.7243	70.72%
4	Control	0.63	2.4734	-

In the present study, the DPPH assay of *L. aspera* in-house sample A and market samples B and C were determined by DPPH radical scavenging assay method and reported as AAE. Study reveals *L. aspera* in-house sample A and market samples B and C were able to decolorize DPPH free radical, and the DPPH scavenging increased with the concentration of the extract. The result showed a greater rate of DPPH scavenging activity found in in-house sample mother tincture as compared to market samples. The percentage of inhibition found in 100 µL volume of *L. aspera* in-house sample A and market samples B and C were 78.50, 45.13, and 70.72% respectively (**Table 5**). The order of DPPH scavenging against *L. aspera* in-house sample A and market sample B and C were found to be sample A > sample C > sample B.

In DPPH assay a significant correlation coefficient (R, 0.9955) was found between the antioxidant activity of alcoholic extracts (mother tinctures) of *L. aspera* in-house sample A and market samples B and C. The proton radical scavenging action is known to be one of the important mechanisms for measuring antioxidant activity. This assay determines the scavenging of stable radical species DPPH by antioxidants compounds present in the mother tincture. The results showed a greater rate of DPPH scavenging activity in in-house sample A as compared to market samples B and C probably due to the presence of high content of Phenolic compound. Our study clearly indicated that the mother tincture of in-house sample A of *L. aspera* exhibited high content of phenolic compound i.e. 9.26mM (**Table 4**) which was significantly correlated with DPPH radical scavenging activity % i.e. 78.50% (**Table 5**).

Table 6 Result of ABTS radical cation scavenging activity against *L. aspera* in-house sample A and market samples B and C.

S.No.	Sample	Concentration in (µg/mL)	Absorbance	% Degradation
1	<i>L. aspera</i> in-house sample A	269.83	0.0003	99.89
2	<i>L. aspera</i> market sample B	267.86	0.0011	99.61
3	<i>L. aspera</i> market sample C	269.04	0.0007	99.75
5	Control	41.76	0.2832	-

In the present study, the ABTS assay of *L. aspera* in-house sample A and market samples B and C were determined by ABTS assay method and reported in terms of Trolox equivalents. A significant correlation coefficient (R, 0.9901) was found between the antioxidant activity of alcoholic extracts (mother tinctures) of *L. aspera* in-house sample A and market samples B and C. Study reveals *L.*

asperain-house sample A and market samples B and C were able to decolorize ABTS⁺ free radical, the ABTS radical cation scavenging activity increased with the concentration of the extract. The result showed the greater rate of ABTS cation scavenging activity found in *L. asperain*-house sample A as compared to market samples B and C. The percentage of inhibition found in 10 µL volume of *L. asperain*-house sample A and market samples B and C were 99.89, 99.61, and 99.75 % respectively (Table 6). The order of ABTS radical cation scavenging activity against *L. asperain* was found to be in the order sample A > sample C > sample B.

Conclusions

The present HPTLC-based qualitative investigation revealed the presence of triterpenes in *L. asperain*-house-mother tincture and commercial market samples. The present study revealed that as part of the preformulation study, the alcohol extract i.e. mother tincture of the whole plant of *L. asperain* showed promising physicochemical characteristics. The antioxidant potential of *L. asperain* mother tincture demonstrated the highest antioxidant activity found in in-house mother tincture sample as compared to market samples. It was concluded that the antioxidant activity of mother tinctures was directly proportional to the phenolic content too. The present physicochemical, HPTLC, and antioxidant study reveals the presence of major phytochemical compounds (including triterpenes ursolic acid and oleanolic acid) are responsible for its overall antioxidant activity.

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