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# Phytochemical profiling and antioxidant potential of *Ailanthus excelsa* Roxb. extracts

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The stem bark of *Ailanthus excels* Roxb., known as 'Aralu', is commonly used as a substitute in trade and raw drug market for several other important medicinal plants like *Oroxylum indicum* and *Holarrhena antidysenterica*. In the present study, its physicochemical parameters, preliminary phytochemical screening, HPTLC fingerprinting and assessment of its antioxidant potential were carried out. The different extracts were analysed for their total phenolic and flavonoid content and subjected to phytochemical analysis using HPTLC and the chemical markers lupeol and stigmasterol were quantified. Lupeol and stigmasterol were found to be present only in the methanolic extract (5.3 µg/mg and 8.1 µg/mg extract respectively). Results indicated that the 50% aqueous methanolic extract contained the highest content of phenolics and flavonoids. The methanolic extract exhibited the best antioxidant potential in the *in vitro* test models used viz., DPPH radical scavenging activity as well as the total antioxidant capacity. The analysis results may thus be used for the routine analysis of the raw drug samples and formulations for the presence of *A. excelsa*. The HPTLC fingerprint profiles are especially useful as they provide a fingerprint of the various phytoconstituents present in the crude drug and can be essentially used for quality control and assessment. They may also be used for confirming the presence of authentic plant material and monitoring the consistency of different batches of finished products where *A. excelsa* has been used as an ingredient.

**Keywords**: *Ailanthus excelsa*, Antioxidant, HPTLC, Phytochemicals

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*Ailanthus excels* Roxb. (Family: Simaroubaceae) is a large deciduous tree occurring in Bihar, Chhota Nagpur, Madhya Pradesh, Uttar Pradesh, forests of Ganjam, Vishakhapatnam and Deccan. It is commonly known as "Indian Tree of Heaven"[1]. In Ayurveda, it is referred to as 'Aralu' which consists of dried stem bark of *Ailanthus excels* Roxb. There are several reports where different parts of this tree have been used as a substitute for other well-known medicinal plants. In Rajasthan and Gujarat, the root and root bark of *Ailanthus excelsa* is commonly used as a substitute for *Oroxylum indicum* which is a constituent of the 'Brihat Panchamoola' group. The bark of *A. excelsa* is used as a substitute for *Holarrhena antidysenterica* which is used for the treatment of dysentery in Ayurveda. Besides this, the leaves are used as an adulterant for *Adhatoda zeylanica* leaves[1]. In the traditional medicine, different parts of this plant are used widely for the treatment of a variety of diseases[2-5]. It is used to cure wounds and skin eruptions as well as in bronchitis, asthma and in conditions of diarrhea and dysentery. The bark also finds use in Asian and Australian medicine where it is used to counteract worms, excessive vaginal discharge, malaria and asthma[6,7]. The Ayurvedic Pharmacopoeia of India indicates the use of stem bark in high fevers and giddiness[8]. It has also been found to possess antiplasmodial activity[9]. The main bioactive constituents isolated from this plant are the bitter quassinoids including ailanthone and excelsin and β-carboline alkaloids including canthin-6-one. Both ailanthone and canthin-6-one have been reported to be responsible for the anti-malarial activity observed for this plant.

This research work deals with the investigation of the stem bark of *A. excelsa* for its physicochemical parameters, preliminary phytochemical screening, HPTLC fingerprinting and assessment of its antioxidant potential. The stem bark of *A. excelsa* was further extracted with three different solvents and they were analysed for their content of total phenolics and flavonoids, antioxidant activity and subjected to phytochemical analysis using HPTLC for the presence of lupeol and stigmasterol.

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## Materials and Methods

### Chemicals and reagents

Reference standards, lupeol and stigmasterol and DPPH were obtained from Sigma-Aldrich. All other solvents and reagents were purchased from Merck Chemicals.

### Plant material

*Ailanthus excelsa* stem bark (Specimen number NBR/PH/227366) was collected from Lucknow, Uttar Pradesh (India). It was authenticated and, for future reference, a sample was deposited in the herbal drug museum of the Pharmacognosy Division, NBRI, Lucknow.

### Physicochemical evaluation and phytochemical screening

The dried and powdered stem bark of *A. excelsa* was evaluated for its physico-chemical parameters. Alcohol- and water-soluble extractives as well as total and acid-insoluble ash values were determined. The different extracts were also subjected to preliminary phytochemical screening. These were conducted as per the methods described in the API.

### Extraction and sample preparation

The dried stem bark of *A. excelsa* was coarsely ground and 20 g each extracted separately with methanol, 50% aqueous methanol and distilled water (50 mL x 3) at 25°C (room temperature) with occasional shaking for 24 h. The extracts were combined and concentrated in a rotatory evaporator (Buchi, Switzerland) under reduced pressure at 45°C. They were further lyophilized to yield dried methanolic, 50% aqueous methanolic and aqueous extracts. The percentage yields of the three extracts were methanolic – 1.78%, aqueous methanolic – 6.10% and aqueous – 7.85%. These extracts were used for HPTLC analysis, evaluation of *in vitro* antioxidant activities and estimation of total phenolic and total flavonoid contents.

### Antioxidant assays

#### *DPPH radical scavenging*

Blois method[10] was used to determine the DPPH radical-scavenging activity of the different extracts of the stem bark of *A. excelsa*. The decrease in the initial concentration of DPPH radicals by the different extracts was measured at the end of 10 min by using two different levels (100 and 200 µL) of test samples of 1 mg/mL concentration. The positive control used was a 0.1 mg/mL solution of Ascorbic acid.

#### *Total antioxidant capacity*

Total antioxidant capacity of the different extracts of the stem bark of *A. excels* was determined by the spectrophotometric method using quantification of the green phosphomolybdenum complex formed as described by Prieto *et al.*[11]. The positive control used was ascorbic acid at 1 mg/mL concentration.

#### *Total phenolic and flavonoid contents (TPC and TFC)*

The Folin-Ciocalteu method[12] was used to quantify the amount of phenolics present in the different extracts. Quantification was done against the calibration curve prepared by using different concentrations of gallic acid. Quantification of the flavonoid content was carried out by aluminium trichloride method using quercetin as reference compound[13].

#### *HPTLC analysis*

HPTLC fingerprint analysis of the methanolic, 50% aqueous methanolic as well as the aqueous extracts of the stem bark of *A. excelsa* was carried out on a Camag HPTLC system using win-CATs 4 software. The test samples were applied on pre-coated silica gel 60 F254 plates (20 x 10 cm, 0.2 mm thickness Merck, Darmstadt, Germany). Plates were developed to a distance of 9 cm in a twin trough glass chamber using Toluene: Ethyl acetate (8:2) as mobile phase. The plates were then dried and observed under UV 254 and 366 nm and HPTLC fingerprint profiles recorded.

Quantitative estimation of the compounds lupeol and stigmasterol in the extracts of *A. excelsa* was also carried out under similar conditions using 15 µL of the extracts of 10 mg/mL concentration along with standard solutions of known concentrations under similar conditions. Plates were developed to a distance of 9 cm using Toluene: Ethyl acetate (9:1) as mobile phase, sprayed with anisaldehydesulphuric acid reagent and heated at 105°C for 10 min till the bands were clearly visible. The Rf values and the spectra of lupeol and stigmasterol standards and corresponding bands in the extracts were compared in order to confirm their presence in the extracts.

## Results

### Physicochemical evaluation and phytochemical screening

Physicochemical evaluation of the stem bark of *A. excelsa* was carried out and the results are given in Table 1. The percentage of alcohol- and water-soluble

Table 1 — Physicochemical parameters of *A. excelsa* stem bark

| S. no. | Parameter | Value |
|---|---|---|
| 1 | Foreign matter | nil |
| 2 | Alcohol-soluble extractive value | 1.13% w/w |
| 3 | Water-soluble extractive value | 6.83% w/w |
| 4 | Total ash value | 7.60% w/w |
| 5 | Acid-insoluble ash value | 0.65% w/w |

Values are means of three determinations each

Table 2 — TPC, TFC, DPPH radical scavenging and total antioxidant capacity of different extracts of *A. excelsa* stem bark

| Sample | Total phenolic content* (mg GAE/g) | Total flavonoid content* (mg QE/g) | % DPPH radical scavenging activity | | Total Antioxidant Capacity by Phosphomolybdate Antioxidant Assay (mg AAE/g) |
|---|---|---|---|---|---|
| | | | 100 µL | 200 µL | |
| AEM | 14.21 | 2.95 | 35.36 | 50.45 | 80.0 |
| AEMW | 18.24 | 4.24 | 33.55 | 41.44 | 45.0 |
| AEW | 9.49 | 2.73 | 27.92 | 31.30 | 23.33 |

*Values are means of three determinations each

extractives was found to be 1.13% and 6.83% w/w dry plant material, respectively. On the basis of this, it can be said that the stem bark of *A. excelsa* contains a greater amount of polar constituents. The total and acid-insoluble ash values were determined to be 7.60% and 0.65% w/w dry plant material, respectively. The results of the preliminary phytochemical screening indicated the presence of alkaloids, sterols, triterpenoids, flavonoids and phenolics.

### *In vitro* antioxidant activity

#### *DPPH radical scavenging*

The results of the DPPH radical scavenging activity exhibited by the different extracts of the stem bark of *A. excels* are given in Table 2. It was observed that the methanolic extract exhibited the highest DPPH radical scavenging activity at both the levels tested (100 and 200 µL of test samples of 1 mg/mL concentration). When 200µl sample was used, it exhibited 50.45% DPPH radical scavenging activity, the highest amongst all the samples tested. The least DPPH radical scavenging activity was exhibited by the aqueous extract (27.92%, 100 µL test sample volume). The percentage activity was found to increase with increase in concentration of the test samples. For all samples, when 200 µL of test sample was used, it exhibited a higher DPPH radical scavenging activity than when 100 µL of test sample was used. Ascorbic acid, a well-known antioxidant, exhibited nearly 90% DPPH radical scavenging activity at the end of 10 min when 30 µL of its 1 mg/mL solution was used.

#### *Total antioxidant capacity*

The results of the total antioxidant capacity of the different extracts of the stem bark of *A. excels* are given in Table 2. Here again, it was observed that the methanolic extract exhibited the highest total antioxidant capacity (80.0 mg/AAE/g) while the least total antioxidant capacity was exhibited by the aqueous extract (23.3 mg AAE/g).

#### *Total phenolic and flavonoid contents*

The results of the estimation of total amount of phenolics and flavonoids present in the different extracts of the stem bark of *A. excels* are given in Table 2. The total phenolic content ranged between 9.49 to 18.24 mg GAE/g dry extract while the total flavonoids ranged between 2.73 to 4.24 mg QE/g dry extract. It was also observed that compared to the methanolic and the aqueous extracts, the 50% aqueous methanolic extract contained the highest amounts of phenolics (18.24 mg GAE/g) and flavonoids (4.24 QE/g).

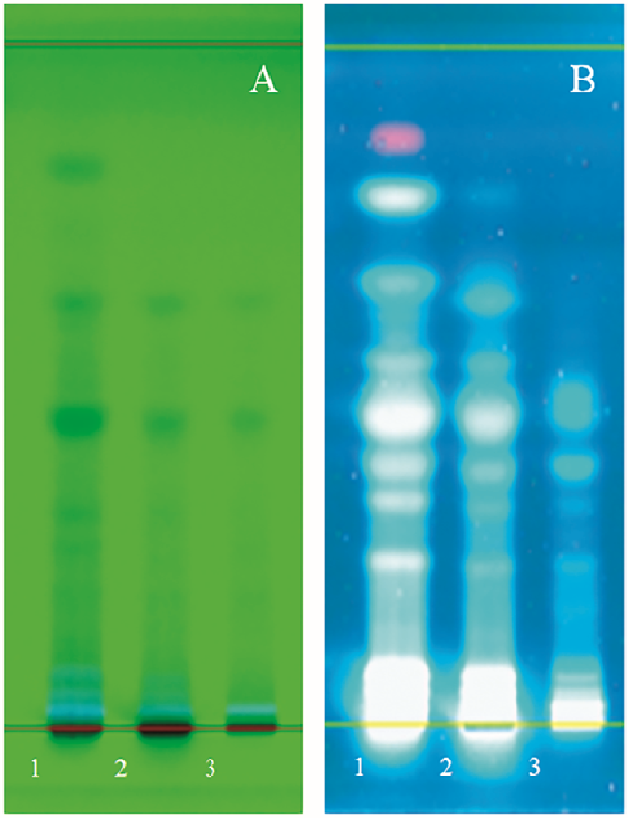


Fig. 1 —HPTLC fingerprint profiles of *A. excels* stem bark (Track 1-Methanolic extract, Track 2-50% Aqueous methanolic extract, Track 3-Aqueous extract; as seen under (A) λ 254, (B) λ 366)

#### *HPTLC analysis*

The different extracts of the stem bark of *A. excelsa* were subjected to HPTLC fingerprint profiling. The HPTLC plates were visualised under UV at 254 and 366 nm and Rf values and colour of the different bands present were recorded. The fingerprint profiles and the Rf values of the major bands seen (Fig. 1,

Table 3) indicate that certain compounds are common to all the three extracts.

Quantitative estimation of lupeol and stigmasterol was carried out under conditions described earlier. Figure 2 shows the HPTLC profiles of the different extracts along with the standard compounds lupeol and stigmasterol observed after derivatisation with anisaldehyde sulphuric acid reagent followed by heating at 105°C for 10 min till the bands were clearly visible. The plate was scanned densitometrically at λ540 nm and peak areas were recorded. Only the methanolic extract of the stem bark of *A. excels* was found to contain these two compounds, lupeol and stigmasterol, which were present at a concentration of 5.3 µg/mg and 8.1 µg/mg extract, respectively. The 50% aqueous methanolic as well as the aqueous extracts did not show the presence of lupeol and stigmasterol. Figure 3 shows the densitometric scan profiles of the standards along with that of the methanolic extract, 50% aqueous methanolic extract and aqueous extract.

## Discussion

The prevailing rise in the demand for herbal drugs is a major cause for their adulteration and substitution. Hence it is imperative to identify and define parameters for establishing the proper identity, purity and quality of the plant materials[14,15]. Considering these facts, in the present study, the physicochemical parameters, preliminary phytochemical screening, HPTLC fingerprinting and assessment of the antioxidant potential of the stem bark of *A. excelsa* were carried out. The different extracts were analysed for their total phenolic and flavonoid contents and subjected to phytochemical analysis using HPTLC and the chemical markers lupeol and stigmasterol were quantified. Results indicate that the 50% aqueous methanolic extract contained the highest content of both, phenolics and flavonoids, which are generally said to be responsible for the antioxidant

Table 3 —Rf values of bands in HPTLC fingerprint profiles of different extracts *A. excelsa* stem bark

| Band Rf | Methanolic extract | 50% Aqueous methanolic extract | Aqueous extract |
|---|---|---|---|
| At λ 254 nm | | | |
| 0.02 | √ | - | √ |
| 0.26 | √ | √ | - |
| 0.30 | √ | √ | - |
| 0.34 | √ | √ | - |
| 0.45 | √ | √ | √ |
| 0.61 | √ | √ | √ |
| 0.71 | √ | - | - |
| 0.81 | √ | - | - |
| At λ 366 nm | | | |
| 0.18 | - | - | √ |
| 0.23 | √ | √ | √ |
| 0.34 | √ | √ | - |
| 0.39 | √ | √ | √ |
| 0.45 | √ | √ | √ |
| 0.54 | √ | √ | - |
| 0.62 | - | √ | - |
| 0.65 | √ | - | - |
| 0.79 | √ | √ | - |
| 0.87 | √ | - | - |

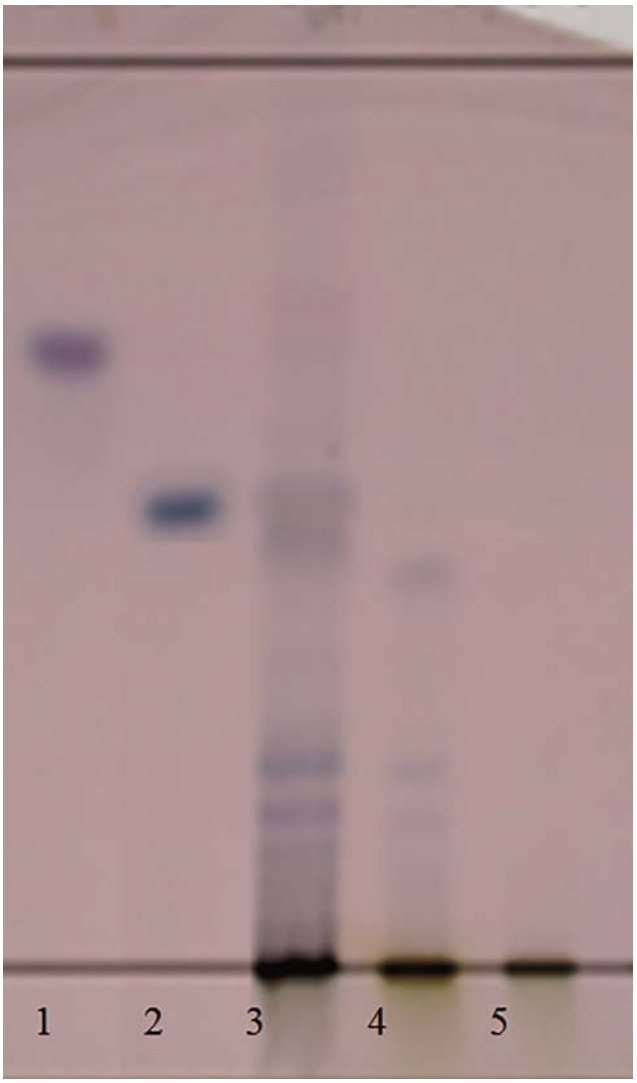


Fig. 2—HPTLC profiles of*A. Excels* stem bark using Lupeol and Stigmasterol as standards (Track 1- Lupeol, Track 2-Stigmasterol, Track 3- Methanolic extract, Track 4- 50% Aqueous methanolic extract, Track 5-Aqueous extract; as seen under visible light after derivatisation)

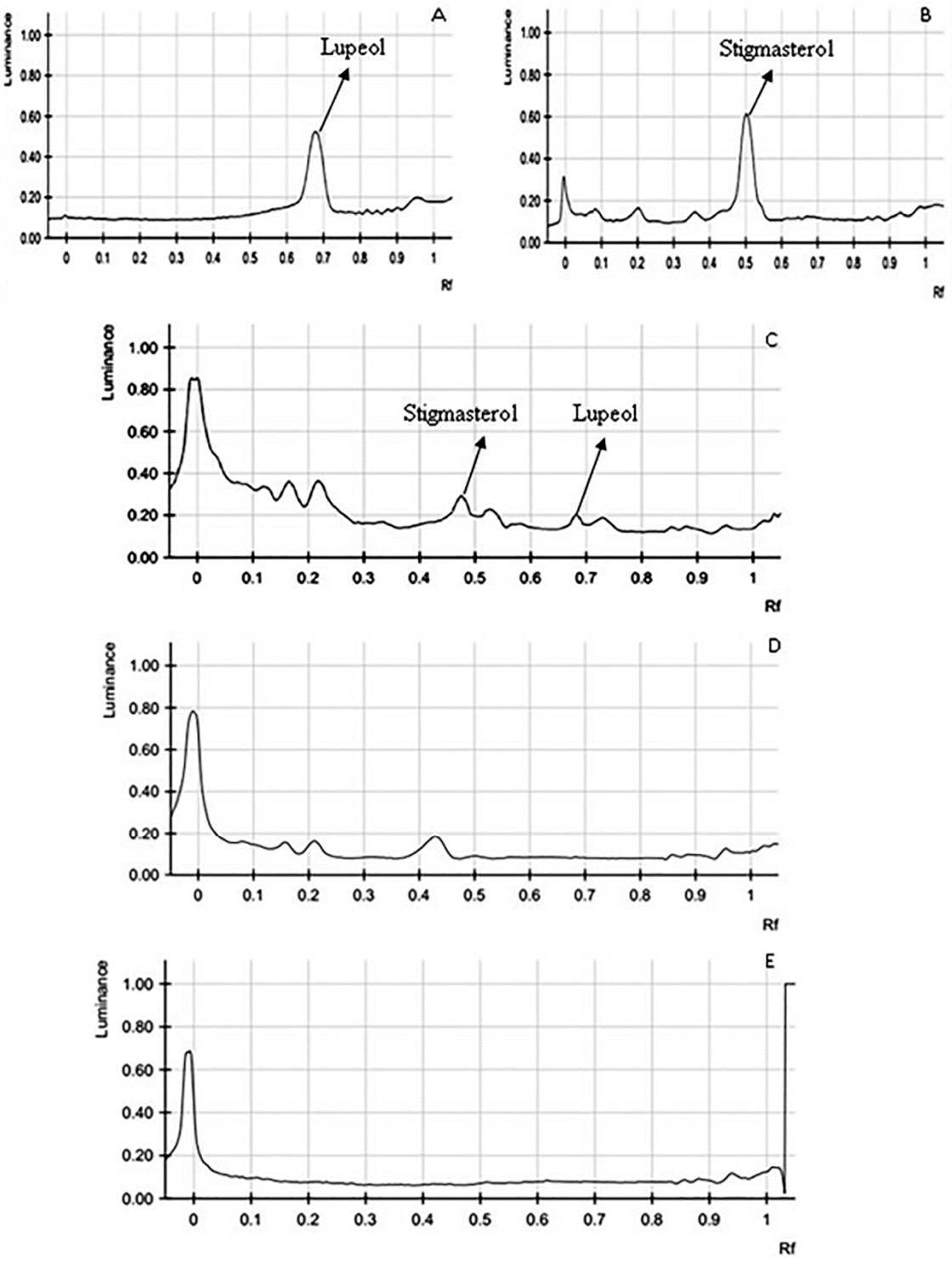


Fig. 3 — HPTLC densitometric scan profiles of the standards along with different extracts of *A. excelsa.* A-Lupeol, B-Stigmasterol, C-Methanolic extract, D-50% Aqueousmethanolic extract, A aqueious extract

activity exhibited by the medicinal plants. However in case of the stem bark of *A. excelsa*, it was the methanolic extract which exhibited the best antioxidant potential in both the *in vitro* test models used. Earlier studies have reported the presence of quassinoids and β-carboline alkaloids in *A. excelsa*.

Also, both the chemical markers, lupeol and stigmasterol, were detected only in the methanolic extract of the stem bark of *A. excelsa*. They are known to possess biological activities. Lupeol is a triterpenoid that has anti-inflammatory and anti-cancer properties while stigmasterol is a phytosterol with antiosteoarthritic and antihypercholesterolemic properties. Thus, it can be conferred that there are compounds other than phenolics and flavonoids which are responsible for the free radical scavenging activity of the stem bark of *A. excelsa*.

## Conclusion

In Ayurveda, the stem bark of *A. excelsa* has been recommended in high fevers and giddiness. Studies have also shown that it possesses anti-plasmodial activity. Hence, it is essential to establish quality control parameters for this important medicinal plant. The stem bark of *A. excelsa*, known as 'Aralu', is commonly used as a substitute for several other important medicinal plants like *Oroxylum indicum* and *Holarrhena antidysenterica* in the raw drug market. Thus, it is very important to develop parameters that will be helpful in identifying and differentiating the stem bark material of *A. excelsa* from other similar materials.

The present study, therefore, will be helpful in establishing the quality control parameters for this important medicinal plant. The results may be used for the routine analysis of the raw drug samples and formulations for determining the presence of *A. excelsa*. The HPTLC fingerprint profiles are especially useful as they provide a fingerprint of the various phytoconstituents present in the crude drug and can be essentially used for quality control and assessment. They may also be used for confirming the presence of authentic plant material and monitoring the consistency of different batches of finished products where *A. excelsa* has been used as an ingredient.

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## Conflict of Interest

The authors have no conflict of interest to declare.

## Author's Contribution

MMP and SR designed experiments. MMP performed experiments and compiled the data. SR processed the data and wrote the manuscript.

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