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Research Article

Qualitative and Quantitative Analysis of Primary and Secondary Metabolites from *Leptadenia pyrotechnica* and *Crataeva nurvala*

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Abstract



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Plants are an abundant source of bioactive compounds that are valuable for medicinal and therapeutic applications. The present study investigates the qualitative presence of primary and secondary metabolites in *Leptadenia pyrotechnica* and *Crataeva nurvala*, two plants widely used in traditional medicine. Phytochemical screening revealed the presence of primary metabolites, including carbohydrates, proteins, and lipids, as well as secondary metabolites, such as alkaloids, flavonoids, and phenolic compounds. Quantitative analysis highlighted variations in metabolite composition between plant parts. Notably, *Leptadenia pyrotechnica* aerial parts exhibited high protein and lipid content, while *Crataeva nurvala* leaf showed significant phenol concentration. Our research demonstrates that *Leptadenia pyrotechnica* and *Crataeva nurvala* possess a rich array of bioactive compounds, validating their traditional medicinal applications. The identified metabolites exhibit potential antioxidant, anti-inflammatory, and antimicrobial properties, necessitating further investigation to characterize and quantify these compounds. This study lays the groundwork for future pharmacological studies and underscores the significance of preserving and sustainably utilizing these plant species in natural medicine. The results have far-reaching implications for the development of innovative therapeutic agents and the promotion of complementary and alternative medicinal practices.

Keywords: *Leptadenia pyrotechnica*, *Crataeva nurvala*, alkaloids, flavonoids, phytochemicals

INTRODUCTION

Plants are recognized as rich sources of metabolites that contribute to their medicinal properties. These metabolites are broadly classified into primary and secondary categories. Primary metabolites, such as carbohydrates, proteins, and lipids, are involved in growth and metabolism. In contrast, secondary metabolites, which include alkaloids, flavonoids, tannins, and phenolics, are involved in defense mechanisms and provide various pharmacological benefits.¹

Leptadenia pyrotechnica (family: Apocynaceae) and *Crataeva nurvala* (family: Capparaceae) are medicinal plants known for their diverse pharmacological applications. *Leptadenia pyrotechnica* is a desert plant commonly used in traditional medicine to treat inflammatory diseases, wounds, and infections.² *Crataeva nurvala* is widely used in Ayurvedic medicine to treat urinary disorders, kidney diseases, and inflammation.³ Despite their traditional uses, there is limited scientific evidence regarding the phytochemical composition of these plants. This study aims to qualitatively analyze the primary and secondary

metabolites present in these species, providing a basis for understanding their medicinal potential.

2. MATERIALS AND METHODS

1. Plant Collection and Identification: The investigational plant matter i.e., *Leptadenia pyrotechnica* (aerial part and root part) and *Crataeva nurvala* (leaf part and fruit part) was used as experimental sample. *Leptadenia pyrotechnica* plant parts were collected from Udaipurwati, Jhunjhunu, Rajasthan India and *Crataeva nurvala* plant parts were collected from areas in front of Albert Hall, Jaipur. The obtained plant was further identified and confirmed and deposited the Herbarium sheet in Department of Botany in the University of Rajasthan.

2. Extract Preparation of the Plant Sample: The plant materials were thoroughly washed with distilled water, air-dried for two weeks in the shade, and then ground into a fine powder using a mechanical grinder. The powdered material was extracted using three solvents: methanol, ethanol, and distilled water, at a ratio of 10 grams of plant material to 100 mL of solvent. The mixtures were allowed to stand for 48 hours with

intermittent shaking, after which they were filtered using Whatman No. 1 filter paper. The filtrates were concentrated by a rotary evaporator, and the resulting extracts were stored at 4°C.

3. Qualitative Evaluation of Phytochemical Compounds

Standard phytochemical tests were employed to identify the presence of primary and secondary metabolites^{1,4}. The protocols by were followed:

Table 1: Qualitative Analysis of Primary and Secondary Metabolites in *Leptadenia pyrotechnica* and *Crataeva nurvalaa*

Phytochemicals	Test Name	Detection
Primary Metabolites		
1. Protein	Biuret's Test	Present
2. Carbohydrates	Molisch's Test	Present
Secondary Metabolites		
3. Flavonoids	Shinoda's Test	Present
4. Tannins	Ferric Chloride Test	Present
5. Phenolic Compounds	Ferric Chloride Test	Present
6. Alkaloids	Mayer's Test	Present

4. QUANTITATIVE EVALUATION

4.1. Protein Estimation

Protein content was estimated using method.⁵ A standard curve was prepared using bovine serum albumin (BSA). The total protein content (TPC) was calculated by measuring the optical density (OD) at 750 nm.

4.2. Carbohydrate Estimation

4.2.1. Total Soluble Sugars (TSS)

TSS was extracted using 80% CH₃OH and estimated using the phenol-H₂SO₄ acid method.⁶

4.2.2. Starch

Starch was extracted using 52% perchloric acid and estimated using the phenol-H₂SO₄ acid method.⁶

4.3. Lipid Estimation

Lipids were extracted using CHCl₃ and CH₃OH, and estimated gravimetrically.⁷

4.4. Phenol Estimation

Phenols were extracted using 80% CH₃OH and estimated using the Folin-Ciocalteu (FC) reagent method.⁵

4.5. Phytosterol Estimation

1. Plant sample preparation: Desiccated plant matter was defatted with petroleum ether (60-80°C, 24h) and hydrolyzed with 30% HCl (4h).

2. Thin-layer chromatography (TLC): The hydrolyzed sample was extracted with benzene and chromatographed on silica gel G plates with reliable sterols as indicators.

4.6. Alkaloid Estimation

1. Plant sample preparation: 5g of powdered plant matter was mixed with 100ml of distilled water and 5ml of 0.05N H₂SO₄. The mixture was deliquesced (3-4h) and bubbled mildly (25min).

2. Thin-layer chromatography (TLC): The extracted sample was mixed with distilled water and chromatographed on stimulated TLC plates with reliable samples of trigonelline.

5. RESULTS AND DISCUSSION

5.1 Secondary metabolite estimation

5.1.1 Phytosterol estimation

Phytosterol screening of *Leptadenia pyrotechnica* (root and aerial part) and *Crataeva nurvala* (leaf and fruit part) was carried out using thin layer chromatography (TLC). The extracts showed different phytosterol profiles (Table 1-4). TLC analysis revealed the presence of stigmasterol (Rf- 0.83), campesterol (Rf- 0.29), and β -sitosterol (Rf- 0.87) in the aerial part of *L. pyrotechnica*, root part of *L. pyrotechnica*, leaf part of *C. nurvala*, and fruit part of *C. nurvala*, respectively. Stigmasterol was used as a standard, showing an Rf value of 0.83.

Table 2: Chromatographic characteristics of Phytosterol compounds isolated from *Leptadenia pyrotechnica* aerial part

Isolated compounds	R _f value	Colour after spray with R
	S	
Stigmasterol	0.83	GY
Campesterol	0.29	GY

Abbreviations: S- Hexane: acetone (8: 2), R- 50% H₂SO₄, GY- Gray.

Table 3: Chromatographic characteristics of Phytosterol compounds isolated from *Leptadenia pyrotechnica* root

Isolated compounds	R _f value	Colour after spray with R
	S	
Campesterol	0.28	GY

Abbreviations: S- Hexane: acetone (8: 2), R- 50% H₂SO₄, GY- Gray.

Table 4: Chromatographic characteristics of Phytosterol compounds isolated from *Crataeva nurvala* leaf

Isolated compounds	R _f value	Colour after spray with R
	S	
β-sitosterol	0.87	PU-BN
Campesterol	0.28	GY

Abbreviations: S- Hexane: acetone (8: 2), R- 50% H₂SO₄, BN- Brown, PU- Purple, GY- Gray.

Table 5: Chromatographic characteristics of Phytosterol compounds isolated from *Crataeva nurvala* fruit

Isolated compounds	R _f value	Colour after spray with R
	S	
Stigmasterol	0.82	GY
β-sitosterol	0.88	PU-BN
Campesterol	0.29	GY

Abbreviations: S- Hexane: acetone (8: 2), R- 50% H₂SO₄, BN- Brown, PU- Purple, GY- Gray.

**Figure 1: Chromatographic characteristics of Phytosterol compounds isolated from (A) *L. pyrotechnica* aerial and root part (B) *C. nurvala* leaf and (C) *C. nurvala* fruit**

5.1.2. Alkaloid estimation

The alkaloids estimation was also performed using the TLC method for all the extracts prepared. As a result, obtained from TLC analysis trigonelline was identified in

the *L. pyrotechnica* root part extract and in both the leaf and fruit part extracts of *C. nurvala* with an R_f value of 0.093, 0.095 and 0.094. However, no alkaloid was identified in the *L. pyrotechnica* aerial part extract.

Table 6: Chromatographic characteristics of Alkaloid compounds isolated from *Leptadenia pyrotechnica* root and aerial part

Isolated compounds	R _f value	Colour after spray with R
	S	
Trigonelline	0.093	BK-RD

Abbreviations: S- Butanol: Acetone: Water (4:1:5), R- Dragendroff's reagent, BK- Brick, RD- Red

Table 7: Chromatographic characteristics of Alkaloid compounds isolated from *Crataeva nurvala* leaf

Isolated compounds	R _f value	Colour after spray with R
	S	
Trigonelline	0.095	BK-RD

Abbreviations: S- Butanol: Acetone: Water (4:1:5), R- Dragendroff's reagent, BK- Brick, RD- Red

Table 8: Chromatographic characteristics of Alkaloid compounds isolated from *Crataeva nurvala* fruit

Isolated compounds	R _f value	Colour after spray with R
	S	
Trigonelline	0.094	BK-RD

Abbreviations: S- Butanol: Acetone: Water (4:1:5), R- Dragendroff's reagent, BK- Brick, RD- Red

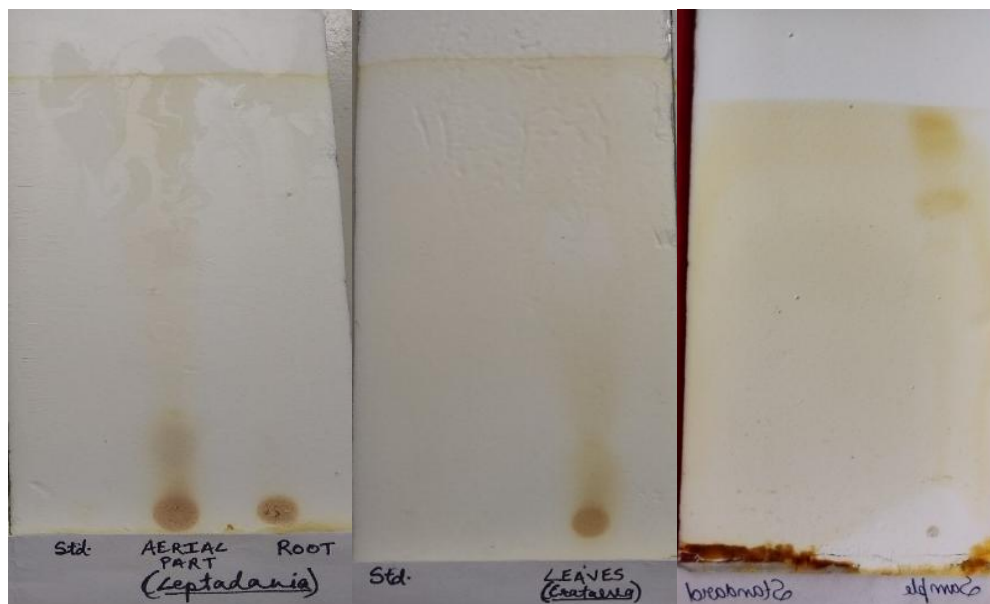


Figure 2: Chromatographic characteristics of alkaloid compounds isolated from (A) *L. pyrotechnica* aerial and root part (B) *C. nurvala* leaf and (C) *C. nurvala* fruit

5.2 Quantitative Evaluation

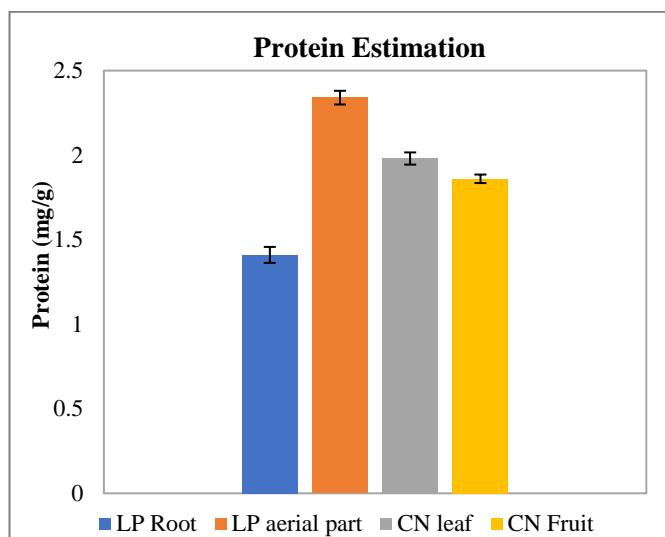
5.2.1. Protein Estimation

The protein content of *L. pyrotechnica* and *C. nurvala* plant part extracts were quantified using Lowry method. [8] The results showed that *L. pyrotechnica* aerial parts

had the highest protein concentration (2.34 mg/g), followed by *C. nurvala* leaf (1.98 mg/g), *C. nurvala* fruit (1.86 mg/g), and *L. pyrotechnica* root (1.41 mg/g) extracts as shown in Figure 3 and table 9.

Table 9: Protein estimation of different plant extract isolated from *Leptadenia pyrotechnica* and *Crataeva nurvala*

Samples	Protein (mg/g)
<i>Leptadenia pyrotechnica</i> Root	1.41±0.04
<i>Leptadenia pyrotechnica</i> aerial part	2.34±0.04
<i>Crataeva nurvala</i> leaf	1.98±0.03
<i>Crataeva nurvala</i> Fruit	1.86±0.02

**Figure 3:** Protein estimation of different plant extract isolated from *L. pyrotechnica* and *C. nurvala*

5.2.2. Carbohydrate Analysis

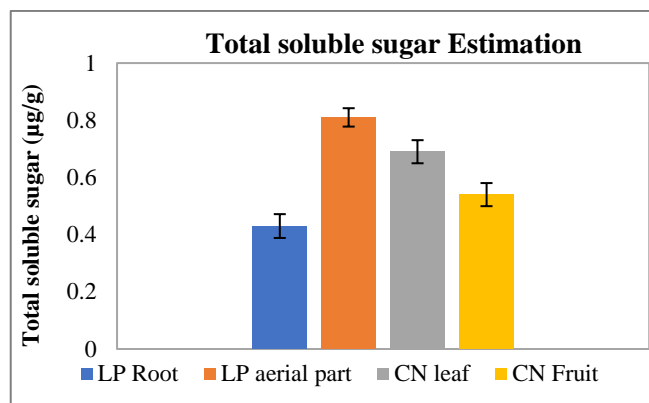
The carbohydrate content of *Leptadenia pyrotechnica* and *Crataeva nurvala* plant part extracts was determined using the phenol-H₂SO₄ acid technique.⁶

5.2.2.1. Total Soluble Sugars (TSS)

The TSS content of the extracts was found to be significantly higher in *L. pyrotechnica* aerial parts ($0.81 \pm 0.01 \mu\text{g/g}$) compared to *C. nurvala* leaf ($0.69 \pm 0.02 \mu\text{g/g}$), *C. nurvala* fruit ($0.54 \pm 0.03 \mu\text{g/g}$), and *L. pyrotechnica* root ($0.43 \pm 0.01 \mu\text{g/g}$) (Table 10, Figure 4).

Table 10: Total soluble sugar estimation of different plant extract isolated from *Leptadenia pyrotechnica* and *Crataeva nurvala*

Samples	Total soluble sugar ($\mu\text{g/g}$)
<i>Leptadenia pyrotechnica</i> Root	0.43±0.04
<i>Leptadenia pyrotechnica</i> aerial part	0.81±0.03
<i>Crataeva nurvala</i> leaf	0.69±0.04
<i>Crataeva nurvala</i> Fruit	0.54±0.04

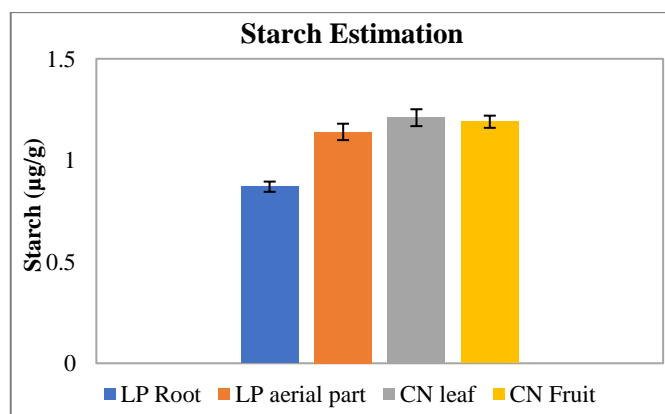
**Figure 4:** Total soluble sugar estimation of different plant extract isolated from *L. pyrotechnica* and *C. nurvala*

5.2.2.2. Starch

The starch content of the extracts was found to be significantly higher in *C. nurvala* leaf ($1.21 \pm 0.02 \mu\text{g/g}$) compared to *C. nurvala* fruit ($1.19 \pm 0.03 \mu\text{g/g}$), *L. pyrotechnica* aerial parts ($1.14 \pm 0.01 \mu\text{g/g}$), and *L. pyrotechnica* root ($0.87 \pm 0.02 \mu\text{g/g}$) (Table 11, Figure 5).

Table 11: Starch estimation of different plant extract isolated from *Leptadenia pyrotechnica* and *Crataeva nurvala*

Samples	Starch ($\mu\text{g/g}$)
<i>Leptadenia pyrotechnica</i> Root	0.87±0.02
<i>Leptadenia pyrotechnica</i> aerial part	1.14±0.04
<i>Crataeva nurvala</i> leaf	1.21±0.04
<i>Crataeva nurvala</i> Fruit	1.19±0.03

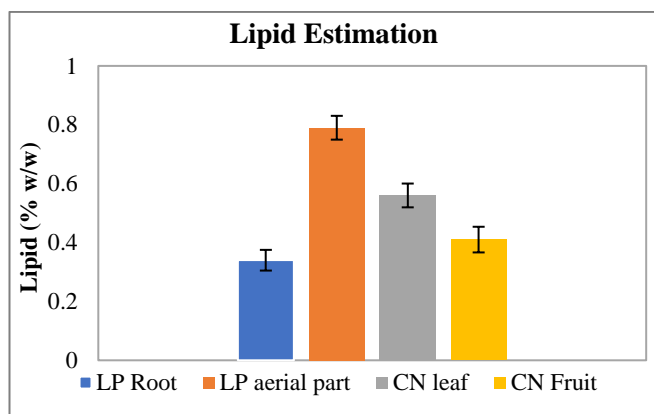
**Figure 5:** Starch estimation of different plant extract isolated from *L. pyrotechnica* and *C. nurvala*

5.2.3. Lipid

The lipid content of *Leptadenia pyrotechnica* and *Crataeva nurvala* plant part extracts was determined. The results showed that *L. pyrotechnica* aerial parts had the highest lipid concentration (0.79% w/w), followed by *C. nurvala* leaf (0.56% w/w), *C. nurvala* fruit (0.41% w/w), and *L. pyrotechnica* root (0.34% w/w) (Table 12, Figure 6).

Table 12: Lipid estimation of different plant extract isolated from *Leptadenia pyrotechnica* and *Crataeva nurvala*

Samples	Lipid (% w/w)
<i>Leptadenia pyrotechnica</i> Root	0.34±0.03
<i>Leptadenia pyrotechnica</i> aerial part	0.79±0.04
<i>Crataeva nurvala</i> leaf	0.56±0.04
<i>Crataeva nurvala</i> Fruit	0.41±0.04

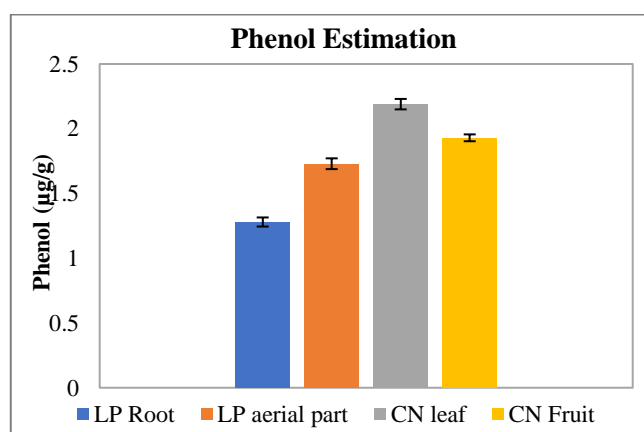
**Figure 6: Lipid estimation of different plant extract isolated from *L. pyrotechnica* and *C. nurvala***

5.2.4. Phenols

The total phenol content of the extracts was assessed using the Bray and Thorpe method.⁸ The results showed that *C. nurvala* leaf had the highest phenol concentration (2.19 µg/g), followed by *C. nurvala* fruit (1.93 µg/g), *L. pyrotechnica* aerial parts (1.73 µg/g), and *L. pyrotechnica* root (1.28 µg/g) (Table 13, Figure 7).

Table 13: Phenol estimation of different plant extract isolated from *Leptadenia pyrotechnica* and *Crataeva nurvala*

Samples	Phenol (µg/g)
<i>Leptadenia pyrotechnica</i> Root	1.28±0.03
<i>Leptadenia pyrotechnica</i> aerial part	1.73±0.04
<i>Crataeva nurvala</i> leaf	2.19±0.04
<i>Crataeva nurvala</i> Fruit	1.93±0.02

**Figure 7: Phenol estimation of different plant extract isolated from *L. pyrotechnica* and *C. nurvala***

6. CONCLUSIONS

The present study reveals that both *Leptadenia pyrotechnica* and *Crataeva nurvala* are rich sources of primary and secondary metabolites, contributing to their medicinal properties. Phytochemical screening confirmed the presence of phytosterols, alkaloids, tannins, and other bioactive compounds that are essential for potential therapeutic applications. Specifically, the detected metabolites suggest significant antioxidant, anti-inflammatory, and antimicrobial activities, which support the traditional uses of these plants in herbal medicine. These findings emphasize the importance of further studies to isolate and quantify these compounds, which could lead to the development of new pharmaceutical agents based on the identified bioactive metabolites. The results align with prior studies on these species, which noted similar phytochemical profiles and biological activities.⁹

Furthermore, this qualitative analysis provides foundational data for future research into these plants' pharmacological effects and supports the conservation and sustainable use of these botanicals in natural medicine. Continued exploration and characterization of bioactive compounds in traditional medicinal plants like *Leptadenia pyrotechnica* and *Crataeva nurvala* could prove invaluable for advancing complementary and alternative medicinal therapies.¹⁰

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