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Pharmacognostic Standardization and Quantitative Phytochemical Assessment of Euphorbia Fusiformis

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ABSTRACT:

Background: Euphorbia fusiformis Buch is a rare medicinal geophyte used traditionally for liver, skin, inflammatory, and gastrointestinal disorders, yet reproducible quality-control data remain limited. Objective: To establish physicochemical and solvent-specific phytochemical characteristics of authenticated E. fusiformis material and quantify total phenolic content (TPC) and total flavonoid content (TFC) in polarity-based fractions.

Methods: Authenticated material (voucher LH/PG/BOT-798) was shade-dried, milled through a No. 22 sieve, and extracted with solvents spanning non-polar to aqueous polarity. Pharmacognostic constants, solvent behaviour, and qualitative reactions for major metabolite classes were recorded. TPC was measured by the Folin-Ciocalteu method against gallic acid at 760 nm; TFC was estimated by the aluminium-chloride method against quercetin at 520 nm. Calibration data were re-fitted by ordinary least squares.

Results: Loss on drying, total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive, and water-soluble extractive were 11.06%, 6.98%, 0.70%, 6.10%, 9.20%, and 6.10% w/w, respectively. The gallic-acid and quercetin models were $A = 0.05609C + 0.01093$ ($R^2 = 0.9914$) and $A = 0.002010C + 0.000067$ ($R^2 = 0.9977$). The hydroalcoholic fraction showed the highest TPC (68.12 ± 2.23 mg GAE/g), whereas the intermediate-polarity fraction showed the highest TFC (45.49 ± 0.26 mg QE/g).

Conclusion: The results define a practical baseline for identity, purity, extraction behaviour, and phenolic/flavonoid standardization of E. fusiformis. Orthogonal chromatographic marker analysis is required before therapeutic or batch-release claims are made.

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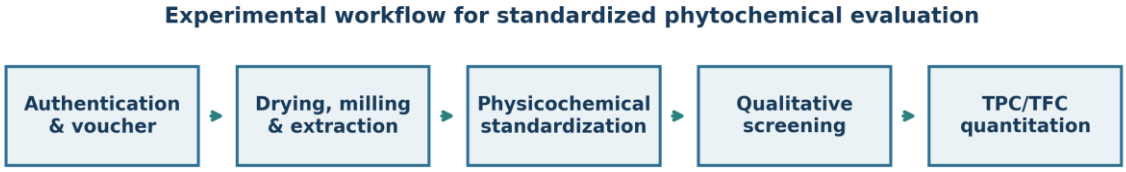

Figure 1. Study workflow used to connect authenticated plant material with quantitative phytochemical standardization.

1. INTRODUCTION

Medicinal-plant investigations are most informative when ethnomedical use is linked to authenticated botanical material, reproducible extraction, and quantitative chemical characterization. *Euphorbia fusiformis* Buch.-Ham. ex D.Don (Euphorbiaceae) is a rare tuberous herb reported from India and used in traditional practice for liver disorders, wounds, fever, rheumatic complaints, and intestinal disorders [1-4]. Traditional use provides a rationale for investigation but does not establish clinical efficacy, dose, or safety.

Experimental studies support several pharmacological hypotheses. Methanolic leaf and rootstock extracts have shown antibacterial activity, with rootstock extracts generally outperforming leaf extracts [2]. An ethanolic tuber extract produced hepatoprotective effects in rifampicin-challenged rats at 250 mg/kg and showed a wide safety margin in the reported toxicological programme [3]. Rhizome investigation has also yielded euphol, beta-sitosterol, caudicifolin, scoparone, and scopoletin; the ethyl-acetate extract displayed notable DPPH-scavenging and antifungal activity [4]. These findings collectively indicate that solvent polarity and plant part can materially alter the chemical and biological profile.

The present study therefore integrates pharmacognostic constants, qualitative phytochemical tests, and calibrated TPC/TFC assays. The objective was to establish a transparent baseline dataset for *E. fusiformis* fractions and identify the fraction types most suitable for subsequent chromatographic marker analysis and pharmacological testing.

2. MATERIALS AND METHODS

2.1 Plant collection and authentication

Euphorbia fusiformis was collected from the Nashik region of Maharashtra, India. A qualified botanist, Dr. S. B. Shisode, authenticated the material, and a voucher specimen was deposited at MGV's Loknete Vyankatrao Hiray Science College, Nashik (authentication no. LH/PG/BOT-798; 17 February 2024). The investigated material comprised the collected whole-plant sample, including the tuberous rootstock.

2.2 Processing and extraction

The material was cleaned, shade-dried, pulverized, and passed through a No. 22 sieve. A 250 g representative portion was extracted in a Soxhlet apparatus using petroleum ether and chloroform as low- and intermediate-polarity solvents, followed by ethanol/hydroalcoholic solvent. A separate aqueous extract was prepared under controlled conditions. Extracts were concentrated at reduced temperature, dried to constant mass, protected from light, and stored in airtight containers. Percentage yield was calculated as 100 × (mass of dried extract / mass of dry starting material).

2.3 Physicochemical and solubility evaluation

Loss on drying, total ash, acid-insoluble ash, water-soluble ash, and alcohol- and water-soluble extractive values were determined using standard pharmacognostic procedures [5,6]. The apparent solubility of the recovered constituents was recorded in water, ethanol, acetone, chloroform, and hexane. Measurements were performed in triplicate where applicable.

2.4 Qualitative phytochemical screening

Acetone, chloroform, water, and ethanol extracts were screened by established colour and precipitation reactions: Shinoda test (flavonoids), foam test (saponins), Borntrager's reaction (anthraquinone-type glycosides), Mayer's test (alkaloids), Benedict's test (reducing carbohydrates), and ferric-chloride reaction (tannins and phenols) [6,7]. Results were recorded as present or absent. These presumptive tests were used for inter-extract comparison rather than compound identification.

2.5 Total phenolic content

TPC was determined using the Folin-Ciocalteu assay [8]. Gallic acid standards (2-20 µg/mL) and extract test solutions were reacted with 10% Folin-Ciocalteu reagent, held for 5 min, treated with 6% sodium carbonate, and incubated in the dark for 90 min. Absorbance was measured at 760 nm against a reagent blank. Results were calculated from the least-squares calibration and expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g).

2.6 Total flavonoid content

TFC was determined by aluminium-chloride complexation using quercetin standards (2-20 µg/mL) [9]. Samples and standards were treated identically and absorbance was recorded at the documented assay maximum of 520 nm. Concentrations were interpolated from the calibration equation and normalized to milligrams of quercetin equivalents per gram of dry extract (mg QE/g).

2.7 Calculation and statistical treatment

For both assays, the standard curve was fitted as $A = mC + b$, where A is absorbance, C is standard-equivalent concentration (µg/mL), m is slope, and b is intercept. Extract content was calculated as cV/m_s, where c is the interpolated equivalent concentration, V is the final extract-solution volume, and m_s is the dry extract mass represented in that volume. Results are reported as mean ± dispersion stated in the supplied study dataset (n = 3). Because individual replicate values were unavailable for independent reanalysis, no new inferential significance testing was added.

3. RESULTS

3.1 Physicochemical characteristics

The physicochemical profile is summarized in Table 1. The alcohol-soluble extractive (9.20% w/w) exceeded the water-soluble extractive (6.10% w/w), suggesting greater recovery of soluble constituents by the alcoholic system. Total ash was 6.98% w/w, while the low acid-insoluble ash value (0.70% w/w) indicated limited siliceous contamination.

Table 1. Physicochemical quality-control parameters of E. fusiformis material.

Parameter	Observed value	Unit
Loss on drying	11.06	% w/w
Total ash	6.98	% w/w
Acid-insoluble ash	0.70	% w/w
Water-soluble ash	6.10	% w/w
Alcohol-soluble extractive	9.20	% w/w
Water-soluble extractive	6.10	% w/w
Extract pH	6.3 ± 0.8	-

3.2 Solvent behaviour and extraction yield

The powdered material/extract showed high compatibility with water, ethanol, and hexane and only sparing compatibility with acetone and chloroform under the recorded screening conditions (Table 2). The hydroalcoholic fraction produced the highest dried yield (17.8%), followed by the aqueous fraction (12.0%) (Table 3).

Table 2. Recorded solvent-compatibility profile.

Solvent	Solubility status
Water	Soluble
Ethanol	Soluble
Acetone	Sparingly soluble

Chloroform	Sparingly soluble
Hexane	Freely soluble

Table 3. Yield and organoleptic characteristics of polarity-based fractions.

Extract/fraction	Yield (%)	Colour	Consistency
Petroleum ether / non-polar	3.5	Yellowish-green to pale yellow	Gummy
Intermediate-polarity	2.9	Dark greenish-brown	Sticky paste
Methanolic/hydroalcoholic	17.8	Dark brown to greenish-black	Thick mass
Aqueous	12.0	Reddish-brown	Sticky mass

3.3 Qualitative phytochemical profile

Flavonoids and carbohydrates were detected in all four screening extracts. Saponins and tannins/phenols were observed in the aqueous and ethanol extracts, while alkaloids were detected only in the ethanol extract. Glycoside reactions were positive in acetone and ethanol extracts (Table 4).

Table 4. Comparative qualitative phytochemical screening of E. fusiformis extracts.

Phytochemical class	Test	Acetone	Chloroform	Water	Ethanol
Flavonoids	Shinoda	+	+	+	+
Saponins	Foam	-	-	+	+
Glycosides	Borntrager	+	-	-	+
Alkaloids	Mayer's	-	-	-	+
Carbohydrates	Benedict's	+	+	+	+
Tannins/phenols	Ferric chloride	-	-	+	+

Key: + = *detected;* - = *not detected under the stated test conditions.*

3.4 Calibration performance

The standard-series readings and recalculated regressions are shown in Table 5 and Figures 2-3. Both models were highly linear over 2-20 µg/mL. Re-fitting the recorded absorbances yielded R^2 values of 0.9914 for gallic acid and 0.9977 for quercetin.

Table 5. Standard calibration readings used for TPC and TFC quantitation.

Concentration (µg/mL)	Gallic acid absorbance (760 nm)	Quercetin absorbance (520 nm)
2	0.123	0.0042
4	0.243	0.0089
6	0.359	0.0123
8	0.458	0.0157
10	0.582	0.0201
12	0.695	0.0235
14	0.739	0.0274
16	0.898	0.0320
18	0.986	0.0364
20	1.196	0.0413

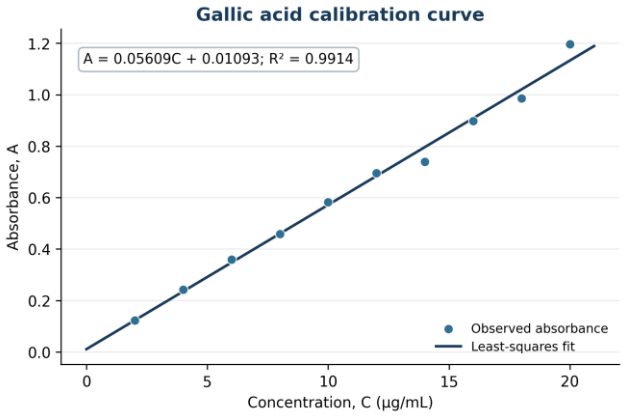

Figure 2. Gallic acid calibration for total phenolic content. Points are observed readings; line is the least-squares fit.

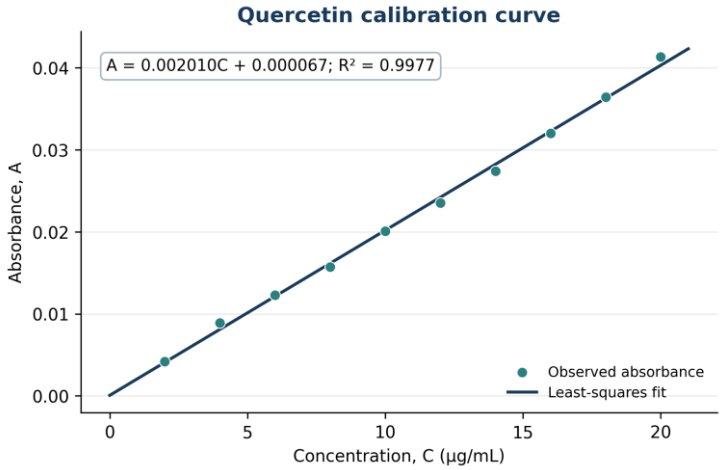

Figure 3. Quercetin calibration for total flavonoid content. Points are observed readings; line is the least-squares fit.

3.5 Total phenolic and flavonoid contents

TPC varied from 20.10 ± 0.63 to 68.12 ± 2.23 mg GAE/g dry extract, whereas TFC ranged from 12.84 ± 0.04 to 45.49 ± 0.26 mg QE/g dry extract (Table 6). The hydroalcoholic fraction had the greatest TPC, approximately 3.39-fold higher than the non-polar fraction. The intermediate-polarity fraction had the greatest TFC, approximately 3.54-fold higher than the non-polar fraction. Thus, phenolic enrichment and flavonoid enrichment did not peak in the same fraction.

Table 6. Total phenolic and flavonoid contents of E. fusiformis fractions.

Extract/fraction	TPC (mg GAE/g dry extract)	TFC (mg QE/g dry extract)
Non-polar	20.10 ± 0.63	12.84 ± 0.04
Intermediate-polarity	54.78 ± 0.97	45.49 ± 0.26
Hydroalcoholic	68.12 ± 2.23	30.06 ± 0.33
Aqueous	49.32 ± 0.62	20.72 ± 0.16

Values are reported as mean ± dispersion in the supplied study dataset (n = 3). GAE, gallic acid equivalents; QE, quercetin equivalents.

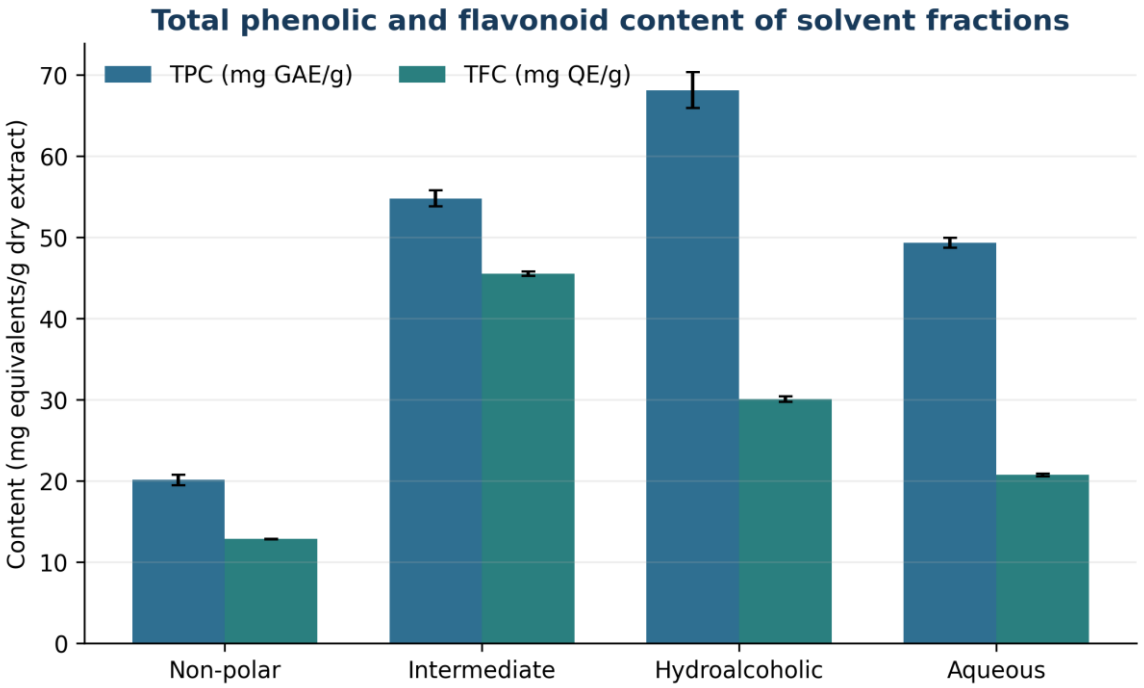

Figure 4. Comparative TPC and TFC of solvent fractions. Error bars reproduce the dispersion values supplied with the dataset.

4. DISCUSSION

Pharmacognostic constants provide a first-line assessment of herbal-drug identity and cleanliness. The observed total ash, extractive values, and loss on drying establish batch-specific reference points for the material studied here. The relatively low acid-insoluble ash is consistent with limited contamination by soil or silica, while the higher alcohol-soluble than water-soluble extractive suggests that an alcoholic system recovers a broader proportion of the soluble constituents. These parameters should nevertheless be treated as provisional internal specifications until several authenticated batches from different seasons and locations have been evaluated [5,6].

The qualitative profile demonstrates a pronounced solvent effect. Universal flavonoid and carbohydrate reactions contrast with the selective recovery of alkaloids in ethanol and of saponins and phenolics in aqueous/ethanolic systems. This pattern is chemically plausible because mixed polar solvents improve penetration of dried matrices and can recover both moderately polar aglycones and polar glycosides. A positive colour reaction remains presumptive, however, and cannot substitute for TLC/HPTLC or LC-MS confirmation [7].

The quantitative data extend the qualitative findings. Hydroalcoholic extraction maximized overall phenolic equivalents, whereas the intermediate-polarity fraction maximized flavonoid equivalents. This divergence indicates that the Folin-Ciocalteu and aluminium-chloride assays are reporting overlapping but non-identical chemical pools. It also argues against choosing a fraction solely on crude extraction yield: the hydroalcoholic fraction gave the highest yield and TPC, but the intermediate fraction had the strongest TFC enrichment. Future fraction selection should therefore align with the intended marker and biological endpoint.

Earlier work on *E. fusiformis* provides biological context. Methanolic extracts showed comparatively strong antibacterial activity [2], and an ethanolic tuber extract showed hepatoprotective activity in vivo [3]. Devendar et al. isolated euphol, beta-sitosterol, caudicifolin, scoparone, and scopoletin and identified the ethyl-acetate fraction as active in antioxidant and antifungal screening [4]. The present TPC/TFC pattern is compatible with solvent-dependent enrichment, but the class-level assays do not demonstrate that any specific constituent caused a therapeutic effect.

The calibration reanalysis also illustrates the importance of retaining primary readings. Ordinary least-squares fitting of the supplied absorbances produced complete slope, intercept, and R^2 estimates, enabling reproducible back-calculation. For

routine quality control, each run should additionally document blanks, replicate absorbances, dilution factors, residual plots, acceptance limits, and instrument identifiers.56. Conclusion

The study establishes a coherent pharmacognostic and quantitative phytochemical profile for authenticated *Euphorbia fusiformis* material. The hydroalcoholic fraction combined the highest extraction yield with the highest total phenolic content, while the intermediate-polarity fraction was richest in flavonoid equivalents. The calibrated TPC and TFC models, together with ash values, extractive values, and solvent-specific screening, provide practical starting specifications for future batches. Chromatographic marker confirmation and bioactivity testing of the same standardized fractions are the essential next steps.

5. DECLARATIONS

Acknowledgements

The authors thank Dr. S. B. Shisode for authentication of the plant material; Dr. D. S. Khandbahale, Department of Botany, K.T.H.M. College, Nashik, for botanical assistance; and the management and laboratory staff of the collaborating pharmacy institutions for facilities and technical support.

Ethics and plant material statement

No human participants or experimental animals were involved in the work reported in this manuscript. Plant collection, authentication, and voucher documentation were performed under institutional procedures (LH/PG/BOT-798; 17 February 2024).

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