

## Formulation and Evaluation of Herbal Capsules from *Mangifera Indica* and *Centella asiatica* Leaves Extract with Anti-Oxidant Activity

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### ABSTRACT

Background: Oxidative stress arising from excessive reactive oxygen and nitrogen species is implicated in the pathogenesis of several chronic disorders, which has renewed interest in plant-derived antioxidants as safer alternatives to synthetic agents. *Mangifera Indica* L. (mango) and *Centella asiatica* (L.) Urban (gotu kola) are two ethnomedicinally important species whose leaves are rich in phenolics, flavonoids and triterpenoids. Objective: This study was designed to extract, characterize and combine the hydroalcoholic leaf extracts of M. Indica and C. asiatica into a single hard-gelatin capsule and to evaluate the resulting formulation for phytochemical composition, antioxidant potential and pharmaceutical quality. Methods: Shade-dried leaf powders were extracted separately by hydroalcoholic (70% ethanol-water) Soxhlet extraction. The extracts were screened qualitatively for phytoconstituents and quantitatively for total phenolic content (TPC, Folin-Ciocalteu method) and total flavonoid content (TFC, aluminium chloride method). A combined powder blend was evaluated for micromeritic properties, filled into hard gelatin capsules, and the capsules were assessed for physical appearance, weight variation, moisture content, disintegration time, content uniformity, in-vitro dissolution, FTIR drug-excipient compatibility and DPPH radical-scavenging activity.

### Introduction

Plants have served as a principal source of therapeutic agents across traditional systems of medicine such as Ayurveda, Siddha and Unani, and continue to inform modern drug discovery because of the structural diversity of their secondary metabolites.[2] Herbal preparations - crude drugs, powders, extracts and finished dosage forms - differ fundamentally from single synthetic molecules in that they deliver a complex mixture of primary and secondary metabolites that may act individually or synergistically.[4] Growing consumer preference for naturally derived healthcare products has intensified research into plant constituents such as alkaloids, flavonoids, phenolics, tannins, terpenoids, steroids, glycosides and saponins, which together underlie antioxidant, anti-inflammatory, antimicrobial, antidiabetic, hepatoprotective, cardioprotective and neuroprotective activities reported for many species.[9]

A substantial proportion of this interest centres on antioxidant potential, since oxidative stress - the excessive generation of reactive oxygen and reactive nitrogen species relative to the body's antioxidant defences - damages cellular lipids, proteins and nucleic acids and is implicated in ageing and in the pathogenesis of cancer, diabetes, neurodegenerative and cardiovascular disease. Phenolic and flavonoid constituents, which donate hydrogen atoms or electrons and chelate pro-oxidant metal ions, are therefore widely investigated as sources of natural antioxidants capable of complementing or replacing

synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene, whose long-term safety remains debated.

#### *Mangifera Indica* L.

*Mangifera Indica* L. (family Anacardiaceae), commonly cultivated for its fruit, also possesses leaves that are traditionally used for their medicinal properties.[7,8] The leaves are a rich source of phenolic acids, flavonoids and, in particular, mangiferin, a xanthone C-glycoside (C<sub>19</sub>H<sub>18</sub>O<sub>11</sub>) widely regarded as the plant's principal bioactive marker.[5,6] Mangiferin and related polyphenols isolated from M. Indica have been reported to possess antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, antidiabetic, immunomodulatory and neuroprotective activity in various experimental models.[12,14,16,18,19,20,21,22] These pharmacological attributes, together with the abundance and low cost of mango leaves as an agricultural by-product,[3] make M. Indica leaf extract an attractive candidate for inclusion in a standardized antioxidant formulation.

#### *Centella asiatica* (L.) Urban

*Centella asiatica* (L.) Urban (family Apiaceae), known as gotu kola or Indian pennywort, is another herb with a long history of traditional use.[24] Its leaves contain characteristic triterpenoid glycosides - principally asiaticoside (C<sub>48</sub>H<sub>78</sub>O<sub>19</sub>), together with madecassoside, asiatic acid and madecassic acid - alongside flavonoids, phenolics and saponins.[25,26] These constituents have

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been associated with antioxidant, anti-inflammatory, wound-healing and antimicrobial activity, and asiaticoside is routinely used as the marker compound for standardising *Centella* preparations.

### Rationale and Objectives

Because *M. Indica* leaf extract is comparatively richer in phenolics and flavonoids while *C. asiatica* contributes triterpenoid and additional phenolic constituents, combining the two extracts was hypothesised to yield a complementary, potentially synergistic antioxidant profile suitable for a single-dosage-form herbal medicine. Hard gelatin capsules were selected as the delivery vehicle because they mask the taste and odour of crude plant extracts, permit accurate and convenient dosing, protect the contents from environmental exposure, and are well suited to the powder characteristics of dried herbal extracts.[5,8] Accordingly, the objectives of the present work were to (i) extract and characterise the hydroalcoholic leaf extracts of *M. Indica* and *C. asiatica*; (ii) quantify their phenolic and flavonoid content; (iii) formulate a combined polyherbal capsule; and (iv) evaluate the resulting formulation for pharmaceutical quality, drug-excipient compatibility and in-vitro antioxidant activity.

### Materials and Methods

#### Plant Material

Fresh, disease-free leaves of *Mangifera Indica* L. (Figure 1A) and *Centella asiatica* (L.) (Figure 1B) Urban were collected from Madurai District, Tamil Nadu, India, and authenticated.



Figure 1A. *Mangifera Indica*.



Figure 1B. *Centella asiatica*

### Chemicals, Excipients and Equipment

Analytical-grade ethanol, methanol, Folin-Ciocalteu reagent, sodium carbonate, aluminium chloride, potassium acetate, DPPH, ascorbic acid, gallic acid, quercetin, ferric chloride and the alkaloid detection reagents (Mayer's, Wagner's, Dragendorff's) were used as received. Microcrystalline cellulose, talc and magnesium stearate served as capsule excipients.[27] Extraction and evaluation were performed using a Soxhlet apparatus, rotary vacuum evaporator, hot air oven, UV-Visible and FTIR spectrophotometers, dissolution and USP disintegration test apparatus, and standard bulk/tap density equipment.

#### Extraction

Shade-dried, powdered leaves (500 g, sieve no. 40) of each plant were extracted separately by Soxhlet apparatus using a 70:30 ethanol-water mixture for 8-10 hours until the siphoning solvent ran colourless. Extracts were concentrated under reduced pressure (40-45 °C) and further dried below 45 °C, and percentage yield was calculated as (practical yield/theoretical yield) × 100.

#### Phytochemical Screening and Quantification

Dried extracts were screened qualitatively for alkaloids (Mayer's/Wagner's test), flavonoids (alkaline reagent test), phenolics and tannins (ferric chloride test), saponins (foam test), glycosides (Keller-Killiani test), terpenoids (Salkowski test), steroids (Liebermann-Burchard test) and carbohydrates/proteins (Molisch's/Biuret test). Total phenolic content was determined spectrophotometrically at 765 nm by the Folin-Ciocalteu method and expressed as mg gallic acid equivalent (GAE)/g extract; total flavonoid content was determined at 415 nm by the aluminium chloride method and expressed as mg quercetin equivalent (QE)/g extract.

#### Formulation and Evaluation of Capsules

The two extracts were combined with microcrystalline cellulose, talc and magnesium stearate to form a uniform powder blend, which was evaluated for angle of repose, bulk and tapped density, Carr's index and Hausner ratio prior to manual filling into size '0' hard gelatin capsules. The finished capsules were evaluated for physical appearance, weight variation (n = 20), moisture content, disintegration time (USP apparatus), content uniformity/assay (n = 6) and in-vitro dissolution profile. FTIR spectra of each extract and of the final blend were recorded to assess drug-excipient compatibility.

#### DPPH Radical Scavenging Assay

Antioxidant activity of both extracts was assessed using the DPPH free-radical scavenging assay over the concentration range 20-100 µg/mL, with ascorbic acid as reference standard, and IC<sub>50</sub> values were derived from the concentration-response curves.

### Results and Discussion

Percentage Yield

Table 1. Percentage yield of hydroalcoholic leaf extracts.

| Plant material           | Powder taken (g) | Dried extract obtained (g) | Percentage yield (%) |
|--------------------------|------------------|----------------------------|----------------------|
| Mangifera Indica leaves  | 500              | 68                         | 13.60                |
| Centella asiatica leaves | 500              | 55                         | 11.00                |

Hydroalcoholic Soxhlet extraction gave a higher yield for M. Indica (13.60%) than for C. asiatica (11.00%), a difference attributable to variation in leaf composition, moisture content, particle size and the relative proportion of polar to moderately polar constituents extractable by the 70% hydroalcoholic solvent system. Both yields were considered appreciable and adequate for subsequent phytochemical and formulation work.

Preliminary Phytochemical Screening



Figure 2. Representative preliminary phytochemical test reactions of the leaf extracts.

Table 2. Qualitative phytochemical screening of the leaf extracts.

| Phytoconstituent   | Test performed           | M. Indica | C. asiatica |
|--------------------|--------------------------|-----------|-------------|
| Alkaloids          | Mayer's/Wagner's test    | Present   | Present     |
| Flavonoids         | Alkaline reagent test    | Present   | Present     |
| Phenolic compounds | Ferric chloride test     | Present   | Present     |
| Tannins            | Ferric chloride test     | Present   | Present     |
| Saponins           | Foam test                | Present   | Present     |
| Glycosides         | Keller-Killiani test     | Present   | Present     |
| Terpenoids         | Salkowski test           | Present   | Present     |
| Steroids           | Liebermann-Burchard test | Present   | Present     |
| Carbohydrates      | Molisch's test           | Present   | Present     |
| Proteins           | Biuret test              | Absent    | Absent      |

Both extracts contained a broad range of secondary metabolites; flavonoids and phenolics were prominent in M. Indica, while phenolics and terpenoids predominated in C. asiatica, consistent with the reported phytochemistry of mangiferin- and asiaticoside-rich species and supportive of

antioxidant potential for both.[5,6,25,26] Absence of protein confirmed that the extraction predominantly concentrated secondary rather than primary metabolites.

Total Phenolic and Flavonoid Content

Table 3. Total phenolic content of the leaf extracts.

| Sample                   | Absorbance at 765 nm (mean $\pm$ SD, n=3) | TPC (mg GAE/g extract) |
|--------------------------|-------------------------------------------|------------------------|
| M. Indica leaf extract   | 0.812 $\pm$ 0.006                         | 168.42 $\pm$ 2.14      |
| C. asiatica leaf extract | 0.756 $\pm$ 0.005                         | 152.76 $\pm$ 1.87      |

Table 4. Total flavonoid content of the leaf extracts.

| Sample                   | Absorbance at 415 nm (mean $\pm$ SD, n=3) | TFC (mg QE/g extract) |
|--------------------------|-------------------------------------------|-----------------------|
| M. Indica leaf extract   | 0.694 $\pm$ 0.004                         | 94.62 $\pm$ 1.56      |
| C. asiatica leaf extract | 0.621 $\pm$ 0.003                         | 82.14 $\pm$ 1.32      |

M. Indica extract showed higher phenolic (168.42  $\pm$  2.14 mg GAE/g) and flavonoid (94.62  $\pm$  1.56 mg QE/g) content than C. asiatica (152.76  $\pm$  1.87 mg GAE/g; 82.14  $\pm$  1.32 mg QE/g), a finding consistent with the qualitative screening and with reports identifying mangiferin and related polyphenols as major contributors to the antioxidant capacity of M. Indica.[6,12,20] The appreciable phenolic content of C. asiatica corroborates its established antioxidant reputation and supports the rationale for combining the two extracts.

UV Spectral Analysis and Standard Curves

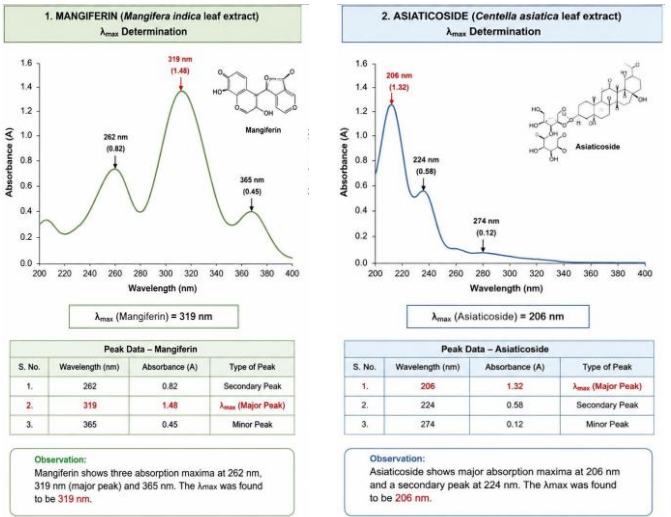


Figure 3. UV absorption maxima used for wavelength selection (mangiferin, left; asiaticoside, right).

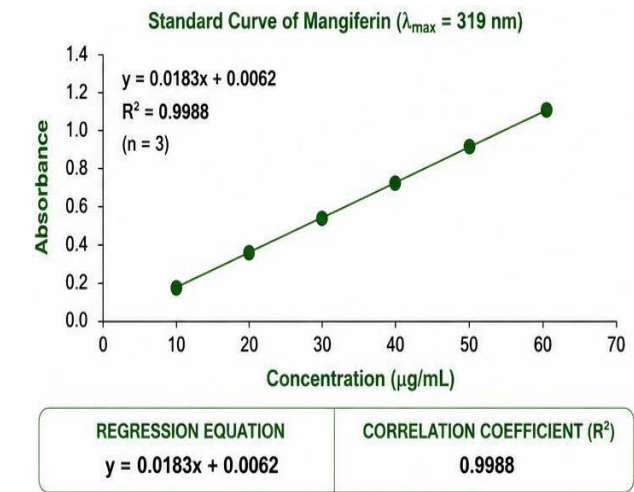


Figure 4. Standard calibration curve for mangiferin.

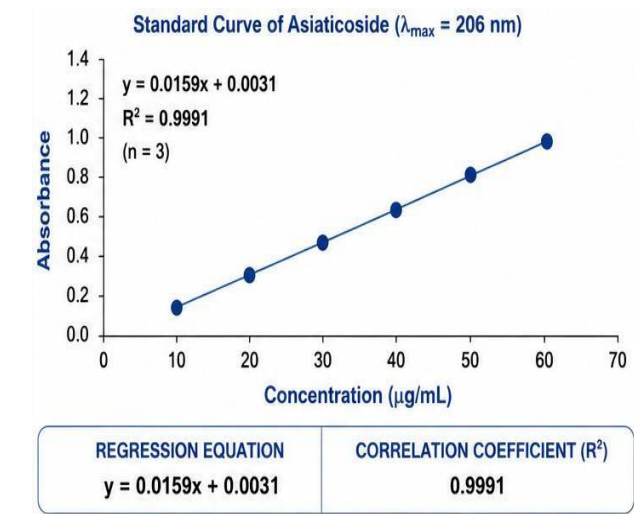


Figure 5. Standard calibration curve for asiaticoside.

Linear calibration curves for the marker compounds mangiferin and asiaticoside were established over the tested concentration range and used for subsequent quantitative estimations related to the formulation.

Powder Blend (Pre-compression) Evaluation

Table 5. Micromeritic properties of the combined powder blend.

| S.No. | Evaluation parameter             | Result | Interpretation |
|-------|----------------------------------|--------|----------------|
| 1     | Angle of repose ( $^{\circ}$ )   | 28.06  | Good flow      |
| 2     | Bulk density (g/mL)              | 0.450  | Satisfactory   |
| 3     | Tapped density (g/mL)            | 0.510  | Satisfactory   |
| 4     | Carr's compressibility index (%) | 11.76  | Good flow      |
| 5     | Hausner ratio                    | 1.13   | Good flow      |

The powder blend displayed satisfactory flow and packing behaviour across all measured parameters (angle of repose 28.06 $^{\circ}$ ; Carr's index 11.76%; Hausner ratio 1.13),

Indicating limited interparticle friction and cohesiveness and confirming its suitability for uniform capsule filling.

FTIR Drug-Excipient Compatibility

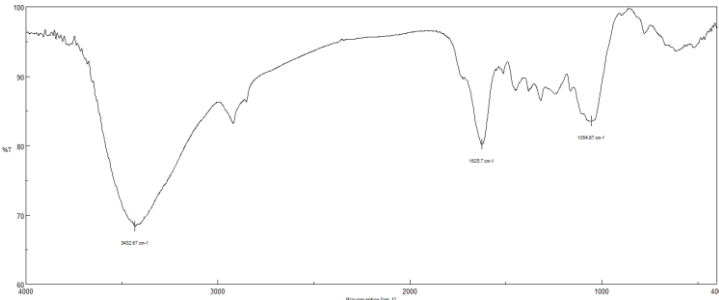


Figure 6. FTIR spectrum of Mangifera Indica leaf extract.

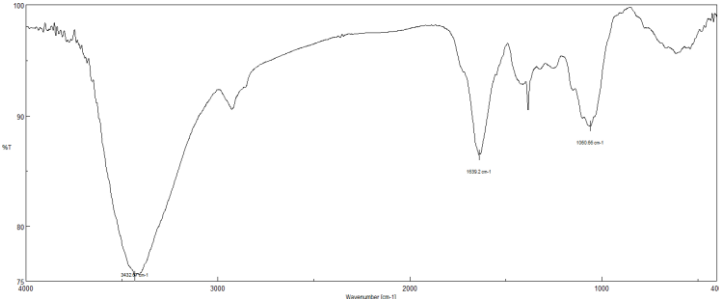


Figure 7. FTIR spectrum of Centella asiatica leaf extract.

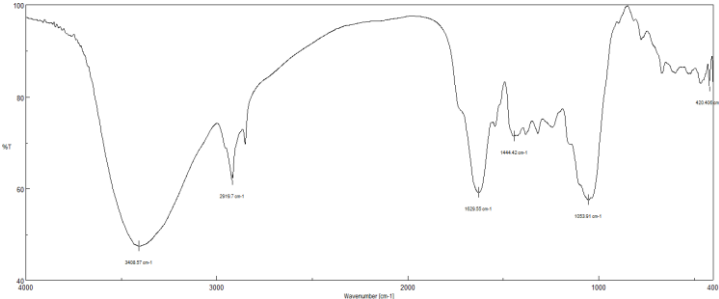


Figure 8. FTIR spectrum of the final herbal formulation.

Table 6. FTIR band assignments for the individual extracts and the final formulation.

| S.No. | Functional group                  | C. asiatica (cm-1) | M. Indica (cm-1) | Formulation (cm-1) |
|-------|-----------------------------------|--------------------|------------------|--------------------|
| 1     | O-H stretching                    | 3431.71            | 3431.71          | 3406.64            |
| 2     | Aliphatic C-H stretching          | 2927.41            | 2921.63          | 2920.66            |
| 3     | Aliphatic C-H stretching          | -                  | 2853.17          | 2852.20            |
| 4     | C=O / C=C stretching region       | 1638.23            | 1624.73          | 1629.55            |
| 5     | Aromatic skeletal / C=C vibration | -                  | 1513.85          | 1543.74            |
| 6     | CH2 bending                       | 1411.64            | 1448.28          | 1444.42            |
| 7     | CH3 bending                       | 1383.67            | 1380.78          | 1381.75            |
| 8     | C-O / O-H bending                 | 1321.96            | 1318.11          | 1319.07            |
| 9     | C-O stretching                    | 1254.47            | 1242.90          | 1243.86            |
| 10    | C-O stretching                    | 1061.62            | 1056.80          | 1053.91            |

The formulation retained the major characteristic absorption regions of both extracts - O-H stretching near 3400-3432 cm-1, aliphatic C-H stretching near 2920-2927

cm<sup>-1</sup>, and the C=C/carbonyl region near 1624-1638 cm<sup>-1</sup> - with only minor peak shifts. The absence of new peaks or loss of characteristic bands indicated no major spectral evidence of drug-excipient incompatibility, supporting the physicochemical compatibility of the two extracts with each other and with the selected excipients.

### Physical Characteristics of the Capsules

**Table 7. Physical characteristics of the finished herbal capsules.**

| Parameter            | Observation                         |
|----------------------|-------------------------------------|
| <b>Capsule shell</b> | Hard gelatin, size '0'              |
| <b>Colour</b>        | Opaque green                        |
| <b>Shape</b>         | Cylindrical with hemispherical ends |
| <b>Odour</b>         | Characteristic herbal odour         |
| <b>Surface</b>       | Smooth and uniform                  |

### Weight Variation, Moisture Content and Disintegration Time

Twenty individual capsules weighed between 597 and 604 mg (mean 600.2 mg), well within pharmacopoeial weight-variation limits, indicating uniform powder filling. Moisture content of the capsule contents was  $3.24 \pm 0.18\%$ , a level low enough to minimise the risk of microbial growth, powder caking or chemical degradation during storage. Disintegration time averaged 8 minutes 24 seconds, with all capsules disintegrating completely within the 30-minute pharmacopoeial limit, consistent with the porous, readily wettable nature of the dried herbal powder blend.

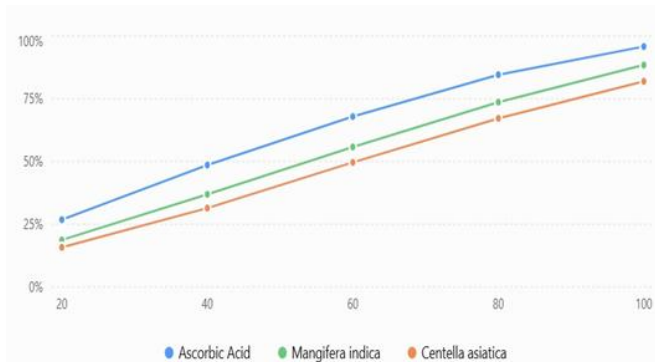
### Content Uniformity and In-vitro Dissolution

Assay of six capsules gave individual drug-content values of 98.8-100.4% (mean  $99.58 \pm 0.61\%$ ), demonstrating good content uniformity attributable to thorough mixing and consistent powder filling. In the dissolution study, extract release increased progressively with time, reaching 36.81% at 10 minutes, 71.54% at 20 minutes, 86.72% at 30 minutes and 95.18% at 45 minutes, indicating rapid wetting and efficient release of the phytoconstituents from the capsule and supporting the suitability of the formulation for oral administration.

### DPPH Radical Scavenging Activity

**Table 8. Concentration-dependent DPPH radical scavenging activity (mean  $\pm$  SD, n = 3).**

| Concentration ( $\mu\text{g/mL}$ ) | Ascorbic acid (%) | M. Indica (%)    | C. asiatica (%)  |
|------------------------------------|-------------------|------------------|------------------|
| 20                                 | $26.84 \pm 0.92$  | $18.73 \pm 0.65$ | $15.82 \pm 0.54$ |
| 40                                 | $48.62 \pm 1.15$  | $36.94 \pm 0.82$ | $31.45 \pm 0.76$ |
| 60                                 | $67.93 \pm 1.28$  | $55.82 \pm 1.04$ | $49.67 \pm 0.88$ |
| 80                                 | $84.61 \pm 1.43$  | $73.68 \pm 1.26$ | $67.21 \pm 1.11$ |
| 100                                | $95.84 \pm 1.51$  | $88.47 \pm 1.37$ | $81.96 \pm 1.24$ |



**Figure 9. DPPH radical scavenging activity of ascorbic acid, M. Indica and C. asiatica leaf extracts as a function of concentration.**

**Table 9. IC<sub>50</sub> values for DPPH radical scavenging activity.**

| Sample                          | IC <sub>50</sub> ( $\mu\text{g/mL}$ ) |
|---------------------------------|---------------------------------------|
| <b>Ascorbic acid</b>            | $41.32 \pm 0.84$                      |
| <b>M. Indica leaf extract</b>   | $55.48 \pm 1.16$                      |
| <b>C. asiatica leaf extract</b> | $61.87 \pm 1.28$                      |

Both extracts showed concentration-dependent free-radical scavenging, with M. Indica ( $\text{IC}_{50}$   $55.48 \pm 1.16$   $\mu\text{g/mL}$ ) consistently more active than C. asiatica ( $\text{IC}_{50}$   $61.87 \pm 1.28$   $\mu\text{g/mL}$ ), though both were less potent than ascorbic acid ( $\text{IC}_{50}$   $41.32 \pm 0.84$   $\mu\text{g/mL}$ ). This ranking parallels the TPC and TFC results and is consistent with reports attributing the antioxidant activity of M. Indica principally to mangiferin and related polyphenols,[12,20] and that of C. asiatica to its triterpenoid and phenolic constituents.[25,26] The complementary phytochemical profiles of the two extracts support the rationale for their combination in a single antioxidant formulation.

### Results

Percentage yields of 13.60% (M. Indica) and 11.00% (C. asiatica) were obtained. Both extracts were positive for alkaloids, flavonoids, phenolics, tannins, saponins, glycosides and terpenoids. M. Indica extract showed higher TPC ( $168.42 \pm 2.14$  mg GAE/g) and TFC ( $94.62 \pm 1.56$  mg QE/g) than C. asiatica ( $152.76 \pm 1.87$  mg GAE/g;  $82.14 \pm 1.32$  mg QE/g). The optimised powder blend showed good flow (angle of repose  $28.06^\circ$ , Carr's index 11.76%, Hausner ratio 1.13). The finished capsules complied with pharmacopoeial limits for weight variation (mean 600.2 mg), moisture content ( $3.24 \pm 0.18\%$ ) and disintegration time (mean 8 min 24 s), with content uniformity of  $99.58 \pm 0.61\%$  and 95.18% in-vitro drug release at 45 minutes. FTIR spectra showed no evidence of major drug-excipient incompatibility. Both extracts exhibited concentration-dependent DPPH scavenging, with  $\text{IC}_{50}$  values of  $55.48 \pm 1.16$   $\mu\text{g/mL}$  (M. Indica) and  $61.87 \pm 1.28$   $\mu\text{g/mL}$  (C. asiatica), against  $41.32 \pm 0.84$   $\mu\text{g/mL}$  for ascorbic acid.

## Conclusion

Hydroalcoholic leaf extracts of *Mangifera Indica* and *Centella asiatica*, rich in phenolic, flavonoid and terpenoid constituents, were successfully combined into a hard-gelatin polyherbal capsule. The formulation met pharmacopoeial requirements for weight variation, moisture content, disintegration time, content uniformity and in-vitro dissolution, and FTIR analysis showed no evidence of significant drug-excipient incompatibility. Both extracts exhibited concentration-dependent DPPH radical scavenging activity, with *M. Indica* showing comparatively stronger activity than *C. asiatica*. Collectively, these findings provide a scientific basis for the further development of this polyherbal combination as a standardized natural antioxidant capsule, and support future work on stability, in-vitro/in-vivo pharmacological evaluation, pharmacokinetics and safety to confirm its therapeutic potential.

The combined hydroalcoholic leaf extracts of *M. Indica* and *C. asiatica* can be successfully formulated into a pharmaceutically acceptable herbal capsule with promising in-vitro antioxidant activity, supporting further pharmacological and clinical evaluation of this polyherbal combination.

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