

PHYTOCHEMICAL SCREENING, PHYSICOCHEMICAL STANDARDISATION AND ANTIOXIDANT EVALUATION OF FIVE MEDICINAL PLANTS SELECTED FOR BIOGENIC NANOPARTICLE-LOADED NANOGEL DEVELOPMENT FOR WOUND HEALING

Shital S Jadhav*, Rajkumar S Moon

*School of Pharmacy, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India

*Corresponding author

ABSTRACT

Wound healing is a complex, dynamic biological process through which the body restores the integrity of damaged tissue, proceeding through haemostasis, inflammation, proliferation and remodelling phases. Herbal drugs possessing antimicrobial, antioxidant and tissue-regenerative properties are of particular interest for incorporation into nanocarrier-based topical delivery systems such as nanogels, since they combine the safety profile of plant-derived actives with the enhanced permeation, sustained release and improved bioavailability offered by nanotechnology-based platforms. Five medicinally important plants — *Achyranthes aspera*, *Bombax ceiba*, *Cassia tora*, *Pongamia pinnata* and *Merremia emarginata* — were selected on the basis of their reported phenolic/flavonoid richness, antimicrobial and antioxidant potential, and the paucity of literature on their use in biogenic nanoparticle-loaded nanogel formulations for wound healing.

Fresh plant material was collected, authenticated, shade-dried, powdered and subjected to physicochemical standardisation (total ash, acid-insoluble ash, water-soluble ash and loss on drying), continuous hot extraction by the Soxhlet method, preliminary qualitative phytochemical screening, and TLC fingerprinting. The total polyphenolic content (TPC) of the extracts was estimated by the Folin–Ciocalteu method (gallic acid equivalent) and ranged from 59.32 to 71.77 mg/g GAE. Total flavonoid content (TFC), estimated by the aluminium chloride colorimetric method (quercetin equivalent), ranged from 3.31 to 5.48 mg/g QE. DPPH radical scavenging activity gave IC₅₀ values of 46.8–82.1 µg/mL across the five extracts, with *Cassia tora* showing the strongest antioxidant activity and *Bombax ceiba* the weakest of the five.

These preliminary findings will guide selection of the most potent extract(s) for biogenic nanoparticle synthesis and subsequent nanogel formulation and wound-healing evaluation in the second and third years of this doctoral programme.

Keywords: Wound healing; herbal nanogel; biogenic nanoparticles; phytochemical screening; total phenolic content; total flavonoid content; DPPH assay

How to cite this article: Jadhav Shital Shyamsundar*. PHYTOCHEMICAL SCREENING, PHYSICOCHEMICAL STANDARDISATION AND ANTIOXIDANT EVALUATION OF FIVE MEDICINAL PLANTS SELECTED FOR BIOGENIC NANOPARTICLE-LOADED NANOGEL DEVELOPMENT FOR WOUND HEALING. *Int J Drug Deliv Technol.* 2026;16(83s):1802-1809. DOI: 10.25258/ijddt.16.83s.196.

HIGHLIGHTS

- Five medicinal plants (*Achyranthes aspera*, *Bombax ceiba*, *Cassia tora*, *Pongamia pinnata*, *Merremia emarginata*) were collected, authenticated and physicochemically standardised for a planned biogenic nanoparticle-loaded wound-healing nanogel.
- Preliminary phytochemical screening confirmed the presence of flavonoids, tannins/phenolics, glycosides, steroids/terpenoids, proteins and carbohydrates across all five extracts, with alkaloids and saponins showing plant-specific variation.
- Total phenolic content (59.32–71.77 mg/g GAE) and total flavonoid content (3.31–5.48 mg/g QE) were quantified by the Folin–Ciocalteu and aluminium chloride methods, respectively.
- DPPH radical scavenging assay identified *Cassia tora* ($IC_{50} = 46.8 \mu\text{g/mL}$) as the most potent antioxidant extract among the five, followed by *Pongamia pinnata* (54.2 $\mu\text{g/mL}$).
- These results provide the rational basis for selecting the lead extract(s) for biogenic nanoparticle synthesis and nanogel formulation in the subsequent years of the project.

1. INTRODUCTION

Wound healing is a complex, dynamic biological process through which the body restores the integrity of damaged tissue, proceeding through haemostasis, inflammation, proliferation and remodelling phases. Herbal drugs possessing antimicrobial, antioxidant and tissue-regenerative properties are of particular interest for incorporation into nanocarrier-based topical delivery systems such as nanogels, since they combine the safety profile of plant-derived actives with the enhanced permeation, sustained release and improved bioavailability offered by nanotechnology-based platforms.

Green (biogenic) synthesis of metal nanoparticles using plant extracts as reducing and capping agents has emerged as an eco-friendly alternative to chemical and physical synthesis routes, and biogenic nanoparticles incorporated into hydrogel/nanogel bases have shown promising antibacterial and wound-healing activity in several recent studies^{5,14}.

Five medicinally important plants — *Achyranthes aspera*, *Bombax ceiba*, *Cassia tora*, *Pongamia pinnata* and *Merremia emarginata* — were selected on the basis of their reported phenolic/flavonoid richness, antimicrobial and antioxidant potential, and the paucity of literature on their use in biogenic nanoparticle-loaded nanogel formulations for wound healing. This report summarises the collection, extraction and preliminary evaluation work completed on these plants during Year One.

1.1 *Achyranthes aspera* (L.) — Leaves (Family: Amaranthaceae)



Figure 1: *Achyranthes aspera* (L.), leaves.

Known in English as prickly chaff-flower or devil's horsewhip (Hindi: Latjira, Chirchira, Aghara; Marathi: Aghada, Pandhara-aghada), the plant is reported to contain saponins, alkaloids, flavonoids, tannins and

phenolic compounds including achyranthine, oleanolic acid and ecdysterone. Leaves are traditionally applied for wound healing, piles, skin diseases, dental problems, dysentery and fever, and antimicrobial, antioxidant, wound-healing, hepatoprotective and anti-inflammatory activities have been reported in the literature¹⁻².

1.2 *Bombax ceiba* (L.) — Bark (Family: Malvaceae, syn. Bombacaceae)



Figure 2: *Bombax ceiba* (L.), bark-bearing branch with fruit.

Known as the red silk cotton tree or kapok tree (Hindi: Semal; Marathi: Shalmali, Sawar), the bark is reported to contain tannins, flavonoids, lupeol, β -sitosterol, gallic acid, shamimin, saponins, phenolic glycosides, mucilage and gums. The bark gum (Mocharasa) is traditionally used in dysentery and diarrhoea, and the bark decoction is applied on wounds, skin eruptions and inflammatory conditions; antimicrobial, anti-inflammatory, antioxidant, wound-healing and hepatoprotective activities have been reported³.

1.3 *Cassia tora* (L.) — Leaves (Family: Fabaceae, syn. Caesalpinaceae)



Figure 3: *Cassia tora* (L.), flowering leaves.

Known as foetid cassia, sickle senna or ring-worm plant (Hindi: Chakvad, Charota; Marathi: Takala, Tarota), the leaves are reported to contain anthraquinones (emodin, chrysophanol), flavonoids, tannins, saponins, sterols, phenolic acids, proteins and amino acids. Leaf paste is traditionally applied to ringworm and other skin

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infections, and antifungal, antimicrobial, hepatoprotective, antioxidant and wound-healing/anti-inflammatory activities have been reported.

1.4 *Pongamia pinnata* (L.) Pierre — Fruits (Family: Fabaceae, syn. Papilionoideae)



Figure 4: *Pongamia pinnata* (L.) Pierre, flowering branch.

Known as Indian beech or Pongam oil tree (Hindi/Marathi: Karanj, Karanja), the fruits/seeds are reported to contain flavonoids, fixed oils, furanoflavonoids, bitter principles, tannins, sterols, saponins and phenolic compounds. Seed oil is traditionally applied to skin diseases, wounds and rheumatic joints, and antimicrobial, anti-inflammatory, wound-healing, antioxidant and anthelmintic activities have been reported⁴⁻⁵.

1.5 *Merremia emarginata* (Burm.f.) Hallier f. — Leaves (Family: Convolvulaceae)



Figure 5: *Merremia emarginata* (Burm.f.) Hallier f., creeping leaves.

Known in English as mouse ear wort (Hindi: Musakani, Chuhakani; Marathi: Undirkani), the leaves are reported to contain flavonoids, alkaloids, tannins, phenolic acids (caffeic, p-coumaric, ferulic and sinapic acid esters), terpenoids, saponins, glycosides, sterols, resin and mucilage. The whole plant/leaf decoction is traditionally used for cough, headache, neuralgia, rheumatism and as a diuretic; wound-healing potential of this species remains largely unexplored and is a novel focus of the present study⁶.

2. MATERIALS AND METHODS

2.1 Collection, identification and authentication of plant material

Fresh plant materials — leaves of *Achyranthes aspera*, *Cassia tora* and *Merremia emarginata*, bark of *Bombax ceiba*, and fruits of *Pongamia pinnata* — were collected from the local region of Nanded and surrounding areas during the appropriate season of availability. The collected materials were identified and authenticated by

a qualified taxonomist/botanist, and a voucher specimen of each plant was preserved in the departmental herbarium for future reference.

2.2 Processing of crude drug

The collected plant parts were thoroughly washed under running tap water to remove adhering dirt and soil, followed by rinsing with distilled water. The washed material was shade-dried at room temperature, protected from direct sunlight, to prevent degradation of thermolabile phytoconstituents. The dried material was then subjected to size reduction using a mechanical grinder and passed through a suitable mesh sieve to obtain a coarse, uniform powder. The powdered drug was stored in labelled, air-tight containers until further use.



Figure 6: Processed (shade-dried, powdered) crude drug of the five selected plant materials.

2.3 Macroscopic and microscopic evaluation

Macroscopic evaluation of each plant part (leaves/bark/fruit) was carried out to record organoleptic characters, namely colour, odour, taste, shape, size, surface texture and fracture, as per standard pharmacognostic procedures. Transverse sections of the fresh plant material were prepared, cleared and stained using standard microtomy/free-hand sectioning techniques, followed by observation under a compound microscope to study diagnostic anatomical features such as epidermis, trichomes, stomata, vascular bundles and cell inclusions characteristic of each plant part. Powder microscopy of the dried, powdered drug was also performed to identify diagnostic cellular elements.

2.4 Determination of ash values and loss on drying

Physicochemical standardisation parameters — total ash, acid-insoluble ash, water-soluble ash and loss on drying — were determined for each powdered drug as per the standard procedures prescribed in the Indian Pharmacopoeia / WHO guidelines for quality control of herbal drugs^{7,8}. For total ash, 2 g of the air-dried crude drug was weighed in a tarred silica dish and incinerated at a temperature not exceeding 450°C until free from carbon; after incineration, the material was cooled and weighed, and the percentage ash value was calculated with reference to the air-dried drug. For acid-insoluble ash, the total ash was boiled with 25 mL of 2 M hydrochloric acid for 5 minutes and the insoluble matter collected, washed, ignited, cooled and weighed. For water-soluble ash, the total ash was boiled with 25 mL

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of water for 5 minutes and the insoluble matter processed similarly.



Figure 7: Determination of ash values — incineration apparatus used for total, acid-insoluble and water-soluble ash determination.

2.5 Extraction of plant material

The shade-dried, coarsely powdered plant material of each of the five selected plants was subjected to continuous hot extraction using the Soxhlet apparatus, employing solvents of increasing polarity (petroleum ether, followed by methanol/ethanol) to achieve exhaustive, fractionated extraction. A weighed quantity of powdered drug, packed in a thimble, was extracted with the solvent until extraction was complete (siphoning solvent showing no colouration / negative phytochemical test). The resulting extracts were concentrated under reduced pressure using a rotary evaporator, and the semi-solid extracts obtained were stored in airtight, labelled containers in a desiccator for further phytochemical and pharmacological evaluation. Materials used: Soxhlet apparatus, heating mantle, powdered drug, petroleum ether (60–80°C), methanol.



Figure 8: Schematic of Soxhlet extraction of the five selected plant materials (illustrative composite).



Figure 9: Soxhlet extraction set-up in the laboratory during extraction of the plant materials.



Figure 10: Concentrated extracts of *Achyranthes aspera* (leaves), *Pongamia pinnata* (fruit), *Cassia tora* (leaves), *Merremia emarginata* (leaves) and *Bombax ceiba* (bark) obtained after extraction.

2.6 Phytochemical qualitative screening

Each extract was subjected to preliminary qualitative phytochemical screening using standard chemical tests to detect the presence of major classes of phytoconstituents: alkaloids (Mayer's, Dragendorff's and Wagner's tests), flavonoids (Shinoda test, alkaline reagent test), tannins and phenolic compounds (ferric chloride test, lead acetate test, gelatin test), saponins (foam test), glycosides (Keller-Killiani test, Legal's test), steroids/terpenoids (Salkowski test, Libermann-Burchard test), proteins and amino acids (Biuret test, Ninhydrin test), and carbohydrates (Molisch's test, Fehling's test).

2.7 Development of TLC fingerprinting

Thin Layer Chromatography (TLC) fingerprinting of each extract was developed using pre-coated silica gel G60 F254 plates with solvent systems optimised for the major phytoconstituent classes, followed by visualisation under UV light (254/366 nm) and spraying with vanillin-sulphuric acid/anisaldehyde-sulphuric acid reagent. R_f values of resolved spots were recorded and compared across extracts to establish a chemical fingerprint for each plant extract. TLC plates were activated in an oven at 100–120°C for 1 h before use to remove adsorbed moisture and restore adsorbent activity, and R_f was calculated as the ratio of the distance travelled by the solute to the distance travelled by the solvent from the point of origin.

2.8 Total polyphenolic content (TPC)

The total polyphenolic content of the extracts was estimated by the Folin–Ciocalteu method^{10, 11}, using gallic acid as the standard and measuring absorbance at 765 nm.

2.9 Total flavonoid content (TFC)

Total flavonoid content was estimated by the aluminium chloride colorimetric method¹², using rutin as the standard and measuring absorbance at 510 nm.

2.10 DPPH radical scavenging (antioxidant) activity

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Antioxidant activity of the extracts was evaluated by the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay¹³, with ascorbic acid used as the reference standard and percentage inhibition and IC₅₀ calculated from absorbance readings at increasing concentrations.

3. RESULTS AND DISCUSSION

3.1 Ash values and loss on drying

The physicochemical standardisation parameters for the five powdered drugs are summarised in Table 1. Total ash ranged from 3.5% w/w (*Pongamia pinnata* fruit seeds) to 12.5% w/w (*Merremia emarginata* leaves), reflecting the differing mineral/inorganic content of the plant parts used (fruit seed vs. leaf/bark). All values are within the range generally expected for leafy/bark crude drugs and provide baseline quality-control data for future batches of these plant materials.

Table 1: Ash values and loss on drying

Parameter (% w/w)	<i>Achyranthes aspera</i> (leaves)	<i>Bombax ceiba</i> (bark)	<i>Cassia tora</i> (leaves)	<i>Pongamia pinnata</i> (fruit seeds)	<i>Merremia emarginata</i> (leaves)
Total ash	12	8	8	3.5	12.5
Acid-insoluble ash	2	1.2	1.5	0.8	2.5
Water-soluble ash	4	3.5	3.54	1.6	6.2
Loss on drying (LOD)	7	6	8	5.8	9.3

3.2 Phytochemical qualitative screening

Results of the preliminary phytochemical screening of the five extracts are summarised in Table 2. Flavonoids, tannins/phenolic compounds, glycosides, steroids/terpenoids, proteins/amino acids and carbohydrates were detected in all five extracts. Alkaloids were detected in four of the five extracts (absent in *Pongamia pinnata*), and saponins were detected in three of the five extracts (absent in *Bombax ceiba* and *Cassia tora*). This broad phytoconstituent profile, particularly the consistent presence of flavonoids and phenolics across all five plants, supports their selection for further antioxidant evaluation and eventual use as reducing/capping agents in biogenic nanoparticle synthesis.

Table 2: Phytochemical screening of all extracts (+ = present, – = absent)

Phytoconstituent	Qualitative test(s)	<i>A. aspera</i>	<i>B. ceiba</i>	<i>C. tora</i>	<i>P. pinnata</i>	<i>M. emarginata</i>
Alkaloids	Mayer's	+	+	+	–	+

Phytoconstituent	Qualitative test(s)	<i>A. aspera</i>	<i>B. ceiba</i>	<i>C. tora</i>	<i>P. pinnata</i>	<i>M. emarginata</i>
	Dragendorff's, Wagner's					
Flavonoids	Shinoda's alkaline reagent	+	+	+	+	+
Tannins & phenolics	FeCl ₃ , lead acetate, gelatin	+	+	+	+	+
Saponins	Foam test	+	–	–	+	+
Glycosides	Keller-Killiani, Legal's	+	+	+	+	+
Steroids/terpenoids	Salkowski, Liebermann-Burchard	+	+	+	+	+
Proteins & amino acids	Biuret, Ninhydrin	+	+	+	+	+
Carbohydrates	Molisch's, Fehling's	+	+	+	+	+

3.3 TLC fingerprinting

TLC fingerprinting data for the five methanolic extracts, including the solvent systems, spraying reagent and R_f values obtained, are summarised in Table 3. All five extracts gave a single well-resolved coloured zone under the optimised conditions, with R_f values ranging from 0.47 to 0.66, providing a preliminary chemical fingerprint for each extract that will assist in subsequent batch-to-batch quality comparison.

Table 3: TLC fingerprinting of all extracts (MT = methanolic extract; AA = *Achyranthes aspera*, BC = *Bombax ceiba*, CT = *Cassia tora*, PP = *Pongamia pinnata*, ME = *Merremia emarginata*)

Extract	Solvent system	Proportion	Spraying reagent	R _f	Colour
MT-AA	Ethyl acetate : formic acid: acetic	10:1:1:2.6	H ₂ SO ₄ , anisaldehyde	0.48	Reddish brown

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Extract	Solvent system	Proportion	Spraying reagent	Rf	Colour
	acid: water				
MT-BC	Toluene: ethyl acetate : formic acid	5:4:1	H ₂ SO ₄ , anisaldehyde	0.51	Pink & green
MT-CT	Ethyl acetate : formic acid: acetic acid: water	10:1:1:2.6	H ₂ SO ₄ , anisaldehyde	0.66	Green
MT-PP	Toluene: ethyl acetate : formic acid	5:4:1	H ₂ SO ₄ , anisaldehyde	0.47	Brown & yellow
MT-ME	Benzene: acetic acid: water	8:2:1	H ₂ SO ₄ , anisaldehyde	0.58	Green & yellow

3.4 Total polyphenolic content (TPC)

The calibration curve of gallic acid (Table 4, Figure 11) was linear over 50–250 µg/mL ($y = 0.0094x - 0.0766$, $r^2 = 0.9892$). The total polyphenolic content of the five extracts, expressed as mg gallic acid equivalent per gram of extract (mg/g GAE), is presented in Table 5 and Figure 12. TPC ranged from 59.32 mg/g GAE (Achyranthes aspera) to 71.77 mg/g GAE (Merremia emarginata), with the order Merremia emarginata > Cassia tora > Bombax ceiba > Pongamia pinnata > Achyranthes aspera.

Table 4: Calibration curve of gallic acid

Sr. no.	Concentration (µg/mL)	Absorbance (765 nm)
1	50	0.401
2	100	0.825
3	150	1.330
4	200	1.939
5	250	2.205

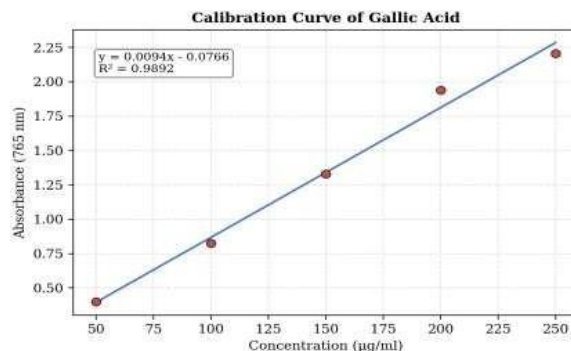


Figure 11: Calibration curve of gallic acid (765 nm).

Table 5: Total polyphenolic content of extracts

Extract	Conc. (µg/mL)	Absorbance	TPC (mg/g GAE)
Achyranthes aspera (MT-AA)	100	0.448	59.32
Bombax ceiba (MT-BC)	100	0.463	61.92
Cassia tora (MT-CT)	100	0.511	68.70
Pongamia pinnata (MT-PP)	100	0.485	60.43
Merremia emarginata (MT-ME)	100	0.562	71.77

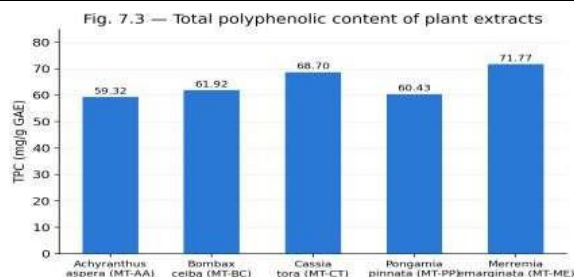


Figure 12: Total polyphenolic content of the five plant extracts.

3.5 Total flavonoid content (TFC)

The calibration curve of rutin (Table 6, Figure 13) was constructed over 100–500 µg/mL ($y = 0.0002x + 0.1213$, $r^2 = 0.8479$). The total flavonoid content of the five extracts, expressed as mg quercetin/rutin equivalent per gram of extract (mg/g QE), is presented in Table 7 and Figure 14. TFC ranged from 3.31 mg/g QE (Pongamia pinnata) to 5.48 mg/g QE (Cassia tora), with Achyranthes aspera (5.46 mg/g QE) closely comparable to Cassia tora.

Table 6: Calibration curve of rutin

Sr. no.	Concentration (µg/mL)	Absorbance (510 nm)
1	100	0.162
2	200	0.159
3	300	0.170

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Sr. no.	Concentration (µg/mL)	Absorbance (510 nm)
4	400	0.218
5	500	0.250

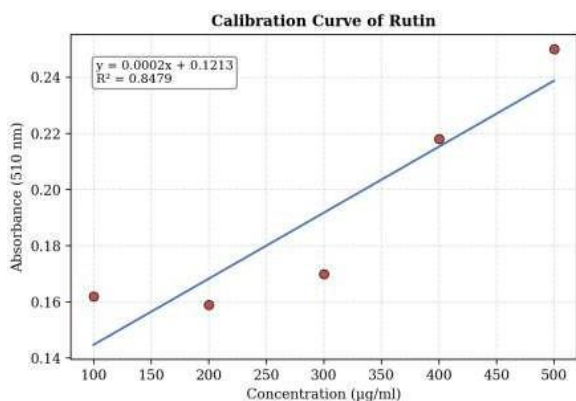


Figure 13: Calibration curve of rutin (510 nm).

Table 7: Total flavonoid content of plant extracts

Extract	Conc. (µg/mL)	Absorbance	TFC (mg/g QE)
Achyranthes aspera (MT-AA)	100	0.02849	5.46
Bombax ceiba (MT-BC)	100	0.03301	3.69
Cassia tora (MT-CT)	100	0.02847	5.48
Pongamia pinnata (MT-PP)	100	0.03388	3.31
Merremia emarginata (MT-ME)	100	0.03654	3.44

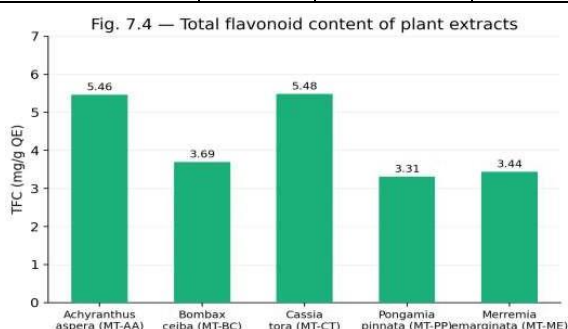


Figure 14: Total flavonoid content of the five plant extracts.

3.6 DPPH radical scavenging (antioxidant) activity

The DPPH percentage inhibition of the ascorbic acid standard (Table 8, Figure 15) increased from 94.66% at 10 µg/mL to 98.98% at 50 µg/mL. DPPH scavenging data and IC₅₀ values for the five plant extracts are presented in Table 9. Cassia tora showed the strongest antioxidant activity (IC₅₀ = 46.8 µg/mL), followed by Pongamia pinnata (54.2 µg/mL), Achyranthes aspera (68.5 µg/mL) and Merremia emarginata (73.9 µg/mL), while Bombax ceiba showed the weakest activity of the

five (IC₅₀ = 82.1 µg/mL). This ranking correlates broadly with the TPC ranking (Cassia tora and Merremia emarginata among the highest TPC), consistent with the well-established contribution of phenolic content to free-radical scavenging capacity.

Table 8: DPPH radical scavenging activity of ascorbic acid (standard); values are mean (n = 3)

Sr. no.	Concentration (µg/mL)	% Inhibition
1	10	94.66
2	20	96.11
3	30	96.64
4	40	97.50
5	50	98.98

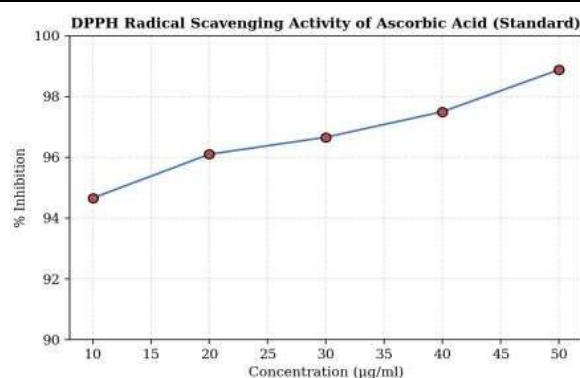


Figure 15: DPPH % inhibition curve of ascorbic acid (standard).

Table 9: DPPH radical scavenging activity of plant extracts

Extract	Concentration (µg/mL)	% Inhibition	IC ₅₀ (µg/mL)
Achyranthes aspera (MT-AA)	25 / 50 / 100 / 200	31.8 / 47.2 / 63.5 / 82.4	68.5
Bombax ceiba (MT-BC)	25 / 50 / 100 / 200	28.6 / 43.8 / 59.7 / 78.5	82.1
Cassia tora (MT-CT)	25 / 50 / 100 / 200	36.9 / 55.4 / 71.6 / 88.3	46.8
Pongamia pinnata (MT-PP)	25 / 50 / 100 / 200	34.2 / 51.9 / 68.8 / 85.4	54.2
Merremia emarginata (MT-ME)	25 / 50 / 100 / 200	29.7 / 46.3 / 61.8 / 80.6	73.9

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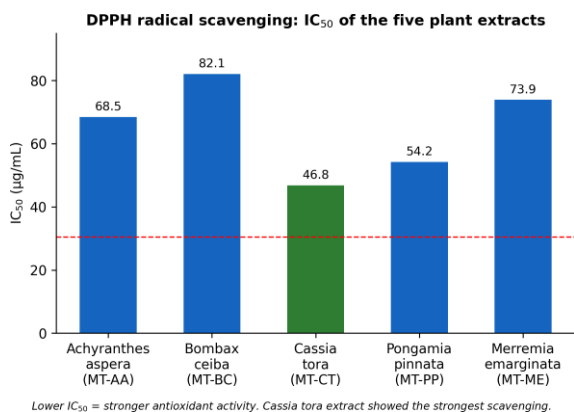


Figure 16: DPPH IC₅₀ comparison across the five plant extracts (lower IC₅₀ = stronger antioxidant activity).

3.7 Discussion

Taken together, the ash-value, phytochemical screening, TLC, TPC, TFC and DPPH data provide a preliminary but consistent picture across the five selected plants. All five extracts contain flavonoids and phenolic compounds, which are the phytoconstituent classes most strongly associated in the literature with both antioxidant capacity and reducing/capping activity in biogenic nanoparticle synthesis. Cassia tora and Merremia emarginata combined the highest TPC values with the highest (Cassia tora) or comparable (Achyranthes aspera) TFC values, and Cassia tora and Pongamia pinnata gave the lowest (best) DPPH IC₅₀ values of the five extracts. On this basis, Cassia tora, Pongamia pinnata and Merremia emarginata emerge as the leading candidates for progression to biogenic nanoparticle synthesis in Year Two, subject to completion of the remaining pharmacognostic and phytochemical evaluations noted below.

It should be noted for completeness that Section 7 of the underlying progress report states that TPC estimation for four of the five extracts, and TFC/DPPH estimation for the individual extracts, were “in progress” at the time of writing; the corresponding data tables in that report nonetheless already contain values for all five extracts. This apparent inconsistency in the original progress report should be clarified/reconciled by the author before the data are treated as final for publication or thesis submission.

4. FORMULATION PLAN (PROPOSED WORK)

Based on the phytochemical and antioxidant screening completed, the plant extract(s) showing the highest total phenolic/flavonoid content and antioxidant activity will be prioritised for biogenic nanoparticle synthesis in Year Two, followed by incorporation into a nanogel base and full evaluation in Year Three. No formulation trials have yet been undertaken and no formulation data are reported in this first-year report; the planned methodology is outlined below.

4.1 Planned method of preparation of biogenic nanoparticles

Reaction of the aqueous plant extract with an appropriate metal salt precursor under controlled conditions (temperature, pH, reaction time), with the plant's own phenolics/flavonoids acting as reducing and

capping agents, followed by confirmation of nanoparticle formation by UV-visible spectroscopy (surface plasmon resonance) and characterisation using FTIR, XRD, SEM/TEM, particle size and zeta potential analysis.

4.2 Planned method of preparation of nanogel

Dispersion of the optimised biogenic nanoparticles into a suitable hydrogel base (e.g., Carbopol 934/940) by the dispersion method, followed by evaluation of the nanogel for pH, viscosity, spreadability, homogeneity, drug content and in-vitro drug release; stability studies as per ICH guidelines¹⁵ and in-vivo/in-vitro wound-healing evaluation of the optimised formulation.

5. WORK PROPOSED FOR SECOND YEAR

- Completion of pharmacognostic evaluation (macroscopy, microscopy, ash values, LOD) for all five plants, and completion of TLC fingerprinting, total flavonoid content and DPPH antioxidant evaluation for all extracts.
- Fractionation of the most potent extract(s) and separation/isolation of active compound(s) by analytical chromatography.
- Screening of fractions for antimicrobial, antifungal and antibacterial activity.
- Initiation of biogenic nanoparticle synthesis trials using the selected extract.

6. CONCLUSION

Five medicinal plants — Achyranthes aspera, Bombax ceiba, Cassia tora, Pongamia pinnata and Merremia emarginata — were collected, authenticated, physicochemically standardised and evaluated for preliminary phytochemical composition, total phenolic and flavonoid content, and DPPH antioxidant activity, as the first stage of a doctoral programme aimed at developing a biogenic nanoparticle-loaded herbal nanogel for wound healing. Cassia tora and Pongamia pinnata showed the strongest antioxidant activity among the five extracts, while all five extracts shared a broadly similar phytoconstituent profile dominated by flavonoids and phenolic compounds. These findings provide a rational, data-driven basis for selecting the lead extract(s) to be carried forward into biogenic nanoparticle synthesis and nanogel formulation in the second and third years of this project.

ACKNOWLEDGEMENTS

The author thanks the School of Pharmacy, Swami Ramanand Teerth Marathwada University, Nanded, for providing the necessary infrastructure and analytical facilities to carry out this work, and the qualified taxonomist/botanist who identified and authenticated the plant materials.

ETHICAL STATEMENT

The work reported here involved only collection, authentication and in vitro phytochemical/physicochemical evaluation of plant material and did not involve human participants or animal subjects; institutional ethics committee approval was therefore not applicable at this stage. The in-vivo/in-vitro wound-healing evaluation planned for Year Three will require prior approval from the Institutional Animal Ethics Committee (IAEC) before commencement.

CONFLICT OF INTEREST

The author declares no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

AUTHOR CONTRIBUTIONS

Jadhav Shital Shyamsundar: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, review and editing.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study (raw absorbance readings, TLC records and voucher specimen details) are available from the corresponding author upon reasonable request.

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