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Extraction, Standardization, and Phytochemical Characterization of Bioactive Compounds from Emblica officinalis, Ocimum sanctum, and Tinospora cordifolia

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ABSTRACT

Background: Emblica officinalis (fruit), Ocimum sanctum (leaves), and Tinospora cordifolia (leaves) are well-known medicinal plants used in traditional systems of medicine. Scientific standardization and characterization of their bioactive constituents are essential to validate their therapeutic potential and to ensure the quality, safety, and consistency of plant-based formulations.

Methods: Authentic plant materials were collected and subjected to quality control and standardization tests, including determination of moisture content, foreign matter, total ash, acid-insoluble ash, and extractive values. Phytochemical screening was conducted to identify major classes of secondary metabolites. Chromatographic analyses using thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) were performed to profile phytoconstituents. Bioactive compounds were isolated through column chromatography and characterized using spectroscopic techniques such as UV–visible spectroscopy, infrared spectroscopy, nuclear magnetic resonance, and mass spectrometry.

Main Findings: Phytochemical analysis revealed the presence of flavonoids, alkaloids, terpenoids, steroids, tannins, and phenolic compounds in the plant extracts. TLC and HPTLC analyses showed multiple Rf values, indicating chemical diversity within the extracts. Column chromatography led to the isolation of rutin from Emblica officinalis, stigmasterol from Ocimum sanctum, and a flavonoid compound from Tinospora cordifolia. Spectroscopic data confirmed the structural identity of these bioactive constituents.

Conclusion: The study successfully standardized and characterized Emblica officinalis, Ocimum sanctum, and Tinospora cordifolia, confirming the presence of important bioactive phytoconstituents. The isolation and spectroscopic identification of key compounds support the medicinal relevance of these plants and provide a scientific basis for their use in herbal formulations and further pharmacological research.

KEYWORDS: Emblica officinalis (Amla), Ocimum sanctum (Tulsi), Tinospora cardifolia, spectral characterization.

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INTRODUCTION

The use of traditional medicines has increased markedly in recent years, with a substantial proportion of the population in developing countries relying on herbal remedies for their primary healthcare needs [1]. Medicinal plants offer several advantages, including a favorable safety profile, cost-effectiveness, and therapeutic efficacy, which have contributed to their widespread acceptance [2]. *Emblica officinalis* (amla) is a prominent medicinal plant extensively utilized in the Ayurvedic and Unani systems of medicine [3]. The fruits of amla are well recognized for their diverse pharmacological activities, particularly their antidiabetic potential [4]. Several studies have demonstrated that herbal agents possessing strong antioxidant properties can effectively neutralize free radicals, thereby preventing or reducing the severity of diabetic complications [5]. All parts of the amla plant are used medicinally; however, the fruit is especially valued in Ayurveda as a potent *rasayana*. Traditionally, it has been employed in the treatment of diarrhea, jaundice, and inflammatory conditions. In addition, the fruit pulp is applied topically to the scalp to relieve headaches and dizziness [6,7]. In Indian Materia Medica, *Ocimum sanctum* (Tulsi) leaf extracts are traditionally used in the treatment of bronchitis, rheumatism, and pyrexia. Other therapeutic applications include the management of epilepsy, asthma, dyspnea, hiccups, cough, skin and hematological disorders, parasitic infections, neuralgia, headache, wounds, inflammation, and oral diseases. The fresh leaf juice is commonly used as ear drops for earache, while tea infusions are employed in the treatment of gastric and hepatic disorders [8]. Phytochemical investigations have revealed that Tulsi is rich in bioactive constituents such as eugenol and ursolic acid, which significantly contribute to its therapeutic effects. However, its medicinal efficacy is attributed not to a single compound but to the synergistic interactions among multiple phytochemicals. Owing to its broad therapeutic potential, historical significance, and increasing validation through modern scientific research, Tulsi remains a promising subject for integrative medicine studies [9]. *Tinospora cordifolia* (TC) has long been used in traditional medicine for the management of general debility, dyspepsia, fever, urinary disorders, jaundice, skin diseases, and diabetes (*madhumeha*). Its antidiabetic properties are well documented in Ayurvedic Materia Medica under terms such as *pramehahara*, *pramehaghna*, *mehahara*, and *mehaghna* [10]. Although the precise mechanisms underlying its antidiabetic action are not fully elucidated, several bioactive constituents—including berberine, palmatine, jatrorrhizine, and mangnoflorine—have been reported to exhibit insulin-mimetic and insulin-

secretagogue activities. The antidiabetic efficacy of *T. cordifolia* is considered holistic, involving both intrapancreatic and extrapancreatic mechanisms of action [11]. Beyond its antidiabetic potential, *T. cordifolia* exhibits a wide range of pharmacological activities, including antiperiodic, antispasmodic, anti-inflammatory, anti-arthritic, antioxidant, anti-allergic, antistress, anti-leprotic, antimalarial, hepatoprotective, immunomodulatory, and antineoplastic effects. Its extensive therapeutic applications, genetic diversity, biologically active constituents, and demonstrated effects in both animal and human studies underscore its significance as a valuable medicinal plant and justify continued scientific exploration [12].

MATERIALS AND METHODS

2.1 Materials:

All the chemicals like Chloroform, Hydrochloric acid, Ethanol, Petroleum ether, Sulfuric acid, 5% ferric chloride, Toluene, Ethyl acetate, Acetic acid, Formic acid, etc. were supplied by Merck annd Cosmo Chem Pvt. Ltd. All the solvents are of analytical grade.

Plant Material: *Emblica officinalis* – Fruit, *Ocimum sanctum* – Leaves and *Tinospora cordifolia* –Leaves

2.2. Methods:

2.2.1. Collections and Drying

The specified plant parts—*Emblica officinalis* (fruit), *Ocimum sanctum* (leaves), and *Tinospora cordifolia* (leaves)—were collected from the Latur region of Maharashtra, India. The collected plant materials were thoroughly cleaned to remove extraneous matter and then shade-dried at ambient temperature, protected from direct sunlight to prevent degradation of thermolabile and photosensitive constituents.

After complete drying, the plant materials were coarsely powdered using a mechanical grinder. The powdered materials were subsequently sieved through a 120-mesh sieve to obtain uniform fine particles, while larger particles were discarded. The resulting fine powders were stored in airtight containers until further use.

2.3 Standardization of plants materials:

2.3.1 Determination of foreign organic matter

A thin layer of 5 g of air-dried, coarsely powdered drug was spread on a clean surface. The sample was examined using a 6 × magnifying lens or with the unaided eye. As thoroughly as possible, foreign organic matter was manually separated, the collected foreign matter was weighed, and the proportion of foreign organic material present in the sample was then calculated.[13]

2.3.2 Determination of moisture content

A glass-stoppered weighing bottle was used to weigh the 2 g sample properly. It was then spread out to a depth of ≤10 mm and dried in an oven until it reached a constant weight. The loss on drying (% w/w) was figured out by taking the weight difference before and after drying and putting it in a desiccator to cool.[14]

2.3.3 Ash value

Ash values are determined by three methods: total ash, acid-insoluble ash, and water-soluble ash. Total ash measures all inorganic residue after burning, including physiological ash (from plant tissue) and non-physiological ash (external matter). Acid-insoluble ash, obtained after treating total ash with dilute HCl and burning, indicates silica content (e.g., sand, soil). Water-soluble ash is the difference between total ash and the residue insoluble in water [15]

2.3.4 Determination of Total ash

An accurately weighed 2 g sample of the air-dried crude drug was placed in a tared silica dish and incinerated at a temperature not exceeding 450 °C until it was completely free from carbon. The residue was allowed to cool, and its weight was recorded. The percentage of ash was calculated with reference to the air-dried powder [16].

2.3.5 Determination of Water- soluble ash

The sample was boiled with 25 ml water for 5 minutes, filtered, and the insoluble residue collected on an ashless filter paper. After rinsing with hot water, it was ignited at ≤450°C for 15 minutes, weighed, and the ash obtained. The difference between the insoluble residue and ash weight gave the water-soluble ash, expressed as a percentage of the air-dried sample [17].

2.3.6 Determination of Acid -insoluble ash

The ash obtained as described above was boiled with 25 mL of 2 M hydrochloric acid for five minutes, then filtered to collect the acid-insoluble matter on an ashless filter paper. The residue was washed with hot water, ignited, cooled in a desiccator, and weighed. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug [18].

2.3.7 Extractive values

Standard technique was used to conduct various extractive values including alcohol soluble extractive, water soluble extractive values [19].

2.3.8 Determination of water-soluble extractive value

1.5 g of air-dried powdered drug was macerated with 100 ml chloroform water in a closed flask for 24 hours, with agitation during the first 6 hours. After filtration, 25 ml of filtrate was evaporated, dried at 105°C, and weighed. The water-soluble extractive value (%) was calculated using the air-dried drug weight as reference [20].

2.3.9 Determination of Alcohol-soluble extractive value

1.5 g of air-dried powdered drug was macerated with 100 ml of ethanol for 24 hours, with frequent shaking during the first 6 hours. After careful filtration, 25 ml of filtrate was evaporated, dried at 105°C, and weighed. The ethanol-soluble extractive value (%) was calculated relative to the air-dried powder [21].

2.4. Extraction

The extraction was carried out sequentially using petroleum ether (60–80 °C), ethanol, and water in increasing order of polarity. Soxhlet extraction was performed in multiple batches to ensure complete extraction with each solvent. Completion of extraction was confirmed by the absence of colored spots on thin-layer chromatography (TLC) plates after exposure to iodine vapor. After extraction, the solvent was removed by distillation under reduced pressure where necessary, and the concentrated residues were air-dried. The dried extracts were stored in airtight containers to preserve their quality. Following petroleum ether extraction, the remaining plant material (marc) was oven-dried to remove residual solvent prior to ethanol extraction. Finally, the dried marc was refluxed with distilled water for three hours to obtain the aqueous extract [22]

2.4.1 Phytochemical Test

The phytochemical tests are given in table 1. [23]

Table 1: Phytochemical Tests

Sr. No	Phytochemical Test	Procedure	Observation
1	**Test for Flavonoids**	In a test tube, add 1–2 mL of the plant extract. add a few drops of concentrated sulfuric acid (H_2SO_4). Observe any color change that occurs.	Yellow/orange color
2	**Test for Terpenoids**	In a test tube, add 1–2 mL of the plant extract. Add 2 mL of chloroform to the extract. Carefully, along the walls of the test tube, add 1–2 mL of concentrated sulfuric acid (H_2SO_4) to form a separate layer. Observe the interface for any color change.	reddish-brown layer
3	**Test for alkaloids**	In a test tube, add 1–2 mL of the plant extract. Add 1 mL of Dragendorff's reagent to the extract. Observe the formation of any precipitate.	orange/reddish-brown precipitate
4	**Test for steroids**	In a test tube, add 1–2 mL of the plant extract. Add 2 mL of chloroform to the extract. Carefully add 2 mL of concentrated sulfuric acid (H_2SO_4) along the walls of the test tube to form a distinct layer. Observe the interface for the formation of a colored ring.	reddish-brown ring
5	**Test for tannins**	In a test tube, add 1–2 mL of the plant extract. Add a few drops of 5% ferric chloride ($FeCl_3$) solution. Observe any color change that occurs.	blue-black/greenish color

2.4.2 TLC- Characterization of Bioactive Fractions: [24,25]

The sole active fraction after pharmacological assessment of ethanol fractions was assessed by thin layer chromatography for phytocomponent identification by following techniques.

Table 2: TLC studies of plant extracts

Sr. No	Extracts	Solvent system (v:v:v)	No. of spots
1	**Amla**	Toluene:ethyl acetate:acetic acid:and formic acid (2:4.5:2:5)	03
2	**Tulsi**	n-hexane:ethyl acetate (2:4)	
3	**Tinospora**	Toluene:acetone:water (5:15:1)	

2.4.3 Column Chromatography of Active Extracts: [26]

Preliminary phytochemical screening of the dried extracts of *Emblica officinalis*, *Ocimum sanctum*, and *Tinospora cordifolia* revealed the presence of several phytochemical constituents in the ethanol and chloroform fractions. Thin-layer chromatography (TLC) analysis of these extracts further confirmed the presence of multiple phytometabolites, as indicated by the appearance of several distinct spots.

2.4.4. High Performance Thin Layer Chromatography (HPTLC) Fingerprint Analysis [27]

1. Preparation of Extract

The all plant materials were dissolved in the respective solvents (5 mg/ml).

2. Application of Extract

The extract dissolved on its respective solvents (5 mg / ml), with the aid of a Linomat syringe sample plated on the HPTLC plate (10-10 cm). The material was added in the form of a 5-6 mm band and a 6 mm width.

3. Development of the Chromatogram

Measure applied plates in the twin chamber of CAMAG. The panels were grown here up to a distance of 80 mm and were removed from the chamber and dried in air after the run was completed.

4. Scanning of the Chromatogram

Using a CAMAG HPTLC Scanner in absorbance mode at wavelengths of 254, 366, and 560 nm, we did high-performance thin-layer chromatography (HPTLC) analysis. Planar Chromatography Manager software (WinCATS) was used to collect and analyze the data. Established fingerprint chromatograms were created to find the samples' unique profiles, and the Rf values that went along with them were noted. The colors of the bands were carefully recorded at each wavelength without using any visualizing agents. To get the best separation, a solvent solution that was designed just for thin-layer chromatography was used.

Quantitative separation of respective marker compounds by HPTLC

- Plate**:** Aluminium precoated with silica gel GF254, 250 µm, 10×10 cm.
- Sample Application**:** 1 µl & 5 µl.
- Solvent System**:** n-Hexane: Toluene: Ethyl acetate (2:4:1.3).
- Detection**:** UV at 366 nm.
- Instrument**:** CAMAG TLC Scanner; densitometry via WINCATS software.
- Standards**:** 1 mg of rutin, stigmasterol, flavonoid, curcumin in 10 ml methanol (100 ppm).
- Sample Fractions: 0.5 g dissolved in 2 ml ethyl acetate.

2.4.5. Pharmacological Screening of Isolated Fractions

These fractions then evaluated for antidiabetic efficacy after gathering all fractions of ethanol extract.

2.5 STRUCTURAL ELUCIDATION:

The above active isolated fractions of TLC spots were further characterized by UV, MS, FTIR, and 1HNMR [28]

RESULTS AND DISCUSSION

3.1. Collections and Drying

The specified plant parts—*Emblica officinalis* (fruit), *Ocimum sanctum* (leaves), and *Tinospora cordifolia* (leaves)—were collected from the Latur region of Maharashtra, India. The collected materials were shade-dried at ambient temperature, protected from direct sunlight. After complete drying, the plant materials were ground into a coarse powder using a mechanical grinder. The powdered materials were then passed through a 120-mesh sieve to remove fine particles and obtain a uniform powder.

3.2. Authentication

The identities of the collected plants were verified by comparing their morphological characteristics. Authentication was confirmed by the Botanical Survey of India, Pune, Maharashtra state, India (BSI/WRC/Iden.Cer./2021).

3.3. Standardization of plants materials*:*

3.3.1. Determination of foreign organic matter:

The foreign organic matter content in the plant materials was found to be 0.5% w/w in *Emblica officinalis* (fruit), 0.2% w/w in *Ocimum sanctum* (leaves), and 0.2% w/w in *Tinospora cordifolia* (leaves).

3.3.2. Determination of moisture content:

The moisture content of the plant extracts was evaluated and found to be within acceptable limits as per standard recommendations. The loss of moisture over time was determined for each plant materials. The *Emblica officinalis* (amla) dry extract exhibited moisture losses of 0.00%, 0.19%, 0.20%, 0.21%, and 0.21% at 0, 1, 2, 3, and 4 hours, respectively. The *Ocimum sanctum* (tulsi) dry extract showed corresponding values of 0.00%, 0.18%, 0.20%, 0.20%, and 0.20%. Similarly, the *Tinospora cordifolia* extract demonstrated moisture losses of 0.00%, 0.20%, 0.21%, 0.21%, and 0.21% at the same time intervals.

3.3.3. Determination of Ash value: -

The evaluation of ash values for the plant extracts revealed that the total ash content was 3% in both Amla dry extract and Tulsi dry extract, while *Tinospora cordifolia* extract showed a slightly higher value of 4%. The acid-insoluble ash values were found to be 0.8% for Amla, 1.2% for Tulsi, and 1.5% for *Tinospora cordifolia*, indicating minor variations in siliceous matter among the extracts. The water-soluble ash values were similar, with Amla and Tulsi extracts showing 0.2% each and *Tinospora cordifolia* extract slightly higher at 0.3%. These results provide an insight into the inorganic content and purity of the plant materials.

3.3.4. Determination of Extractive values: -

The extractive values of the plant extracts were determined in different solvents. The ethanol-soluble extractive values were 12.1% for Amla dry extract, 11.3% for Tulsi dry extract, and 12.4% for *Tinospora cordifolia* extract. The water-soluble extractive values were 6.1% for Amla, 8.7% for Tulsi, and 5.6% for *Tinospora cordifolia* extract.

3.4. EXTRACTION

Extraction was carried out in several batches using different solvents. The whole plant material was extracted sequentially with various solvents, and the appearance and percentage yield of each extract were recorded. All extracts obtained using different solvents were dark green in color and semisolid with a sticky consistency. The petroleum ether extract showed a percentage yield of 8.45% w/w, while the ethanol extract exhibited the highest yield of 11.58% w/w. The chloroform and ethyl acetate extracts yielded 7.41% w/w and 6.23% w/w, respectively. The aqueous extract demonstrated a yield of 7.44% w/w.

Yield of Various Extracts Obtained from leaves Powder (***Ocimum sanctum*** – Tulsi)

The extracts obtained using different solvents were dark green in color and semisolid with a sticky consistency. The petroleum ether extract exhibited a percentage yield of 5.40% w/w, while the ethanol extract showed the highest yield at 9.68% w/w. The chloroform and ethyl acetate extracts yielded 4.12% w/w and 3.28% w/w, respectively. The aqueous extract demonstrated a yield of 5.01% w/w.

Yield of Various Extracts Obtained from leaves Powder (***Tinospora cordifolia***)

All extracts obtained using different solvents were dark green in color and semisolid with a sticky consistency. The petroleum ether extract exhibited a percentage yield of 6.23% w/w, while the ethanol extract showed the highest yield at 10.48% w/w. The chloroform extract yielded 9.58% w/w. The ethyl acetate and aqueous extracts demonstrated yields of 7.45% w/w and 9.87% w/w, respectively.

Yield of Various Extracts Obtained from fruit Powder (*Emblica officinalis)*

The yield of various extracts obtained from the whole plant powder (Amla dry extract) was evaluated using different solvents. All extracts were dark green in color and exhibited a semisolid, sticky nature. Among the solvents used, the ethanol extract showed the highest percentage yield (11.58% w/w), indicating better extraction efficiency compared to other solvents. Petroleum ether extract yielded 8.45% w/w, followed by aqueous extract (7.44% w/w) and chloroform extract (7.41% w/w). The ethyl acetate extract showed the lowest yield (6.23% w/w). These results suggest that ethanol was the most effective solvent for extracting phytoconstituents from the whole plant powder.

3.5. Preliminary Phytochemical Screening: -

Active ingredients including Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Proteins, Free Amino Acids, Carbohydrate and Vitamin C were examined for presence in extracts.

3.5.1. Phytochemical test of Amla Extract

Phytochemical screening of the *Emblica officinalis* (amla) extract revealed the presence of several bioactive constituents. Flavonoids were detected by a positive reaction in the H_2SO_4 test, terpenoids were identified using the chloroform test, alkaloids were confirmed by Dragendorff's reagent, steroids were detected by the Salkowski test, and tannins were identified using the ferric chloride ($FeCl_3$) test. All tests showed positive results, indicating the presence of these phytoconstituents.

3.5.2. Phytochemical test Tulsi Extract

Phytochemical screening of the *Ocimum sanctum* (tulsi) extract indicated the presence of flavonoids, as evidenced by a positive H_2SO_4 test. In contrast, terpenoids, alkaloids, steroids, and tannins were absent, as demonstrated by negative results in the chloroform, Dragendorff's, Salkowski, and $FeCl_3$ tests, respectively.

3.5.3. Phytochemical test *Tinaspora Cardifolia* of Extract

Phytochemical analysis of the *Tinospora cordifolia* extract revealed the presence of flavonoids, confirmed by a positive H_2SO_4 test. However, terpenoids, alkaloids, steroids, and tannins were not detected, as indicated by negative results in the chloroform, Dragendorff's, Salkowski, and $FeCl_3$ tests, respectively.

The phytochemical evaluation of the whole plant extract powder comprising amla dry extract (30%), tulsi dry extract (10%), and *Tinospora cordifolia* extract demonstrated the presence of flavonoids as the major constituent across all extracts. Additionally, terpenoids, alkaloids, steroids, and tannins were predominantly detected in the amla extract, while tulsi and *Tinospora cordifolia* extracts primarily showed the presence of flavonoids.

3.6. Thin layer chromatography of extract

Thin-layer chromatographic (TLC) studies were carried out to isolate and identify active components with potential pharmacological activity. Phytochemical analysis using TLC revealed the presence of major classes of constituents, including alkaloids, flavonoids, tannins, and steroids. These compounds were identified using appropriate mobile phases and visualization reagents.

Alkaloids produced violet-blue spots upon spraying with 10% sulfuric acid in ethanol, with Rf values ranging from 0.71 to 0.87 in different solvent systems. Flavonoids developed yellowish-green spots after derivatization with anisaldehyde–sulfuric acid and exhibited Rf values between 0.73 and 0.89. Tannins were visualized as black spots following treatment with ferric chloride,

showing an Rf value of 0.62 in ethanol. Steroids produced pink spots after spraying with vanillin–sulfuric acid, with Rf values ranging from 0.46 to 0.83 depending on the solvent system used.

These findings confirm the presence and distinct chromatographic behavior of key phytochemical groups in the sample, supporting their potential contribution to the observed pharmacological activities.

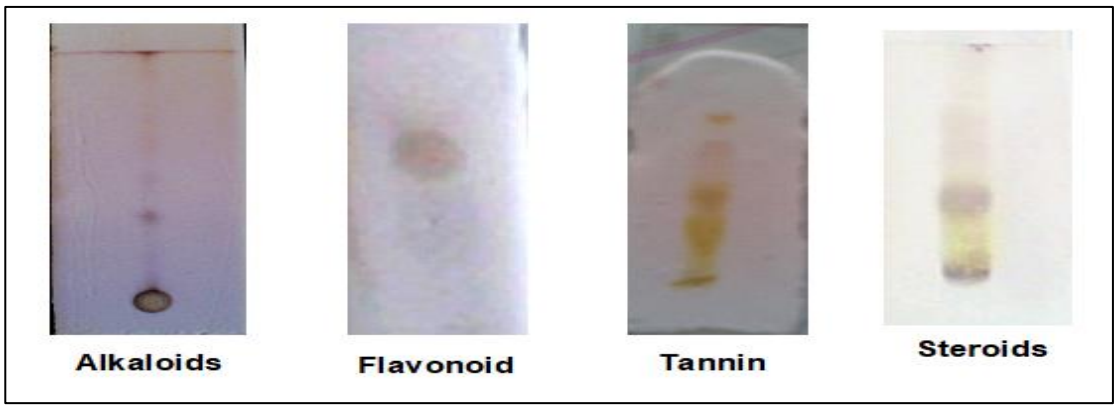

Figure 1: TLC chromatography for Alkaloid, flavonoid, tannins and steroids

3.7. Column Chromatography of Active Extract of All Plant Extract

Preliminary phytochemical screening of the whole plant ethanol and chloroform extracts revealed the presence of alkaloids, steroids, flavonoids, and terpenoids. Thin-layer chromatography (TLC) analysis further confirmed the presence of several phytometabolites in these extracts. The ethanol and chloroform extracts demonstrated a notable diversity of potential phytoconstituents. Based on these findings, column chromatography was employed for the isolation of phytocompounds from the ethanol extract.

Ethanol Extract Column Chromatography

Table 3: Appearance and percent yield of all fractions.

Plant Name	Mobile phase	Fraction Designation	Weight of fraction (gm.)	% Yield w/w
Amla dry extract (30%)	n-Hexane	AEF1	0.89	3.5
		AEF2	0.84	4.1
		AEF3	0.78	3.6
Tulsi dry extract (10%)	n-Hexane: Ethyl acetate (5: 5)	TEF1	0.74	4.2
		TEF2	0.76	3.9
		TEF3	0.81	4.2
Tinospra cardifolia extract	Ethyl acetate	TCEF1	0.82	4.1
		TCEF2	0.88	3.8
		TCEF3	0.78	3.4

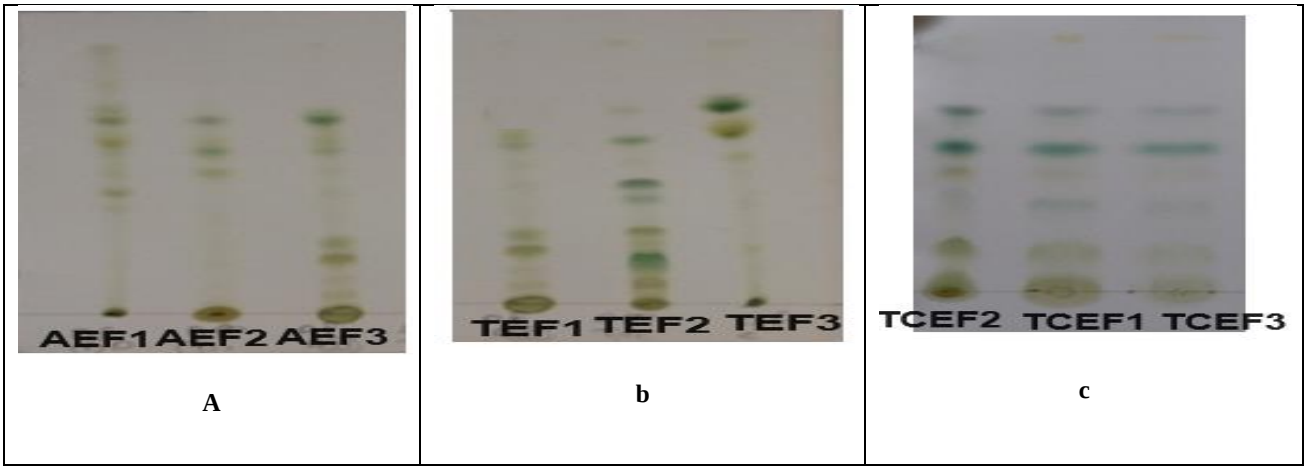

Figure 2: [a] TLC chromatograph of Amla dry extract [b] TLC chromatograph of Tulsi dry extract [c] TLC chromatograph of *Tinospra cardifolia* extract

The Rf values of the extracts were recorded as follows: Amla showed Rf values of 0.32, 0.44, and 0.55; Tulsi exhibited Rf values of 0.36, 0.72, and 0.84; and Tinospora displayed Rf values of 0.38, 0.47, and 0.63.

3.8. High Performance Thin Layer Chromatography (HPTLC)

HPTLC fingerprints study

Table 4: HPTLC fingerprints study of various extracts

Sr. No	Plant name	Extracts	Detection Wavelength (nm)	No. of spots	R_f values
1	Amla	AEF1	254	05	0.06, 0.18, 0.21, 0.33, 0.46, 0.52, 0.66.0.80.
2	Tulsi	TEF2	366	12	0.02, 0.08, 0.07, 0.32, 0.42, 0.51, 0.56, 0.59, 0.66, 0.71. 0.80, 0.87,
3	Tinospora	TCEF1	560	08	0.04, 0.16, 0.21, 0.32, 0.42, 0.47, 0.57, 0.66.

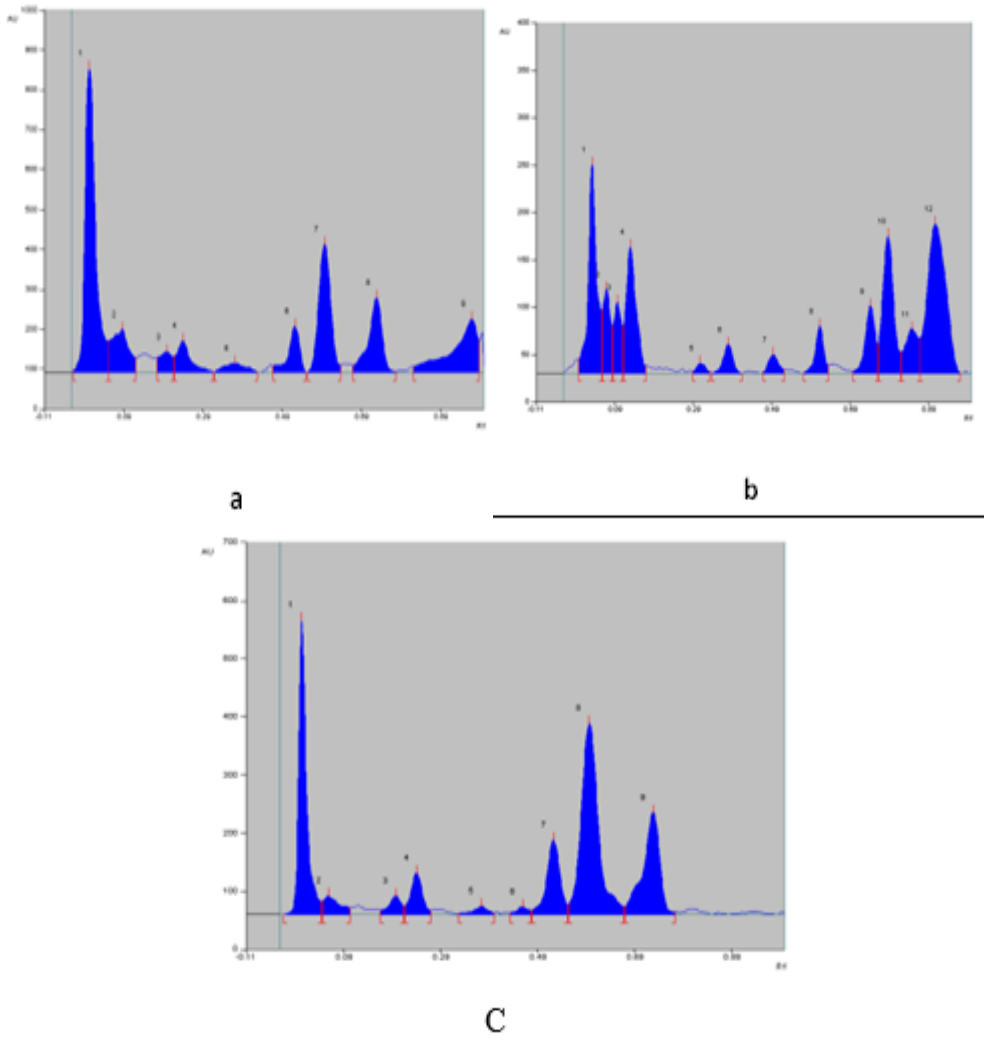

Figure 3: [**a]** HPTLC chromatogram of AEF1 extract measured at 254nm **[b]** HPTLC chromatogram of TEF2 extract measured at 366nm [**c]** HPTLC chromatogram of TCEF1 extract measured at 560nm

TLC analysis showed distinct Rf values for each extract: Amla (254 nm) had 8 spots (Rf: 0.06–0.80), Tulsi (366 nm) showed 12 spots (Rf: 0.02–0.87), and Tinospora (560 nm) had 8 spots (Rf: 0.04–0.66), confirming the presence of multiple phytoconstituents.

3.9. Spectroscopic data of isolated Compound-I.

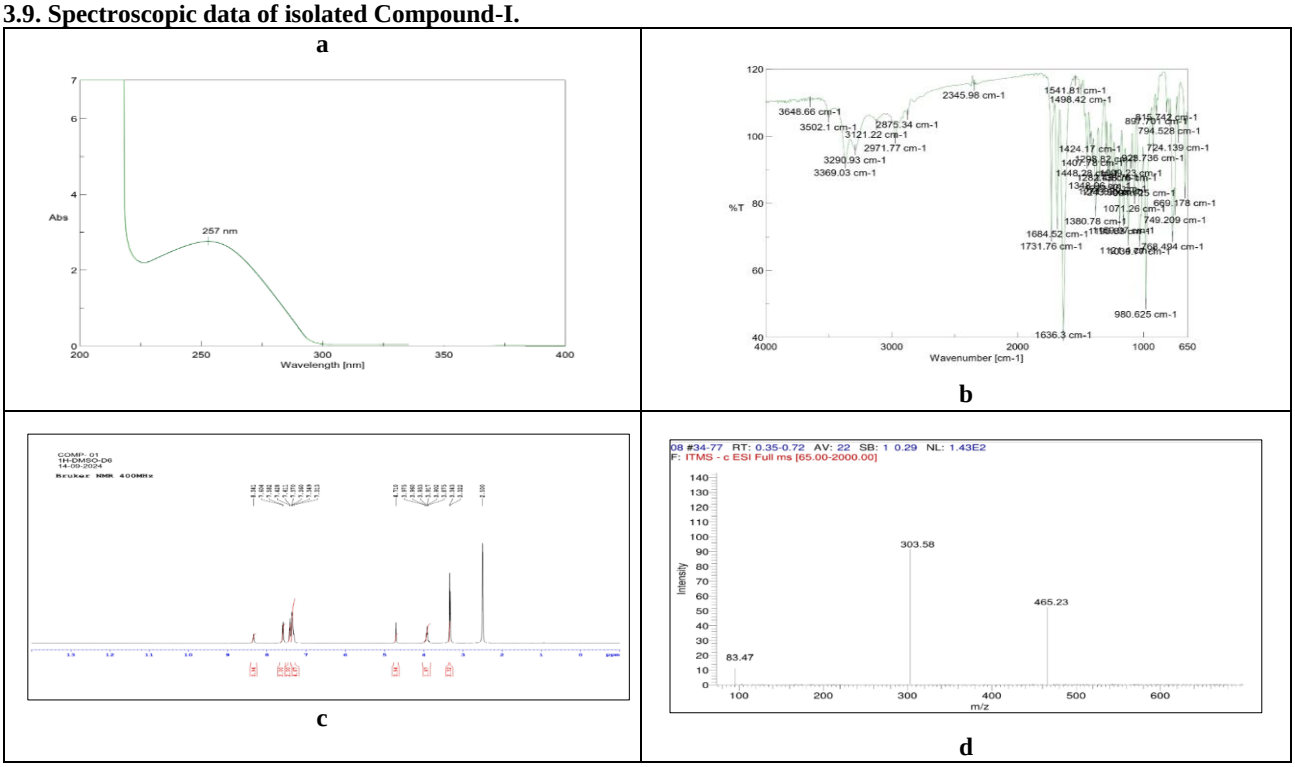

Figure 4: [a] UV spectrum of Amla extract [b] IR spectra of compound I [c] Proton NMR spectrum of Compound – I [d] MASS spectrum of Compound – I

On the basis of chromatographic, IR, NMR and MASS spectra data of compound and as per literature elucidated structure is match with spectral data of Rutin. So, we can confirm that the elicited compound is analogue of Rutin.

Compound 2

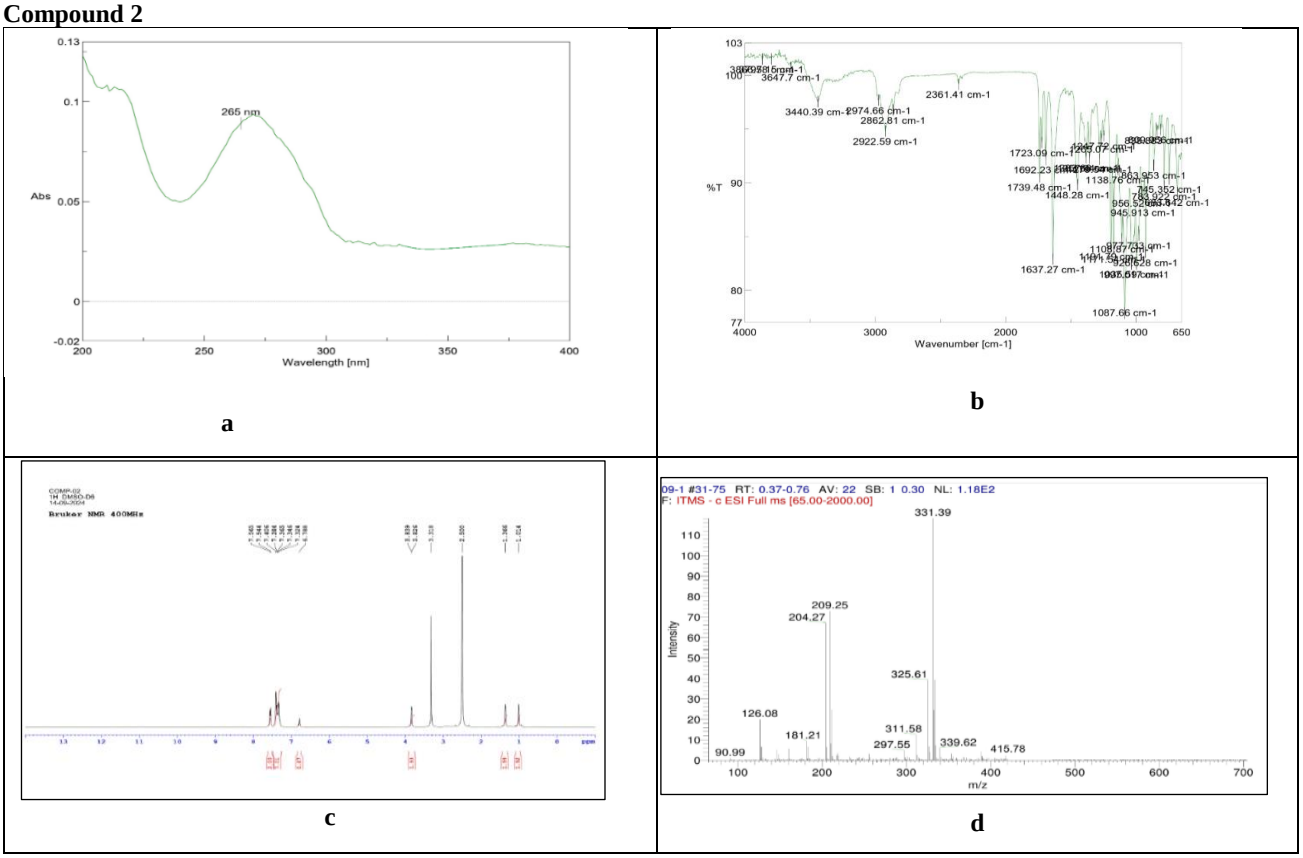

Figure 5: [a] UV spectrum of Tulsi extract in n-hexane: ethyl acetate [b] IR spectra of compound II [c] Proton NMR spectrum of Compound – II [d] MASS spectrum of Compound – II

Structure of elucidated Compound-II

On the basis of chromatographic, IR, NMR and MASS spectra data of compound and as per literature elucidated structure is match with spectral data of plant sterol. So, we can confirm that the elicited compound is analogue of stigmasterol.

Compound 3

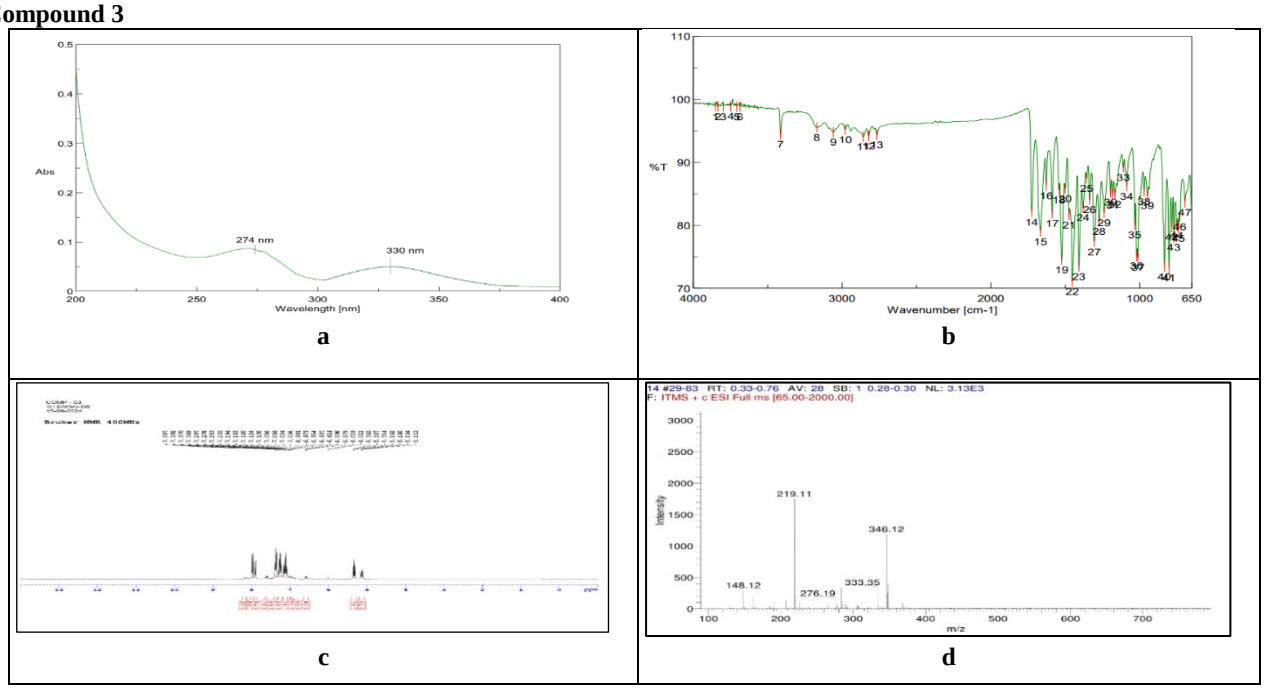

Figure 6: [a] UV spectrum of Tinospora extract [b] IR spectra of compound III [c] Proton NMR spectrum of Compound – III [d] MASS spectrum of Compound – III

On the basis of chromatographic, IR, NMR and MASS spectra data of compound and as per literature elucidated structure is match with spectral data of flavonoid. So, we can confirm that the elicited compound is analogue of flavonoid.

CONCLUSION

The present study successfully extracted and standardized *Emblica officinalis* (Amla), *Ocimum sanctum* (Tulsi), and *Tinospora cordifolia* extracts, demonstrating good physicochemical properties. Phytochemical screening revealed the presence of flavonoids, terpenoids, alkaloids, steroids, and tannins. TLC and HPTLC analyses confirmed the presence of distinct phytoconstituents with characteristic Rf values. Column chromatography enabled the isolation of three major compounds, which were identified as analogues of rutin, stigmasterol, and flavonoids. Structural elucidation using UV, IR, NMR, and mass spectrometry supported these identifications. Based on spectral analysis and comparison with reported literature, fractions II and III were confirmed as analogues of stigmasterol and flavonoid, respectively. These findings highlight the presence of bioactive phytoconstituents in these medicinal plants, supporting their traditional therapeutic uses and providing a foundation for future pharmacological and drug development studies.

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Conflict of Interest

The authors declare no Conflict of Interest.

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