

**Investigation of Anti-Inflammatory Activities in Traditionally Used Indigenous Plants****Shirole Nitin L.<sup>1</sup>, Laware Sandip G.<sup>2</sup>**<sup>1</sup>Professor, DCS's A.R.A. College of Pharmacy Nagaon, Dhule<sup>2</sup>Associate Professor, PIMS-DU College of Pharmaceutical Sciences, Loni

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Conflict of interest: Nil

**Abstract:**

Since 1900, the pharmaceutical industry has profited from natural resource extraction. Accessibility, cost, and absorption into broader belief systems are the main reasons herbal medicine is widely used in economically challenged cultures. Lack of quality control for natural goods and production methods is a major issue for the herbal industry. Herbal medicine standards and active substances are commonly linked. Many researchers are having trouble administering or targeting drugs to induce systemic effects. This work was built on prior research in analytical chemistry, pharmacology, phytochemistry, applied medicinal chemistry, formulation science, and quality assurance. This thesis investigates three medicinal plants: the Indian *Nyctanthus arbortristis* Linn, also known as Night-flowering Jasmine or Coral Jasmine; the Canadian *Solanum xanthocarpum*, also known as yellow-berried nightshade; and the Indian *Clerodendrum serratum*, also known as Brahmannayastika. Modern analytical methods were used to verify these drugs' validity, purity, safety, and efficacy, standardizing them. These drugs were also tested for standardization, biological screening, and conversion into classic and innovative topical dose forms with specific effects. This study employed molecular docking to assess the extract's anti-inflammatory potential. The extract was used to find and extract analytical and chemical markers with similar features, and chromatographic procedures were employed to establish the indicators. In addition to biological screening, spectroscopic and chromatographic procedures standardized the components and extracts that served as chemical and analytical markers in the original and new formulations. The standardized formulations used in this study were analytically stable. This study investigates historically significant plants from three civilizations to educate people about inflammatory treatment options.

**Keywords:** Anti-Inflammatory activity and Endogenous plants.

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**Introduction**

A significant portion of the things that we use and consume today are the result of our predecessors' understanding of plants. It is both fascinating and unsettling to consider the possibility that study on traditional wisdom may be carried out with the intention of treating more prevalent diseases.

In order to investigate the possibility of using plant-based medicines as safer alternatives to conventional medications, research is now being conducted on a rising number of plant-based herbal remedies. Therapeutic plants are those that are commonly thought to have dependable and effective medicinal characteristics. This is the definition of therapeutic plants in the medical profession. Due to the fact that these plants are crucial in the treatment of a wide range of diseases and disorders, they are frequently utilized in the treatment and prevention of these conditions. There is a connection between this term and the fields of agriculture and the improvement of natural

resources development. There is a solution to every problem that people get themselves into in the natural world [1,2]. There are a number of natural remedies that have the potential to treat problems that conventional medicine has been unable to treat due to unforeseen adverse effects. Some examples of natural remedies include herbs, plants, and algae. People have relied on plants and products produced from plants for the preservation of their health throughout the course of human history.

It is possible for the medical business to fulfill its requirements with the assistance of new chemical entities (NCEs) that are derived from plants. Around 5000 B.C., the Rig-Veda and the Atharva-Veda were the first texts to elucidate the conditions that were necessary for health and the illnesses that were prevalent at the time. All of the key elements that make up India's healthcare system were established during this time period [3-5]. Approximately one thousand years before the

Common Era, the "Charaka Samhita" and the "Sushruta Samhita" were the texts that formalized the utilization of plants and Polyherbal remedies for therapeutic purposes. One of the most important aspects of India's cultural past is the way in which Ayurveda and other plant-based medicinal therapies have developed throughout time as a result of the experiences that people have had in their everyday lives. A little over eighty percent of the population in poor countries receives the majority of their medical care from traditional healing practices. A significant portion of the conventional medical procedures are founded on the use of herbal supplements. Among the more than two thousand natural treatments that are included in the Indian Materia Medica, the majority

of them are based on traditional medical practices and folklore [6].

### Materials and Methods

#### Collection and Authentication of Plant Material:

The following plant herbarium specimens were sent to the Pune regional office of the Botanical Survey of India to verify the study's findings.

- The initial item on our list is *Solanum xanthocarpum*.
- Our team will assign the *Nyctanthus arbortristis* to the second position.
- The plant in dispute is *Clerodendrum serratum*, which is the third item on the inventory.



Figure 1: Roots of *Clerodendrum serratum* and *Solanum xanthocarpum*



Figure 2: Barks of plant *Nyctanthus arbortristis*

### Pharmacognostical Study

**Macroscopic and microscopic analysis:** Determination of Moisture content for *Clerodendrum serratum*, *Solanum xanthocarpum* Schradt. & Wendl and *Nyctanthus arbortristis* L.:

The Indian Pharmacopoeia (IP) 1996 defines "loss on drying" as the mass loss as a percentage of the substances total weight after drying. Each alternative is plausible and should be considered: The box includes a dryness and Stoppard-weighed glass. A little cup can be used to weigh. After the next processes, the bottle was filled to 10 mm. After measuring, scattering, and pouring one grammes of sample into the container, this was

done. After filling, the cork was removed and the bottle was baked. Drying the sample ensured its weight would remain constant after disposal. After drying, a desiccator lowered the temperature to room temperature.

The weighted and predicted drying loss is shown as a percentage of the total weight after computation [7].

**Determination of Ash Value for *Clerodendrum serratum*, *Solanum xanthocarpum* Schradt. & Wendl and *Nyctanthus arbortristis* L.:** The assessment of ash value in plants entails incinerating a specified mass of plant material to a stable weight, often at a temperature ranging from

500 to 600°C. The residual ash is subsequently weighed and expressed as a percentage of the initial sample. The ash value denotes the total quantity of mineral residue remaining post-combustion of organic matter [8].

**Preparation of Extracts:** We used tap water to clean three plants' roots and bark. *Nyctanthus arbortristis*, *Solanum xanthocarpum*, and *Clerodendrum serratum* were involved. We air-dried the roots in the shade for 22 days after chopping them into tiny pieces. The dried plant material was placed in small plastic containers and marked with paper after being blended into a fine powder with an electric blender. Powder was then applied on container coverings. The Soxhlet extraction method yielded crude plant extracts in acetone, petroleum ether, chloroform, and water over 20 hours. It was decided to store crude extracts in hermetically sealed vials at 4°C until needed [9].

**Qualitative Chemical Investigation of Extracts:** To determine the phytoconstituents, a qualitative

analysis was performed on the root extracts of *Solanum xanthocarpum* and *Clerodendrum serratum*, as well as the bark and bark extracts of *Nyctanthus arbortristis*. This was done in order to determine the phytoconstituents [10].

**Chromatographic Study of *Clerodendrum serratum*, *Solanum xanthocarpum* Schradt. & Wendl and *Nyctanthus arbortristis* L. Extract:**

**Thin Layer Chromatography:** Thin layer chromatography was employed to detect phenolic chemicals in *Clerodendrum serratum*, *Solanum xanthocarpum*, and *Nyctanthus arbortristis* plant extracts. The TLC technique uses silica gel G as an adsorbent. The TLC plate slurry needs filtered or distilled water. An applicator applied this mixture of slurries to a dry, clean 10x20-centimetre glass plate, creating a 0.4-millimetre layer. The plate reached 110°C after an hour of heating. TLC solvents consist of toluene, acetone, and acetic acid. 3606 nanometres is the absorption wavelength. This experiment used optical detection and vaniline-sulfuric acid [11].

**Table 1: Solvent System for TLC Development of Extracts**

Sr. No	Extracts	Composition of Mobile Phase
1.	<i>Clerodendrum serratum</i> Extracts (CSE)	Chloroform : Methanol (4.7:0.25)
2.	<i>Solanum xanthocarpum</i> Extracts (SXE)	Toluene : Ethyl acetate : Glacial Acetic Acid (0.8:0.1:1.0)
3.	<i>Nyctanthus arbortristis</i> Extract (NAE)	n-Hexane : Chloroform : 1-2,Dichloromethane (0.4:0.2:1.4)''

#### High Performance Liquid Chromatographic Assessment:

By referring to the guidelines established by the International Council for Harmonisation (ICH), the HPLC techniques that were used in the examination of rutin derived from *Nyctanthus arbortristis*, saponins derived from *Clerodendrum serratum*, and ellagic acid derived from *Solanum xanthocarpum* were assessed. Therefore, it was necessary for us to adhere to all of the ICH

requirements in order to guarantee that the analytical procedure would provide accurate results. During the course of the testing procedure, a number of criteria that are associated with validation were evaluated. Some of these attributes were specificity, linearity, range, accuracy, precision (system, method, and intermediate), resilience, and solution stability. Other characteristics included reliability and stability [12].

**Table 2: HPLC Chromatographic Conditions**

Sr. No	Particulars	<i>Clerodendrum serratum</i> Extracts (CSE)	<i>Solanum xanthocarpum</i> Extracts (SXE)	<i>Nyctanthus arbortristis</i> Extract (NAE)
1.	Column	Hypersil C18 column, 5 $\mu$ (4.6 X 250 mm)	Sunfire C18 column, 5 $\mu$ (4.6 X 250 mm)	Phenomenox, 5 $\mu$ (4.6 X 250 mm)
2.	Flow rate	1.1 mL/minute	1.0 mL/minute	1.0 mL/minute
3.	Column Temperature	32°C $\pm$ 5°C	30°C $\pm$ 5°C	35°C $\pm$ 5°C
4.	Sample Temperature	25° C $\pm$ 5°C	25° C $\pm$ 5°C	25° C $\pm$ 5°C
5.	Injection volume	20 $\mu$ L	10 $\mu$ L	10 $\mu$ L
6.	Detector	UV at 425 nm	UV at 280 nm	UV at 257 nm
7.	Run Time	40 minutes	35 minutes	40 minutes
8.	Retention Time	Saponins -I: 12 minutes Saponins -II: 13 minutes Saponins: 14 minutes	Ellagic acid:16 minutes	Rutin: 11 minutes
9.	Diluent	Methanol	Methanol	Methanol''

**Pharmacological Screening of *Clerodendrum serratum*, *Solanum xanthocarpum* Schradt. & Wendl and *Nyctanthus arbortristis* L. Extract:** Albino Wistar rats in this study weighed 130 to 220 grams throughout the experiment, regardless of gender. Acute toxicity, pain relief, antipyrexia, and anti-inflammatory effects were examined. Along with plenty of water, the animals were fed a commercial laboratory meal. All animals in the experiment were treated to the same strict protocols. Six rats were housed in clean, 25°C cages.

Rats had a 12-hour day/night cycle. Every day, the cage mattresses were replaced. The IAE Committee of the consented to the inquiry, which may be

morally acceptable. Six plant extracts were tested for anti-inflammatory properties (13).

## Result and Discussion

### Pharmacognostical Study:

#### *Clerodendrum serratum* L.:

**Physical properties of root:** In spite of the fact that it is smooth and has fine grains, the solid, light-yellow wood does not have any fragrance or aroma.

Due to the very minute size of the pores, it is impossible to distinguish them; yet, the growth rings are readily apparent.

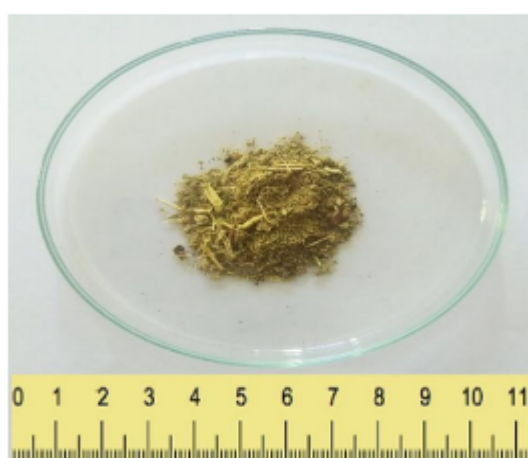


Figure 3: Macroscopy of *Clerodendrum serratum* L root powder

**Anatomy of the root:** There is a thickness of 3.8 mm in the specimen that demonstrates the lateral root. On the root, as shown in Figure 4, there are a few small fissures and some thin peelings of phloem tissue occurring. This particular root is shown in the illustration.

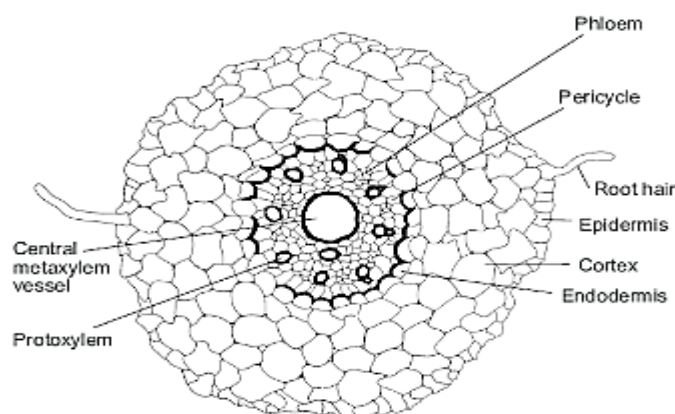


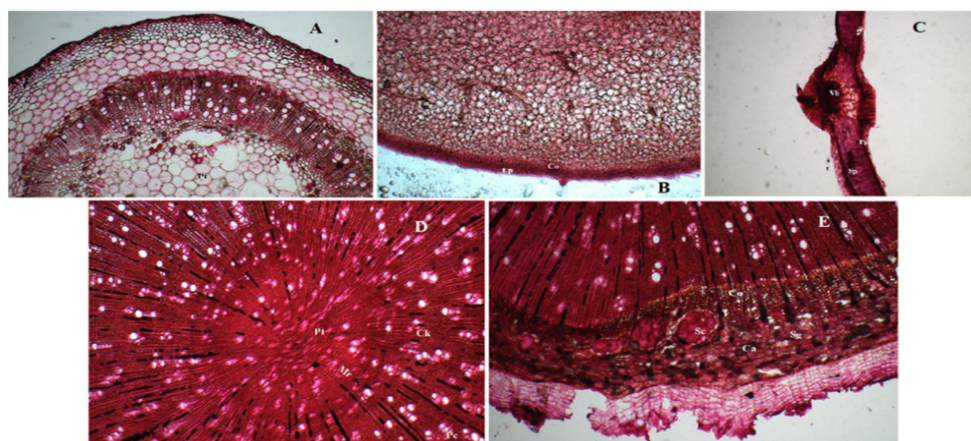
Figure 4: T. S of young root in ground plan

#### *Solanum xanthocarpum* Schradt. & Wendl.

**Microscopic:** The cross-section of a fully grown root exhibits the following traits: layers of thin-walled, tangentially elongated, oval or circular parenchymatous cells; solitary or clustered stone cells;

secondary phloem, which consists of parenchyma and sieve elements with medullary rays scattered throughout; and xylem, which contains vessels, tracheids, fibre tracheids, parenchyma, and transverse medullary rays.

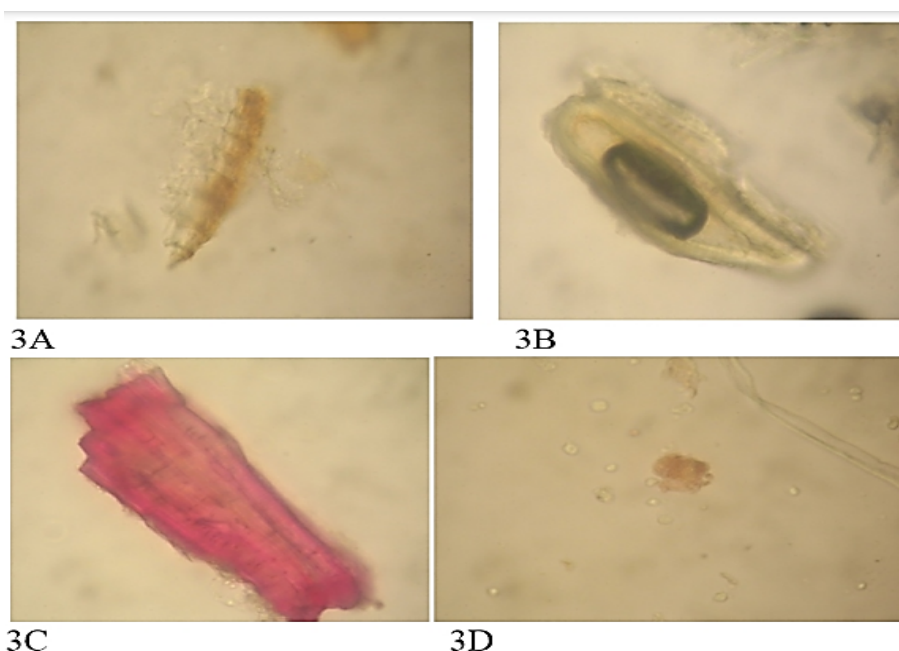




**Figure 5: Microscopic evaluation of the whole plant of *Solanum xanthocarpum* Schrad. & Wendl.**

Fibrous root powder is a velvety, odourless, and flavourless powder-like substance that you will encounter. The substance contained both simple and complex granules of starch. The morphology of these particles varied from oval to cylindrical, and they were arranged in a pattern. When the

environment contained elevated levels of hydrochloric acid and phloroglucinal, Fibrovascular bundles were observed. Figure 7 illustrates the presence of calcium oxalate crystals and stone cells.



**Figure 6: Powder characteristics of *Solanum xanthocarpum* Schradt. & Wendl root. 3A: Cork cells; 3B: Stone cell; 3C: Vascular element; 3D: simple and compound starch grains and calcium oxalate crystal**

#### ***Nyctanthes arbortristis* L.**

**Microscopy of stem bark:** Ten to fifteen layers of tangentially expanded cork cells were seen when the mature bark was split transversely. These cork cells measured 19.99–26.66–39.99 inches broad and 26.66–66.65–79.98 inches long. Also, the upper level walls are fully submerged. Cells of cork trees contain tannin. Multiple places exhibit scleren-

chymatous threads. Phellogen possesses thin-walled, compacted cells. These cells may form one or two layers across the phellogen.

The phelloderm, cortex, and phollogen have 15–20 layers of loosely aligned thin-walled cells.

These cells are 53.32–16.65–79.98–93.31 by 39.99–53.32–66.65 inches. Category similarities exist.

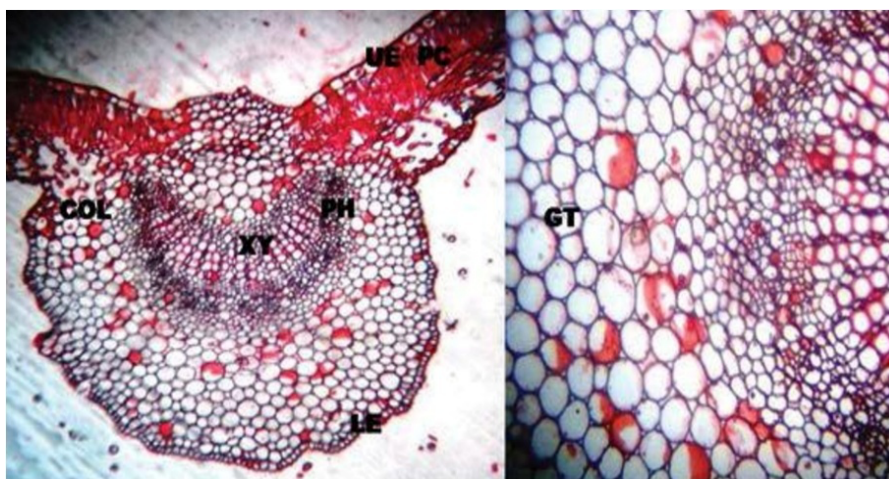


Figure 7: T.S. of *Nyctanthos arbor-tristis* bark

Tannins, starch, and acicular calcium oxalate crystals are lower than predicted. Under the cortex, secondary phloem has stronger walls and polygonal to hexagonal cells. It measures 39.99-53.32-66.65-79.98-106.64 inches.

Lignified sclerenchymatous fibres partitioned these cells into hexagons and polygons. Narrow lumens and surface striae identify fibres. Secondary phloem can be detected by starch grains, calcium oxalate crystal acicular raphides, and tannin-packed phelloderm and cortex (Fig. 8).

#### Powder study

Aside from having a colour that was similar to a creamy brown, the powdered stem also had a scent and smell that was bitter. Following exposure to a chloral hydrate solution, staining with 1% saffranin for five to ten minutes, and mounting with 50% glycerine, the data shown in Figure 9 reveals the presence of tannin-filled cells, cork, stone, starch, and calcium oxalate crystals. These crystals were found when the samples were mounted with 50% glycerine.

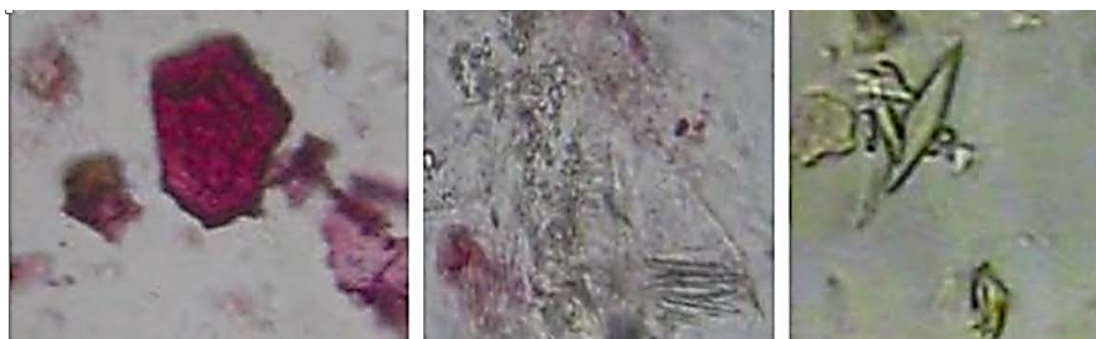


Figure 8: Stone cell, Starch grains and Calcium oxalate crystals

#### Phytochemical Parameters:

Ash values identify samples with calciferous and inorganic components. Extractive values let one comprehend the object's amount and chemical makeup. The drying loss method may measure sample moisture. Water-soluble extractives outnumber alcohol-soluble extractives, indicating

that plants have more water-soluble components. Considering this extraction value, the data support our assumption that plants contain more water-soluble chemicals than previously thought. The facts backed scientific consensus. These methods may be used to standardize plant-based drugs.

**Table 3: Physical constants for Clerodendrum serratum, Solanum xanthocarpum and Nyctanthus arbortristis**

Parameters	Results		
	Clerodendrum serratum	Solanum xanthocarpum	Nyctanthus arbortristis
<b>Ash Values</b>	3.2 % w/w	12.9 % w/w	5.80 % w/w
Water Soluble Ash	0.79 % w/w	2.2 % w/w	2.20 % w/w
Acid Insoluble Ash	80.90 % w/w	12.2 % w/w	18.06 % w/w
Total Ash			
<b>Extractive Values</b>	6.20 % w/w	18.6 % w/w	12.8 % w/w
Alcohol Soluble Extractives	7.20 % w/w	27.5 % w/w	30.60 % w/w
Water soluble extractive			
Loss on Drying	3.54 % w/w	16.5 % w/w	15.5 % w/w"

#### Qualitative Chemical Investigation of Extracts Percentage Yield of Extraction:

The powdered roots of *Clerodendrum serratum*, *Solanum xanthocarpum*, and *Nyctanthus arbortristis* were extracted using the Soxhlet device, a continuous hot extraction technique. However, *Clerodendrum serratum*'s alcoholic (methanol) and aqueous extracts have 10.45% and 12.20%

percentages, respectively. *Nyctanthus arbortristis* extracts included 17.55 percent methanol and 18.15 percent water. Both methanol and water percentages were noted.

According to studies, the methanolic and aqueous concentrations had 20.75 percent and 19.1 percent *Solanum xanthocarpum*. Table 6 details percentage yields and extracts.

**Table 4: Percentage yield of extracts of Clerodendrum serratum, Solanum xanthocarpum and Nyctanthus arbortristis**

Sr. no.	Plant name	Extracts	% yield w/w	Physical state of Extract
1	Clerodendrum serratum	Aqueous (200 g)	10.45 %	Semisolid Viscous
		Methanol (200 g)	12.20 %	Semisolid Viscous
2	Solanum xanthocarpum	Aqueous (200 g)	18.15 %	Semisolid Viscous
		Methanol (200 g)	17.55 %	Semisolid Viscous
3	Nyctanthus arbortristis	Aqueous (200 g)	20.75 %	Semisolid Viscous
		Methanol (200 g)	19.1 %	Semisolid Viscous"

#### Physicochemical Evaluations

**Table 5: Physicochemical Evaluation of Extracts**

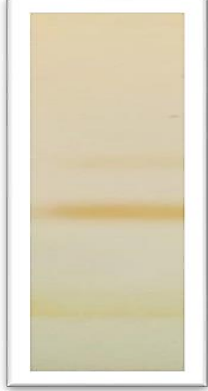


Sr. No.	Parameters	Observations		
		CSE	SXE	NAE
1.	Total Polyphenolic content (mg GAE/100 gm)	0.682 ± 0.0044	26.24 ± 0.5268	16.43 ± 0.5608
2.	Total Flavonoid content (mg/gm)	-	18.33 ± 0.8819	13.46 ± 0.6767
3.	Pesticide Residues	Nil	Nil	Nil
4.	Total Aflatoxins, (B1, B2, G1, G2)	< 5 µg/kg	< 5 µg/kg	< 5 µg/kg
5.	Microbial Load, E coli, Salmonella, Ps. aeruginosa, S. aureus Aerobic Microbial Count	Absent	Absent	Absent
	Total Bacterial Count	91 CFU/GM	85 CFU/GM	80 CFU/GM
	Total Fungal Count	< 10 CFU/GM	< 10 CFU/GM	< 10 CFU/GM
	Total Yeast and Mould count	< 10 CFU/GM	< 10 CFU/GM	< 10 CFU/GM
6.	Heavy Metals			
	Lead	0.512 ppm	0.571 ppm	0.514 ppm
	Arsenic	Nil	Nil	Nil
	Mercury	Nil	Nil	Nil
	Cadmium	Nil	Nil	Nil"

**Chromatographic Characterization of Extracts****Thin Layer Chromatography (TLC):**

The technique of thin-layer chromatography (TLC), which was developed by combining toluene and

ethyl acetate in a solvent solution where the ratio of the solvent was 85:15, may be used to determine the amount of saponin components that are present in extracts. Diosgenine of this particular kind served as the benchmark.

**Table 6: Details of TLC Development for Extracts**

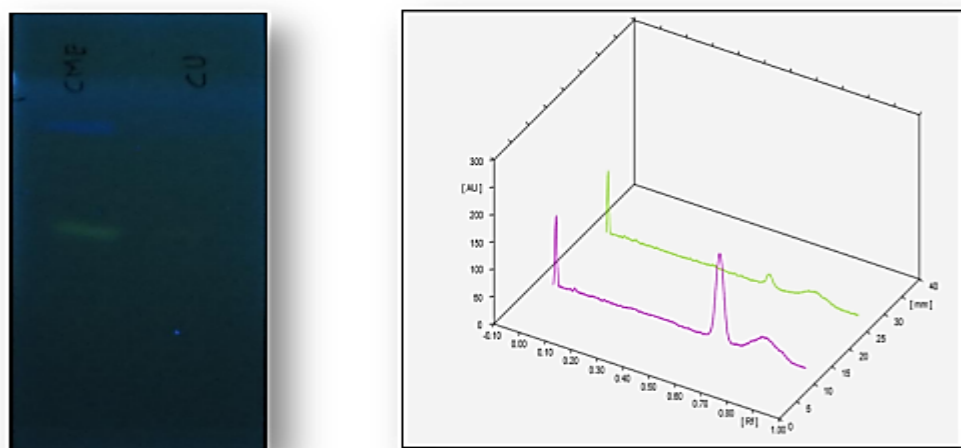
Sr. No.	Extracts	Composition of Mobile Phase	Images
1.	Clerodendrum serratum Extracts (CSE)	Chloroform : Methanol (4.7:0.25)	
2.	Solanum xanthocarpum Extracts (SXE)	Toluene : Ethyl acetate : Glacial Acetic Acid (0.8:0.1:1.0)	
3.	Nyctanthus arbortristis Extract (NAE)	n-Hexane : Chloroform : 1-2, Dichloromethane (0.4:0.2:1.4)	

**High Performance Liquid Chromatographic Assessment:**



**Table 7: Results for HPLC Method Development and Validation**

Parameters	Acceptance Criteria	Result		
<b>Extracts</b>		<b>CSE</b>	<b>SXE</b>	<b>NAE</b>
<b>Analytical marker</b>		Saponins	Ellagic acid	Rutin
<b>Specificity</b>	No Interference for Analyte Peak	Specific	Specific	Specific
<b>Linearity</b>	$r^2 > 0.999$	0.9999	0.999	0.999
<b>% Assay</b>		94.25	1.42	0.393
<b>Precision</b>				
<b>System Precision</b>	% RSD < 2.0	1.92	0.30	0.89
<b>Method Precision</b>	% RSD < 2.0	1.01	1.41	0.33
<b>Intermediate Precision</b>	% RSD < 2.0	0.78	1.86	0.31
<b>Accuracy</b>				
<b>80%</b>	Average % Recovery should be in the range of 98% - 102 %	101.32	101.40	99.03
<b>100%</b>	Average % Recovery should be in the range of 98% - 102 %	101.27	100.29	100.53
<b>120%</b>	Average % Recovery should be in the range of 98% - 102 %	100.87	100.99	99.56
<b>Solution Stability</b>	% relative change of Analytical Marker in standard and Test solution w.r.t initial < 5.0 %	The Standard and Test solution were stable for 24 hrs.	The Standard and Test solution were stable for 24 hrs.	The Standard and Test solution were stable for 24 hrs.
<b>Robustness</b>	System suitability parameters should comply	Robust	Robust	Robust"

**High Performance Thin Layer Chromatographic****Figure 9: HPTLC Image and Scanning Graph for CSE, Saponins and Isolated Saponins Fraction**

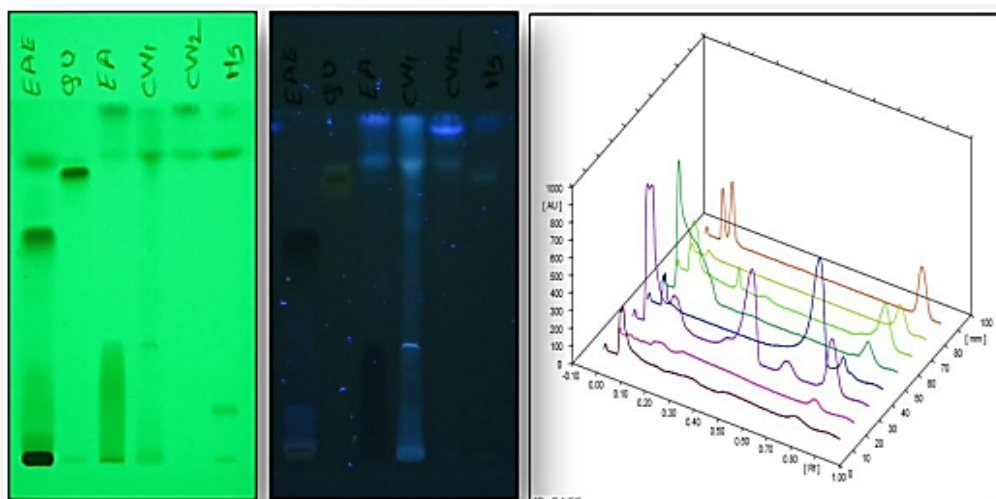


Figure 10: HPTLC Image and Scanning Graph for SXE, Analytical Markers and Elutes (Chemical Markers)

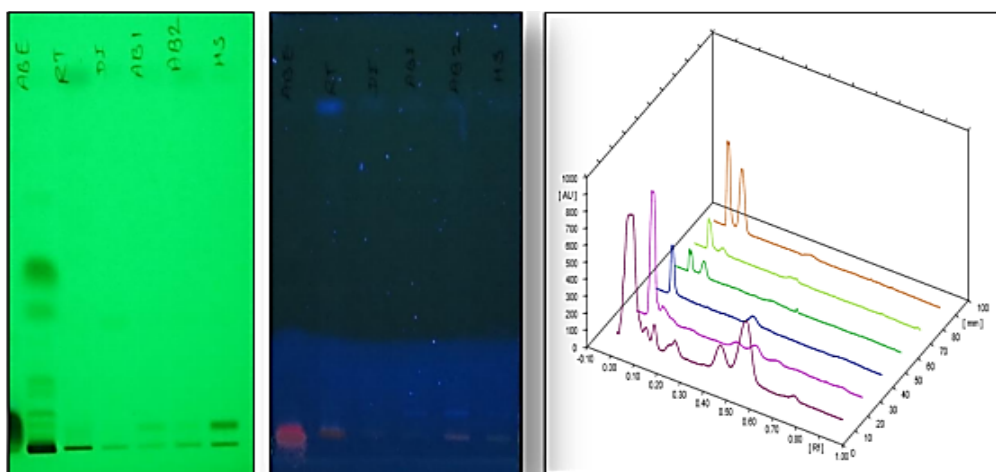


Figure 11: HPTLC Image and Scanning Graph for NAE, Analytical Markers and Elutes (Chemical Markers)

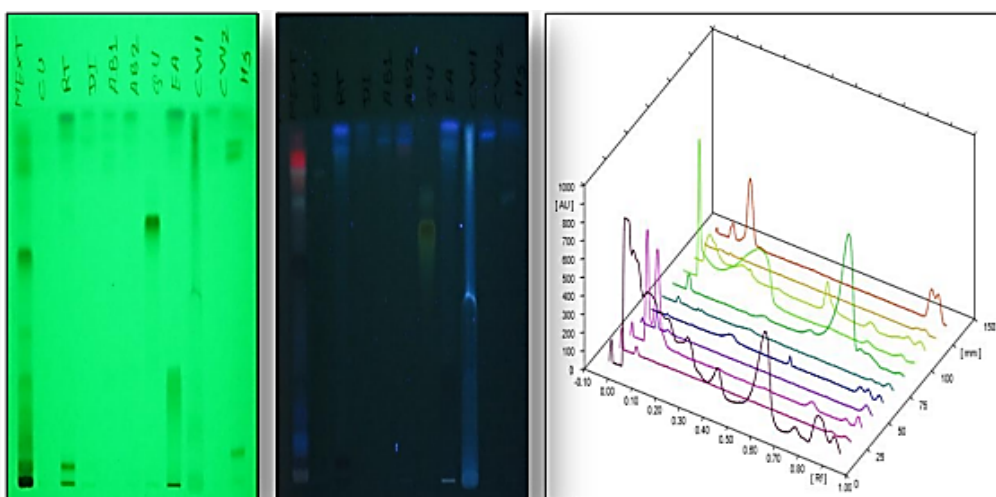


Figure 12: HPTLC Image and Scanning Graph for MEX, Extracts, Analytical Markers and Elutes (Chemical Markers)

**Table 8: Quantification of Analytical and Chemical Markers from Extracts by HPTLC**

Sr. No	Extracts	Analytical Markers / Chemical Markers	% Assay	RSD
1.	CSE	Saponins	98.20	0.16
2.	SXE	Quercetin	0.82	0.01
		Ellagic acid	1.08	0.04
		Hesperidin	0.65	0.01
		CW-1	0.44	0.05
		CW-2	1.97	0.07
3.	NAE	Diosmin	11.07	0.04
		Rutin	0.63	0.04
		AB-1	0.45	0.11
		AB-2	0.55	0.19
4.	MEXE	Saponins	60.32	0.38
		Quercetin	0.61	0.01
		Ellagic acid	3.96	0.13
		Hesperidin	0.82	0.20
		CW-1	0.28	0.11
		CW-2	0.56	0.22
		Diosmin	2.64	0.10
		Rutin	0.55	0.31
		AB-1	0.25	0.18
		AB-2	0.55	0.06"

### Pharmacological Screening

#### LD<sub>50</sub> values:

Root fluids that have been concentrated in either methanol or water from three different plants: *Nyctanthus arbortristis* L., *Solanum xanthocarpum*,

and *Clerodendrum serratum*. Additionally, the LD<sub>50</sub> has been established in order to determine the LD<sub>50</sub>. These findings from the acute toxicity research are presented in table 11 for your perusal and consideration.

**Table 9: Finalized Effective dose and obtained LD<sub>50</sub> (lethal dose)**

Sr. No.	Extract	LD <sub>50</sub> value (Mg / kg b.wt.)	Effective dose (Mg / kg b.wt.)
1	Methanolic extract of <i>Clerodendrum serratum</i>	3000	300
2	Aqueous extract of <i>Clerodendrum serratum</i>	3000	300
3	Methanolic extract of <i>Solanum xanthocarpum</i>	2000	200
4	Aqueous extract of <i>Solanum xanthocarpum</i>	2000	200
5	Methanolic extract of <i>Nyctanthus arbortristis</i>	4000	400
6	Aqueous extract of <i>Nyctanthus arbortristis</i>	4000	400"

1/tenth of this deadly measurements was taken as viable measurement (effective dosage) for its succeeding pharmacological action or therapeutic action.

#### Acute Toxicity Study

Researchers examined numerous plant extracts for short-term hazards. The dose was based on each animal's birth weight. The maximum dose was 3,000 mg/kg during the trial. After treatment,

animals were monitored for four hours for adverse effects. Three days following observation, the animals were toxically tested. The extract at 5,000 to 3,000 milligrams per kilogram of body weight did not cause toxicity or death in rats. Maximum dosages were tolerated by all animals. The treatment did not impact animals' behavior, respiration, skin, water and food intake, or temperature (Table 12).

**Table 10: General appearance & behavioral observations of acute toxicity study for control and treated groups after treatment**

Parameter	Control Group		Test Group	
	4 hr.	72 hr.	4 hr.	72 hr.
Body weight	Normal	Not change	Not change	Not change
Temperature	Normal	Normal	Normal	Normal
Food intake	Normal	Normal	Normal	Normal
Urination	Normal	No effect	No effect	No effect
Rate of respiration	Normal	No effect	No Effect	No effect
Change in skin & Fur	No effect	No effect	No effect	No effect
Eye color	No effect	No effect	No effect	No effect
Digestion	Not observed	Not observed	Not observed	Not observed
Diarrhea	Not present	Not present	Not present	Not present
General physique	Normal	Normal	Normal	Normal
Death	None	None	None	None

No organ weight changes were seen following extract administration. The extract therapy did not impair the vital organs of the study—the small intestine, pancreas, heart, kidneys, and liver. The investigation indicated that animals could tolerate dosages over 3,000 mg/kg. Since the animals showed no clinical indications of toxicity, the extract appeared harmless. Each plant extract cumulatively altered the test group's general observation, behavioural observation, autonomic function, and neurologic function. Organs vary in weight.

### Summary and conclusion

The findings from the current investigation on polyherbal formulations indicate possible anti-inflammatory action. These actions may result from the significant presence of phenolic chemicals, alkaloids, flavonoids, tannins, and steroids. The study's findings suggest that the polyherbal formulation including *Clerodendrum serratum*, *Solanum xanthocarpum* Schrad. & Wendl, and *Nyctanthus arbortristis* L. is a significant source of physiologically active chemicals with anti-inflammatory properties (28-30).

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