



**INTERNATIONAL JOURNAL OF
PHARMACEUTICAL SCIENCES**
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: <https://www.ijpsjournal.com>



Review Article

A Comprehensive Review On: Phytochemical Screening, Standardization, & Pharmacological Activities of Polyherbal Plants

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ARTICLE INFO

Published: 21 May. 2026

Keywords:

Senna siamea, Achyranthes aspera, Tagetes erecta, Phytochemical Screening, Standardization, Extraction, Anti-inflammatory, Antioxidant, wound healing activity.

DOI:

10.5281/zenodo.20323184

ABSTRACT

A medicinal plants Senna siamea (Siamese cassia), Achyranthes aspera (chaff flower), and Tagetes erecta (African marigold) have been traditionally used in ethnomedicine for their anti-inflammatory, antimicrobial, and antioxidant properties. The goal of the current study is to conduct a thorough inquiry that includes Senna siamea, Achyranthes aspera, Tagetes erecta Authentication, isolation, extraction, separation, phytochemical Screening, and standardization. To separate and isolate Bioactive components from the crude extracts, chromatographic Techniques such as High-Performance Liquid chromatography (HPLC), HPTLC, and column Chromatography were used. All three plants had alkaloids, flavonoids, tannins, saponins, phenols, and terpenoids, according to phytochemical analysis; Senna siamea had a high flavonoid content, Achyranthes aspera had a lot of alkaloids and saponins, and Tagetes erecta had a lot of terpenoids and phenols as Phytoconstituents. Sennosides (in Senna siamea), achyranthine (in Achyranthes aspera), and patuletin/quercetin (in Tagetes erecta) are examples of marker compounds that were identified and quantified using quantitative standardization using HPTLC and HPLC. No acute toxicity up to 2000 mg/kg, these standardized extracts demonstrated notable pharmacological properties, such as antioxidant, anti-inflammatory, antibacterial, analgesic, and antidiabetic actions, confirming their ethnomedical applications and validating conventional usage for wound-healing, anti-arthritic, and laxative purposes. The study supports the use of Senna siamea, Achyranthes aspera and Tagetes erecta in both traditional and contemporary medicine by offering a scientific foundation for its identification, quality assurance, and therapeutic application.

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



INTRODUCTION

Senna siamea Linn. belongs to the family **Fabaceae**(Caesalpinioideae). It is commonly called kassod tree or yellow cassia, was introduced to Africa from tropical Asia. It is widely grown throughout tropical Africa, India. Many of the parts such as leaves, pods, roots, and fruits of the natural plants have beneficial pharmacological properties against diseases. Traditionally *Senna siamea* is used for the treatment of typhoid fever, jaundice, abdominal pain, menstrual pain and is also used to reduce sugar level in the blood. Ethno medicinally *Senna siamea* is used as laxative, blood cleaning agent, cure for digestive system and genitourinary disorders, herpes. *Achyranthes aspera* Linn. belongs to the family **Amaranthaceae** is a perennial stiff, erect herb, 2.0 m high is growing up to 1000 m in height. *Achyranthes aspera* is a rough flowered stalk is described as in Sanskrit synonyms. The medicinal plants are used for the treatment of various diseases because of their safety and effectiveness. Though almost all of its parts are used in traditional systems of medicines, seeds, roots, and shoots are the most important parts which are used medicinally. *Achyranthes aspera* L. also known as Prickly chaff flower in English. The plant is Highly esteemed by traditional healers and used in Treatment of asthma, bleeding, in facilitating Delivery, boils, cold. *Tagetes erecta* Linn. belongs to family **Asteraceae** containing about 50 species of annual or perennial herbaceous plant. The plant *Tagetes Erecta* L. is locally known as Genda Phool (Marigold). *Tagetes erecta* shows diverse pharmacological activities like, Antibacterial, Anti-inflammatory, Hepatoprotective activity, Anticancer activity, Anti-epileptic, Anti-fungal, Wound-healing activity. Different parts of *Tagetes erecta* plant including flower are used as a traditional medicine to cure Various diseases. Leaves of this plant are

used as Antiseptic, in kidney troubles, muscular pain, piles. There also includes a number of analytical and standardization factors; such as macroscopic and microscopic evaluation, physicochemical analysis, extractive values, ash values, moisture content, microbial contamination and chromatographic techniques. The study of crude pharmaceuticals derived from natural sources, including plants, animals, And minerals, with a focus on their macroscopic, microscopic, physicochemical, and Phytochemical properties, is known as **Pharmacognosy**. As a result, the pharmacognostic study of *Senna siamea*, *Achyranthes aspera*, *Tagetes erecta* is crucial for determining the Plant material's phytochemical components, standardization criteria, and diagnostic characteristics. These investigations support its safe and efficacy.

Synergistic Effects Of Polyherbal Combinations:-

Because *Senna siamea*, *Achyranthes aspera*, and *Tagetes erecta* contain a variety of bioactive phytoconstituents, including flavonoids, alkaloids, tannins, saponins, terpenoids, phenolic compounds, glycosides, and carotenoids, the polyherbal combination shows notable synergistic effects. Compared to separate extracts, the pharmacological properties of these plants are enhanced when they are combined in a polyherbal formulation, which improves therapeutic efficacy. This polyherbal combination's synergistic anti-inflammatory action is primarily ascribed to the suppression of inflammatory mediators like prostaglandins, cytokines, histamine, and nitric oxide. additionally, by encouraging collagen production, epithelialization, angiogenesis, and quicker wound contraction, the combination exhibits synergistic wound-healing efficacy. All three plants include antioxidant components that enhance cellular regeneration and shield injured



tissues from damage caused by free radicals. Furthermore, because the three plants' phenolic and flavonoid constituents work together to scavenge reactive oxygen species and lessen oxidative damage, the polyherbal mixture exhibits increased antioxidant activity. Phytochemicals work in concert to boost the formulation's stability, therapeutic potency, and bioavailability

while lowering the necessary dosage and potential adverse effects of single-herb therapy. For anti-inflammatory, antibacterial, antioxidant, and wound-healing purposes, the polyherbal combination of *Senna siamea*, *Achyranthes aspera*, and *Tagetes erecta* can therefore be a potential natural therapeutic system.

Parameter	<i>Senna siamea</i>	<i>Achyranthes aspera</i>	<i>Tagetes erecta</i>
Image			
Synonyms	<i>Cassia siamea</i>	Prickly Chaff Flower	African Marigold
Common Names	Marathi- Kashid Hindi- Kassode Tree Sanskrit- Rajataru	Marathi- Aghada Hindi- Apamarga Tamil- Nayuruvi	Marathi- Zendu Hindi- Genda Tamil- Sammanti
Kingdom	Plantae	Plantae	Plantae
Genus	Senna	Achyranthes	Erecta
Species	Siamea	Aspera	Tagetes
Order	Fabales	Caryophyllales	Asterales
Family	Fabaceae	Amaranthaceae	Asteraceae
Chemical Constituents	Major:- Flavonoids(Quercetin) Minor:- Saponin,Tannins, Anthraquinones	Major:- Alkaloids (Achyranthin) Minor:- Triterpenoids, Phenolic compounds	Major:- Carotenoids (Lutein) Minor:- Glycosides, Saponins, Phenolic compounds
Habit	Medium evergreen tree	Annual/perennial herb	Aromatic annual herb
Geographical Distribution	Found in India, Thailand, Sri Lanka, and tropical regions	Found in India, Nepal, Sri Lanka, and other Asian countries.	Widely cultivated In India and tropical countries.
Category	Antioxidant properties	Anti-Inflammatory	Wound-healing

Plant Profile:-

Macroscopic Characteristics:-

1. ***Senna siamea***:- The leaves were examined with unaided senses (naked eyes). The color is examined using An untreated sample under diffuse daylight. Artificial Light source similar



to those of daylight was also used Occasionally. The organoleptic Properties such as color, texture, odor, and taste of the Plant material were also observed.

- **Leaves:-** Compound, paripinnate leaves with 6–12 pairs of leaflets.
- **Flowers:-** Bright yellow, arranged in terminal clusters.
- **Fruits:-** Flat, elongated pods, dark brown to black when mature.
- **Height:-** About 10–20 m
- **Odor:-** Slight characteristic odor.
- **Taste:-** Slightly bitter.

2. ***Achyranthes aspera:-*** Plants are erect, woody, tall herb, leaves Elliptical, ovet, elliptic lanceolate, 3-6 x 2-3.5cm. Membranous, acute or rounded at base silky Beneath, flower pale green in terminal, Simple and Branched spike reaching 30cm long in fruit, utricles At apex, rounded at base, brown. The dried leaves had a characteristics odor and were slightly Mucilaginous in taste.

- **Leaves:-** Simple, opposite, ovate or elliptic leaves.
- **Flowers:-** Small, greenish-white flowers in long spikes.
- **Fruits:-** Small utricle containing a single seed.
- **Height:-** About 0.5–1 m
- **Odor:-** Slight characteristic odor.
- **Taste:-** Bitter and slightly acrid.

3. ***Tagetes erecta:-*** The plant usually grows up to 30–90 cm in height and possesses a bushy, branched appearance. The leaves are pinnately divided, dark green in color, and arranged oppositely at the lower part and alternately at the upper part of the stem.

- **Leaves:-** Pinnately divided, lanceolate leaflets with serrated margins.
- **Flowers:-** Large yellow to orange flower heads.
- **Fruits:-** Black, slender achene.
- **Height:-** About 30–90 cm
- **Odor:-** Strong aromatic odor.
- **Taste:-** Slightly bitter and pungent.

Materials & Methods:-

❖ Plant Collection and Authentication:-

The verification of the plants under research is a crucial component of the technique because there are multiple species from the same genus and they may resemble other plants physically. The proper plant species and plant parts utilized as raw materials for scientific research on therapeutic plants are guaranteed by authentication. The healthy plants was collected aseptically with the help of secateurs, photographed, documented, and placed in paper bags. The collected plants of *senna siamea*, *Achyranthes aspera*, *Tagetes erecta* was Identified & verified based on their taxonomic features by referring the standard floras and manuals by Botanist through Botanical Survey.

❖ Preparation of plant material:-

The collected plant samples was washed Thoroughly with the running tap water to remove The adhered soil and peripheral dust particles. The



Completely dried plant material was pulverized in Mechanical grinder to get the fine powder. The Pulverized plant material was stored in zip lock Polythene covers at room temperature until Subjected for the extraction process.

❖ **Extraction of Active Constituents from *S. siamea*, *A. aspera*, *T. erecta* leaves:-**

Powered leaves weighing about fifty (50) grams each were macerated for twenty-four hours at room temperature in 500 milliliters of distilled water and methanol, respectively. After filtering each preparation through Whatman filter paper, the aqueous filtrate was dried in a water bath at 400C while the methanol extract was dried in a rotary evaporator at 500C. The residue was then diluted with 10% Dimethylsulphoxide (DMSO) to create 100 mg/ml of the extract, from which different concentrations of 50, 40, 30, 20, and 10 mg/ml.

Phytochemical Screening Of Crude Ethanol & Aqueous Extracts (*senna siamea*, *Achyranthes aspera*, *Tagetes erecta*) leaves:-

A] Test for *Senna siamea* leaves :-

1. Test for Phenols (Ferric Chloride Test):-

About 2ml of plant extract was treated with 5% of FeCl₃ solution, the formation of deep blue or black Colour indicates the presence of phenols.

2. Test for Tannins (Gelatin Test):-

About 2 ml of aqueous solution of plant extract Was mixed with 1% of gelatin solution to this Mixture around 10% NaCl solution was added. The formation of white precipitation specifies the Presence of tannins.

3. Test for Alkaloids:- (Mayer's Test):-

To the 2 ml of extract, 2 ml of Mayer's reagent Was added. An organic dull white or cream colour Precipitate indicates the presence of alkaloids.

4. Test for Flavonoids (Alkaline Reagent Test):-

About 2 ml of plant extract was treated with 20% NaOH solution. A formation of intense yellow Colouration confirms the presence of flavonoids.

5. Test for Terpenoids (Salkowski's Test):-

To 2 ml of extract, 2 ml of chloroform was added. To this mixture around 2 ml of concentrated H₂SO₄ was added gently along the sides of test Tube. The formation of reddish-brown monolayer Colouration at the interface, confirms the presence of terpenoids.

B] Test for *Tagetes erecta* leaves :-

1. Test for alkaloids:- Solvent free extracts, 50 mg was stirred with few ml of dilute Hydrochloric acid and filtered separately. The filtrate was tested carefully with various Alkaloid reagents as follows:-

❖ **Wagner's test:-**

To 1 ml filtrate of the extract, 0.5 ml of Wagner's reagent was added by the Side of the test tube. Reddish brown precipitate was formed and that was indicated as the Presence of alkaloids.

❖ **Hager's test:-**

To 1 ml filtrate of the extract, 0.5 ml of saturated picric acid solution (Hager's Reagent) was added by the side of the test tube. Yellowish precipitate was formed and that Was indicated as the presence of alkaloids.

❖ **Dragendroff's test:-**



To 1 ml filtrate of the extract, 0.5 ml of Dragendorff's reagent (bismuth Nitrate) was added by the side of the test tube. A prominent yellow or orange brown Precipitate was formed and that was indicated as the presence of alkaloids.

2. Test for Tannins:-

❖ Potassium dichromate test:-

2 ml solution of the extract was taken in a test tube. Then 0.5 ml Of 10% potassium dichromate solution was added. A yellow precipitate was formed indicates The presence of tannins.

3. Test for saponins:-

The extracts were diluted with 20 ml of distilled water separately and Further shaken for 15 min in a graduated cylinder. A layer of foam measuring about 1 cm was Formed which indicated the presence of saponins.

4. Test for glycosides:-

Extracts were hydrolyzed with dilute HCl and then subjected to test for Glycosides. Extracts were treated with Ferric Chloride solution and immersed in boiling water For about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose pink Colour in the ammonical layer showed the presence of glycosides.

5. Test for steroids:-

The extracts were treated with chloroform and filtered. The filtrates were Treated with few drops of acetic anhydride boiled and cooled concentrated sulphuric acid was Added through the sides of the test tube. The formation of brown coloured ring at the junction Of two liquids confirmed the presence of steroids.

C] Test for *Achyranthes aspera* leaves:-

1. Test for Carbohydrates:-

❖ Molisch's test:-

2ml f the extract was dissolved in 5 ml of Distilled water and filtered. To the filtrate Solution, A few drops of alcoholic alpha-Naphthol were added and subsequently Followed by concentrated sulfuric acid from the Sides of the test tube. The Violet ring visible at The junction indicated the presence of Carbohydrates.

2. Test for Flavonoids:-

❖ Shinoda test:-

The extract was dissolved in alcohol and a few Magnesium turnings were added to it, followed by the addition of concentrated hydrochloric acid Drop by drop. The development of pink color was Indicative of the presence of flavonoids.

❖ Alkaline reagent test:-

A small portion of the extract was dissolved in Alcohol and treated with a few drops of sodium Hydroxide solution. Observation of an intense Yellow color, which vanished upon the addition of dilute hydrochloric acid, was an indication of the presence of flavonoids.

3. Test for Saponins:-

❖ Foam tests:-

A small amount of the extracted sample was shaken with water. The persistence of form above the liquid surface for a few minutes indicated the presence of saponins.

4. Test for Cardiac Glycosides:-

❖ Legal's test:-



The extract solution was dissolved in 1 ml Pyridine and 1 ml alkaline sodium nitroprusside. Blood red color was taken as an indication of the Presence of cardiac glycosides.

❖ Keller- kilani Test:-

1 ml extract + mixed with Few drops of glacial acetic acid and Boiled for a Minute and cooled. To this solution add 2 drops of Ferric chloride Solution. The contents were Transferred to another tube containing Conc. Sulphuric Acid. Formation of reddish brown ring at the junction of 2 layers that indicates Presence of Cardiac Glycosides.

5.Test for Steroids:-

❖ **Libermann-Buchard's Test:** 1 ml extract + acetic Anhydride + Conc. H₂SO₄ Along the sides of tube. Formation of red ring at the junction Of two layers that indicates Presence of Sterols.

Phytochemical Screening Of Aqueous & Ethanol Extracts Of (*senna siamea*, *Achyranthes aspera* & *tagetes erecta*) LEAVES:-

Senna siamea, *Achyranthes aspera*, and *Tagetes erecta* aqueous and ethanol extracts were subjected to phytochemical screening, which identified a number of significant bioactive components that underlie their pharmacological actions. Carbohydrates, proteins, glycosides, tannins, phenolic compounds, flavonoids, and saponins were found in the aqueous extracts according to preliminary phytochemical analysis. In contrast, ethanol extracts showed a comparatively higher concentration and a wider range of phytochemicals because the active ingredients were more soluble in ethanol. *Senna siamea*'s anti-inflammatory, antioxidant, and antibacterial qualities are mostly attributed to the presence of alkaloids, flavonoids, anthraquinones,

tannins, and phenolic compounds. Saponins, alkaloids, steroids, terpenoids, flavonoids, and glycosides all of which have anti-inflammatory, analgesic, and wound-healing properties. were found in *Achyranthes aspera*. Also flavonoids, carotenoids, tannins, triterpenes, and essential oils all of which are important for antioxidant and wound-healing properties were also found in *Tagetes erecta* extracts. Comparative phytochemical screening revealed that ethanol extracts typically had stronger responses for the majority of phytoconstituents than water extracts, indicating that ethanol was more effective in extracting secondary metabolites. Steroids are useful in therapeutic settings because of their well known anti-inflammatory activity. The therapeutic potential of these medicinal plants is supported by the existence of these bioactive chemicals, which also justify their usage in polyherbal formulations for increased pharmacological action.

Standardization Of Plants Extracts:-

Standardization of plants is the process of establishing or prescribing a set of peculiar identities, specific characteristics which are generally unique and of ushered qualities. Pharmacognostic standardization of a plant is a process involving a series of a laboratory experiment which reveal and assemble a set of inherent peculiar characteristics such as, constant parameter, definite qualitative and quantitative values or specific and unique features on the bases of which similar herbal medicine, claimed to be the same can be compared for the purpose of authenticity, efficacy, purity, reproducibility and overall quality assurance.

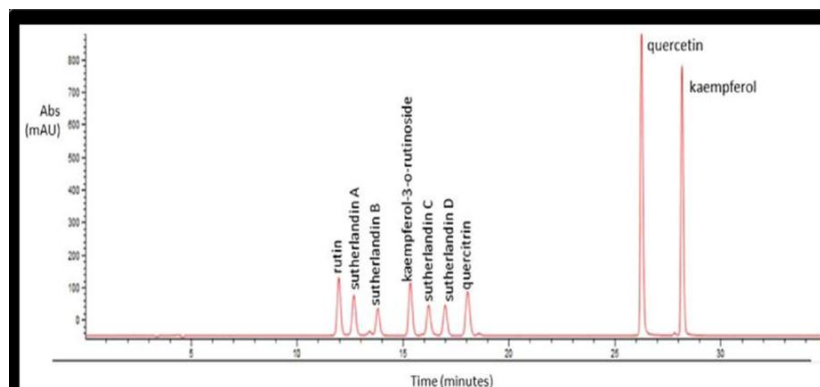
❖ Standardization of *Senna siamea* plant leaves extract:-

The standardization of leaf extracts of *Senna siamea* by High-Performance Thin-Layer



Chromatography (HPTLC) is an important analytical approach used to ensure the quality, purity, and consistency of herbal preparations. In *Senna siamea*, flavonoids, anthraquinones, and phenolic compounds such as kaempferol, chrysophanol, Quercetin are commonly selected as marker constituents. Before injection, precisely weighed extract is diluted in HPLC-grade methanol and passed through a 0.45 μ m membrane filter for HPLC standardization. A C18 column is frequently used as the stationary phase in reverse-phase HPLC (RP-HPLC). Depending on the target marker chemicals, the mobile phase often consists of acetonitrile and water containing 0.1%

orthophosphoric acid or methanol:water mixtures in optimum ratios, such as acetonitrile:water (60:40 v/v) or methanol:water (70:30 v/v). For phenolic and flavonoid chemicals, gradient or isocratic elution is performed at a flow rate of 1.0 mL/min with detection wavelengths between 254 and 280 nm. Chromatographic fingerprinting is established based on retention time, peak area, peak symmetry, and resolution of marker points. To confirm that kaempferol, chrysophanol, Quercetin was present in the extracted sample compared to the standard, The sample's R_f value, accuracy, Precision, LOD, LOQ was calculated.

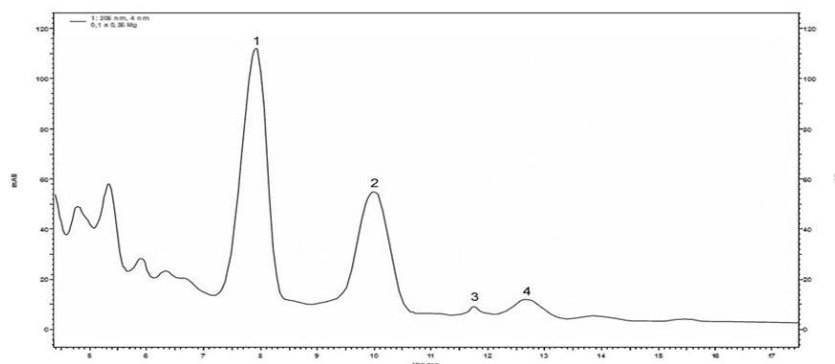


• Fig. 1- Standardization of *Senna siamea* Plant Extract by HPLC method

❖ Standardization of *Achyranthes aspera* Plant leaves extract :-

In *Achyranthes aspera*, oleanolic acid, ecdysterone, quercetin, rutin, or β -sitosterol are commonly marked Constituent. Before injection, a predetermined amount of the dried extract is dissolved in HPLC-grade methanol and passed through a 0.45 μ m membrane filter. A C18 column is frequently used for reverse-phase HPLC (RP-HPLC). Acetonitrile and water acidified with 0.1% orthophosphoric acid or formic acid in ratios like

acetonitrile:water (55:45 v/v) or methanol:water (70:30 v/v), optimized based on the polarity of target phytoconstituents, often make up the mobile phase. Depending on the marker compounds chosen, isocratic or gradient elution is carried out at a flow rate of 1.0 mL/min, and detection is often done at wavelengths between 210 and 280 nm. To confirm that oleanolic acid, ecdysterone, quercetin, rutin was present in the extracted sample compared to the standard, sample's R_f value, accuracy, Precision, LOD, LOQ was calculated.

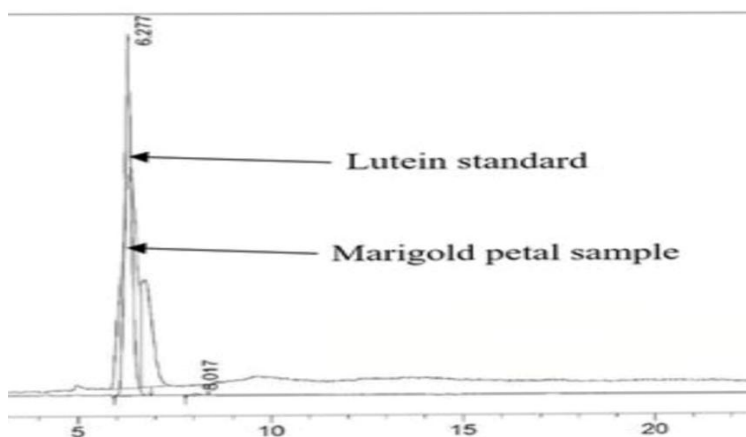


• Fig. 2- Standardization of *Achyranthes aspera* extract by HPLC method

❖ Standardization of *Tagetes erecta* plant leaves extract:-

In *Tagetes erecta*, Lutein, quercetin, patuletin, and quercetagitrin are commonly marked Constituents. Prior to analysis, the dried extract is precisely weighed, diluted in HPLC-grade methanol, and filtered through a 0.45 μm membrane filter for HPLC standardization. A C18 column is frequently used as the stationary phase in reverse-phase HPLC (RP-HPLC). Depending on the polarity of the marker chemicals, the mobile phase often consists of acetonitrile and water acidified

with 0.1% orthophosphoric acid or methanol:water mixtures in optimum ratios, such as acetonitrile:water (65:35 v/v) or methanol:water (75:25 v/v). Flavonoids and carotenoids, especially lutein, are typically detected at wavelengths between 254 and 450 nm using gradient or isocratic elution at a flow rate of 1.0 mL/min. To confirm that Lutein, quercetin, patuletin, quercetagitrin was present in the extracted sample compared to the standard, sample's R_f value, accuracy, Precision, LOD, LOQ was calculated.



• Fig. 3- Standardization of *Tagetes erecta* extract by HPLC method

Physico- Chemical Analysis:-

❖ For *Senna siamea*:-

Physicochemical Analysis of *Senna siamea* is the purpose of this analysis is to determine the crude

drug's legitimacy, purity, and quality control standards. These metrics aid in determining stability, identifying adulteration, and guaranteeing the repeatability of herbal compositions.

1. Moisture Content:- The amount of moisture and volatile materials in the powdered medication is determined by the loss during drying. Microbial growth and phytoconstituent breakdown may be facilitated by excessive dampness. Senna siamea powdered leaves are dried at 105°C until they reach a consistent weight. The moisture content and appropriate drying and storing conditions are reflected in the percentage decrease in weight.

2. Total Ash Value:- The total amount of inorganic and mineral content in the crude medication after full incineration is represented by the total ash value. It contains both non-physiological ash from outside pollutants like dust and soil and physiological ash from plant tissue. Senna siamea's purity and quality can be evaluated by measuring its total ash. **3. Acid-Insoluble Ash:-** The amount of siliceous matter in the plant sample, such as sand, silica, and earthy components, is measured by acid-insoluble ash. After treating the entire amount of ash with diluted hydrochloric acid, the insoluble part is gathered and weighed. A low acid-insoluble ash value suggests that the crude medication is of higher quality and has less contamination.

4. Analysis of Fluorescence:- By applying several chemical reagents to the powdered medication and monitoring color changes under visible and ultraviolet light, fluorescence analysis is carried out. Because Senna siamea contains a variety of phytochemicals, it displays distinctive fluorescence behavior. This technique acts as a quick identifying tool for adulteration detection and authentication.

❖ For *Achyranthes aspera*:-

Achyranthes aspera physicochemical analysis is a crucial component of pharmacognostic standardization, which establishes the crude drug's identity, purity, quality, and authenticity. These

criteria guarantee uniformity in herbal formulations while assisting in the detection of adulteration, contamination, and incorrect management of plant resources.

1. Moisture Content:- The amount of moisture and volatile materials in the crude medication is determined by the loss during drying. Microbial development, fungal contamination, and the breakdown of active phytoconstituents can all result from excessive dampness. Until a consistent weight is reached, the powdered plant material is dried at 105°C. The moisture content and appropriate drying and storing conditions are indicated by the percentage weight loss.

2. Total Ash Value:- It comprises non-physiological ash from outside pollutants like dust, soil, and sand as well as physiological ash from plant tissues. This metric aids in assessing *Achyranthes aspera*'s quality and purity.

3. Ash Soluble in Water:- The amount of inorganic components that are soluble in water is represented by water-soluble ash. It is calculated by deducting the weight of ash that is insoluble in water from the overall ash value. This characteristic tells us whether the plant material contains inorganic salts and water-soluble minerals.

4. Analysis of Fluorescence:- In order to do fluorescence analysis, the powdered medication is treated with different chemical reagents, and color changes under visible and ultraviolet light are observed. *Achyranthes aspera*'s phytochemical components lead it to exhibit distinctive fluorescence behavior. This method works well for both adulteration detection and authentication.

❖ For *Tagetes erecta*:-



A crucial component of pharmacognostic standardization that determines the identification, purity, quality, and authenticity of the crude medicine is the physicochemical analysis of *Tagetes erecta*. In order to ensure consistency and safety in herbal formulations, these criteria aid in the detection of adulteration, contamination, and variances in plant components.

1. Moisture Content:- The amount of moisture and volatile materials in the crude medication is ascertained by measuring the loss on drying. Excessive moisture can encourage fungal contamination, microbial growth, and the breakdown of active phytoconstituents. This process involves drying the powdered plant material at 105°C until a consistent weight is achieved. The moisture content and appropriate drying and storage conditions of *Tagetes erecta* are reflected in the percentage of weight loss.

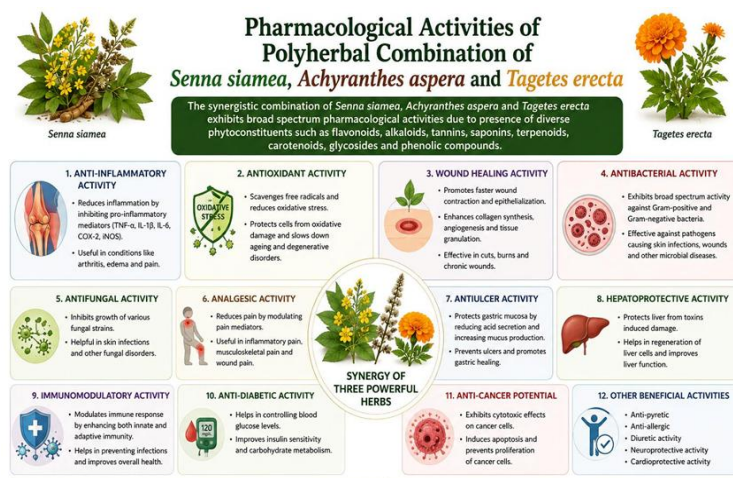
2. Total Ash Value:- The entire amount of inorganic wastes left over after the plant material has been completely burned is represented by the total ash value. It comprises non-physiological ash from outside pollutants including sand, dust, and soil as well as physiological ash from plant tissues. Total ash measurement aids in assessing *Tagetes erecta*'s quality and purity.

3. Acid-Insoluble Ash:- The amount of siliceous materials, including silica, sand, and earthy contaminants, in the crude medicine is measured by acid-insoluble ash. After treating the entire ash

with diluted hydrochloric acid, the insoluble residue is gathered and weighed. Less contamination and higher-quality plant material are indicated by a low acid-insoluble ash value

Pharmacological Activity:-

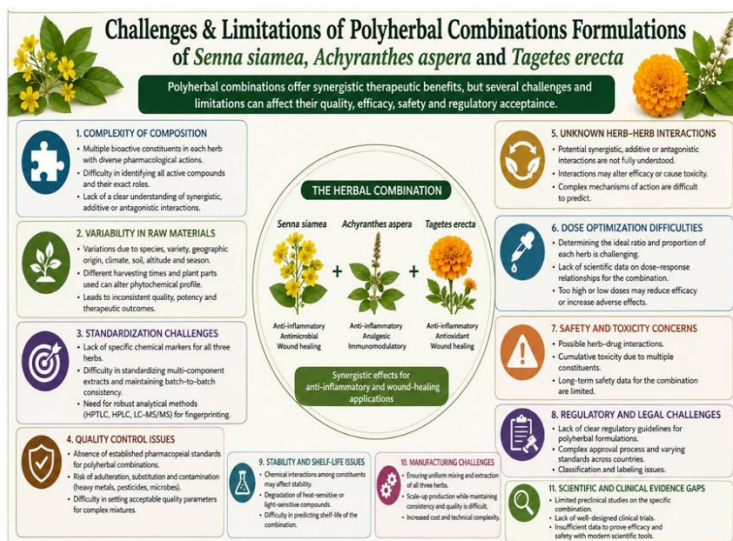
- ❖ **Antioxidant Activity:-** An antioxidant is defined as any substance that when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate. Many antioxidant compounds such as barakol, vitamin C, Vitamin E, carotenoids, flavonoid, phenolic acids, and diverse enzymes.
- ❖ **Antibacterial Activity:-** *Senna siamea* (leave) has been valued for its use in the treatment of infectious diseases. Recently, interest in *Senna siamea* has focused on its antibacterial activity evaluated against various Gram positive and Gram negative bacteria species by using cylinder plate assay.
- ❖ **Anti-inflammatory, Analgesic, Antipyretic Activity:-** Ethanol and aqueous extracts of *Senna siamea*'s leaves and stem bark (100 – 400 mg/kg, po, for 4 hours) had significant dose-dependent anti-inflammatory, analgesic and antipyretic activities using experimental rat models ($p < 0.01$). The analgesic and antipyretic effects of these extracts were more important than paracetamol (50 mg/kg, po) and morphine (2 mg/kg, po) ($p < 0.001$).



Challenges & Limitations Of Polyherbal Combinations:-

The synergistic anti-inflammatory, antibacterial, antioxidant, and wound-healing properties of polyherbal preparations containing *Senna siamea*, *Achyranthes aspera*, and *Tagetes erecta* are

extensively investigated. The creation, standardization, safety assessment, and clinical application of these herbal combinations present a number of difficulties and restrictions, despite the fact that they offer improved therapeutic efficacy when compared to single-herb formulations.



CONCLUSION:-

In Conclusion, the presence of these significant secondary metabolites is confirmed by preliminary phytochemical screening of alcoholic and aqueous extracts, showing their function in hepatoprotective, immunomodulatory, anti-inflammatory, antioxidant, antibacterial, analgesic, and wound-healing properties.

Flavonoids, alkaloids, tannins, saponins, glycosides, terpenoids, phenolic compounds, carotenoids, anthraquinones, and essential oils are just a few of the many physiologically active phytoconstituents found in these plants. Reliable quality control methods for the authentication and repeatability of crude pharmaceuticals and polyherbal formulations are provided by parameters including ash values, moisture content,

pH determination, and chromatographic fingerprinting by HPTLC and HPLC. *Senna siamea*'s flavonoids and anthraquinones are primarily responsible for anti-inflammatory, antimicrobial, antioxidant, laxative, and hepatoprotective properties. Alkaloids, saponins, and ecdysteroids are responsible for *Achyranthes aspera*'s strong anti-inflammatory, analgesic, and wound-healing effects. The antioxidant, antibacterial, anti-inflammatory, and tissue-regenerative properties of *Tagetes erecta* are attributed to its abundance of flavonoids, carotenoids, lutein, and essential oils. By working through several biochemical routes, increasing bioavailability, lowering toxicity, and improving overall efficacy in comparison to single-herb therapy, these plants may elicit synergistic therapeutic effects when combined in a polyherbal formulation. As a result, the polyherbal combination of *Tagetes erecta*, *Achyranthes aspera*, and *Senna siamea* represents an important natural therapeutic approach with substantial pharmacological potential. The creation of safe, efficient, stable, and standardized herbal formulations for a variety of therapeutic uses, especially in the treatment of inflammation and wound healing, can be facilitated by appropriate phytochemical screening, standardization, and scientific validation.

ACKNOWLEDGEMENT: -

I would like to express my sincere gratitude to all those who have supported and guided me throughout the completion of this study on Phytochemical Screening standardization, and pharmacological activities of *Senna siamea*, *Achyranthes aspera*, *Tagetes erecta*. I am deeply thankful to my guide for their invaluable guidance, constant encouragement, and insightful suggestions that greatly contributed to the successful completion of this work. Their

expertise and motivation helped me stay focused and dedicated throughout the research Process.

RESULT: -

In result, number of significant bioactive components, such as flavonoids, alkaloids, tannins, saponins, glycosides, phenolic compounds, terpenoids, steroids, anthraquinones, carotenoids, and essential oils, were found in preliminary phytochemical screening of aqueous and ethanolic extracts. Because phenolic and somewhat polar chemicals are more soluble in alcohol, ethanolic extracts demonstrated a richer phytochemical profile and a higher extraction efficiency than water extracts. The three therapeutic plants' phytoconstituents may interact synergistically, as evidenced by the increased phytochemical diversity of the mixed polyherbal extract. All quality control values were within acceptable pharmacognostic ranges, according to physicochemical standardization studies. Proper drying and low water content were indicated by moisture content or loss on drying values, which decreased the possibility of microbial contamination and the deterioration of active ingredients. The values of total ash, acid-insoluble ash, and water-soluble ash attested to the crude pharmaceuticals' purity and low levels of inorganic matter, silica, or earthy material contamination. Higher aqueous and alcoholic extractive values were found in extractive value assays, suggesting the presence of significant amounts of water-soluble and alcohol-soluble phytochemicals. In order to ensure appropriate standardization, authentication, and batch-to-batch consistency of the polyherbal formulation, chromatographic studies employing HPLC revealed distinctive fingerprint profiles and verified the presence of key phytochemical markers like flavonoids, phenolic compounds, and terpenoids.



Significant anti-inflammatory, antioxidant, antibacterial, analgesic, immunomodulatory, and wound-healing properties were found in the polyherbal combination's pharmacological examination. Prostaglandins, cytokines, nitric oxide, and cyclooxygenase enzymes are examples of inflammatory mediators that the formulation successfully suppressed, reducing inflammation, edema, discomfort, and tissue damage. The findings imply that the synergistic interaction of several phytoconstituents gives the polyherbal combination of *Senna siamea*, *Achyranthes aspera*, and *Tagetes erecta* exceptional therapeutic efficacy. The results demonstrate the potential use of these plants in the creation of safe, efficient, and standardized herbal formulations for the treatment of inflammatory disorders, microbial infections, oxidative stress-related conditions, and wound-healing management.

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HOW TO CITE: Sayali Panmand*, Kajal Walunj, Payal Padwal, Nisha More, Ansari shabboparvin, A Comprehensive Review On: Phytochemical Screening, Standardization, & Pharmacological Activities of Polyherbal Plants, Int. J. of Pharm. Sci., 2026, Vol 4, Issue 5, 5366-5381.
<https://doi.org/10.5281/zenodo.20323184>