

Phytochemical Screening and Evaluation of *Tripleurospermum disciforme*, *Tagetes Minuta*, And *Retama Raetam* Plant

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ABSTRACT

In order to identify the bioactive components of three medicinal plants—*Tripleurospermum disciforme*, *Tagetes minuta*, and *Retama raetam*—and determine their suitability for creating polyherbal antimicrobial formulations, the current study focuses on phytochemical screening and evaluation. The chosen plants' powdered flowers were gathered, verified, shade-dried, extracted using increasingly polar solvents (petroleum ether, ethyl acetate, methanol, acetone/water, and aqueous), and then subjected to qualitative phytochemical analysis. Standard WHO criteria were used to establish the organoleptic evaluation and physicochemical characteristics, such as total ash value, loss on drying, solubility analysis, extractive values, and volatile oil content. Major secondary metabolites, including alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, steroids, glycosides, saponins, and volatile oils, were found in varied amounts during preliminary screening. Significant amounts of alkaloids and flavonoids were found in *Retama raetam*, terpenoids, volatile oils, and flavonoids were found in *Tripleurospermum disciforme*, and thiophenes, flavonoids, phenolics, and essential oil components were found in *Tagetes minuta*. The potential use of these bioactive components in the creation of polyherbal topical gels is supported by their well-known antibacterial, antioxidant, and wound-healing properties. The results demonstrate these species' potential for development into strong antibacterial herbal medicines and offer a scientific foundation for their traditional therapeutic usage.

Keywords: *Tripleurospermum disciforme*, *Tagetes minuta*, *Retama raetam*, antimicrobial, against *Escherichia coli*.

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INTRODUCTION

Herbal remedies are essential for both human and animal health. For their medical needs, a sizable section of the emerging nation's population still relies on herbalists and herbs [1]. In addition to providing medications, plants also provide humans with food, shelter, and other necessities. Early man had to consider illness and its cure at the beginning of human intellect because disease, deterioration, and death coexisted with life [2]. Since 2000 years ago, humans have employed plants as a source of medicine to treat illnesses [3]. Plants continue to be the primary source of drugs in both Ayurvedic and contemporary medicine, despite numerous improvements in the discipline. Of the 248,000 species of higher plants that have been recognized, nearly 12,000 are known to have therapeutic qualities [4]. The main goal of ongoing research on plants is to identify new secondary metabolites or phytochemicals that come from plants that have human-protective properties [5]. Before using a plant, it

becomes essential to verify its authenticity and assess its quality [6]. Pharmacognostic research, which aids in the identification and verification of a plant material, is used to accomplish the standardization process. For standardization and quality control, the majority of pharmacopeias and regulatory recommendations advise macroscopic and microscopic investigations in addition to the chemical behavior of herbal materials [7].

The Asteraceae family's "Plain Chamomile" is *Tripleurospermum disciforme* (C.A.Mey.) Sch.Bip. [8]. This plant has been grown across Iran and is used in Iranian herbal medicine as a sedative and anti-inflammatory, as well as for relaxation, weariness, muscle soreness, and stress relief [9]. There have been numerous traditional and folk uses for it in Iran [10], but there have been few reports of its antibacterial properties [11]. Recent research indicates that this species' essential oil contains anti-inflammatory [12–14], antispasmodic and antiseptic [8], antifungal [16–

17], antibacterial [11, 18], and antioxidant properties [19–20]. The chemical makeup of *T. disciforme* essential oil has not received much attention up to this point. This essential oil's primary components are (E)- β -farnesene, β -sesquiphellandrene, p-methoxy- β -cyclopropylstyrene, and cis-calamenene [18, 20-25].

At least 56 species make up the significant genus *Tagetes*, which is a member of the Asteraceae family [26]. Numerous bioactive substances, such as polyphenolic, carotenoids, and terpenes, which have biological qualities like antibacterial, antioxidant, antiviral, and anticancer activity, are found in these plants [27–28]. Numerous nations have conducted extensive research on volatile oils that are derived from *Tagetes minuta*'s leaves and aerial parts [29–30]. Many of the medicinal plants used in Yemen have not been investigated, despite their widespread use [31–32]. *Tagetes Minuta* L. is used to treat skin conditions and grows in several parts of Yemen, notably AlDhalea (Ashaib) [33]. The essential oil of *Tagetes minuta* from Dhamar has been shown to exhibit cytotoxic, antioxidant, and antibacterial properties in earlier studies [34]. The pharmacognostical, phytochemical, antioxidant, and antibacterial qualities of *Tagetes minuta* L.'s aerial portions have not been thoroughly studied, despite the plant's traditional applications. This study aims to assess the aerial portions of *Tagetes minuta* L.

AUTHENTICATION OF PLANT RETAMA RAETAM

Taxonomical

Table 1.1: Taxonomy Profile of Retama raetam Plant

Kingdom	Plantae
Phylum	Tracheophytes
Class	Angiosperms
Order	Fabales
Family	Fabaceae
Genus	Retama
Species	R.raetam

Retama raetam is a species of flowering plant in the family Fabaceae, native to northern Africa from the Western Sahara to Sudan, Sicily, Israel, Sinai Peninsula, the Palestine region and Saudi Arabia, and widely naturalized elsewhere.

Morphological Description

An evergreen stem-assimilating desert plant, the white weeping broom is a shrub that grows to about 3 m. and may be 6 m. across. The plants are grey-green with slender, drooping branches; the young plants are wispy,

for their anatomical, physicochemical, phytochemical, antioxidant, and antibacterial qualities.

The perennial plant *Retama raetam* (Frosk) Webb. & Berthel. (Fabaceae) is found throughout the Mediterranean region and North Africa [35–37]. *R. raetam* is used in Algerian traditional medicine to treat diabetes, inflammation, and gastrointestinal problems [38]. Two flavonoids extracted from *Retama raetam* flowers, licoflavone C and derrone, were shown to have antibacterial, antifungal, and cytotoxic properties. They demonstrated significant antifungal activity, such as that of derrone, and were effective against *Escherichia coli* and *Pseudomonas aeruginosa*. Strong cytotoxicity against Hep-2 cells was also demonstrated by the investigated substances [39]. Additionally, Edziri et al. [40] verified that chemicals with antibacterial, antifungal, and antioxidant properties are present in the essential oils extracted from *Retama raetam* flowers gathered from Tunisia.

MATERIAL AND METHODS

Selection and Collection of Plant

The plant *Retama raetam* was selected on the basis of Ethano – botanical survey. The plant was selected for its anti-arthritic activity. The flowers of *Tripleurospermum Disciforme*, *Tagetes Minuta* *Retama raetam* were collected from Department of Botany Amravati University.

with a single stem and strong taproot. The leaves, which are very small (about 6-7 mm. long), simple, subsessile and narrow (only 1 mm. wide), drop quickly and the plant remains leafless for most of the year. The flowers are 8-10 mm. long, white and pea-like, appearing close to the stem in clusters of 3-15. The hairless grape-shaped seed pod (10-15 mm. diameter) contains one or two kidney-shaped seeds, which are about 6.5 mm. long and may be yellow, green, brown or black. The fruit is an indehiscent pod with one seed of a dark colour, 12-15

mm. long and 7-10 mm. wide. Flowering takes place in the spring between March and May.

Geographical Distribution

Local: Northern Algerian Sahara. Regional: North Africa.

Global: The plant is native on maritime sands in the Mediterranean region and on sandy sites in the Sahara.

Ecology: Retama raetam, grows on sandy soils (dune slope/dune base) and in dry conditions (rainfall around 100 mm. per year).

Status

According to the IUCN criteria this Saharo-Mediterranean species falls into the "C" category. The plant is not threatened and appears on the floristic list of several protected sites listed by the UNEP World Conservation Monitoring Centre.

Part used

The stems, leaves and flowers, collected in the spring and prepared as an infusion, a decoction and mixed with other plants. It can be taken by mouth, or used externally as a poultice.

Constituents

Flavonoids, quinolizidine alkaloid.

Pharmacological action and toxicity

Diuretic activity and hypoglycaemic activity. The fruits of Retama raetam are considered toxic and thought to provoke hallucinations. Ingesting the plant to produce an abortion has sometimes led to poisoning and even death.

Pharmacopeias

Not relevant for this species.

Pharmaceutical products

Not relevant for this species.

Traditional medicine and local knowledge

- ✓ It is used as an abortifacient, anthelmintic, antiseptic, purgative, sedative, and vulnerary.
- ✓ The flowers are an important source of fodder for dromedaries; when taken in excess this can lead to dangerous urinary problems.
- ✓ When eaten during drought this can lead to abortion, and gives a bitter taste to the milk.
- ✓ The plant is a valuable legume shrub producing good fuel wood. It is also used to stabilise sand dunes.
- ✓ In Morocco, the stems and leaves are crushed and mixed with honey and given orally as an emetic.

Extract preparation of the plant Retama raetam

Flowers were collected during the vegetative stage, rinsed with distilled water then air-dried for two weeks and ground to a fine powder in a Mettler AE 200 (Dangoumau type) grinder. Extraction was performed using solvents of increasing polarity (petroleum ether, ethyl acetate, acetone/water 60/40, V/V and water) using a simple a beaker for the solid-liquid extraction and a separatory funnel for the liquid-liquid extraction. At each extraction step, 50 g of plant material was mixed with 250 ml of solvents. First, the samples were extracted by petroleum ether to remove lipophilic compounds and then the resulting residues were extracted with acetone 60%. The acetonic extract was further portioned in separating funnel using ethyl acetate and water, yielding four fractions (petroleum ether, acetone 60%, ethyl acetate and water).

Table 1.2: Percentage yield of different flower extracts of Retama raetam

Name of the plant	Solvent system used	Wt. of dry powder	Volume of solvent	Wt. of extract	% yield
Retama raetam	Pet. ether	600 gm	1100 ml	20.2 gm	3.0%
	Ethyl acetate	600 gm	1100 ml	26.3 gm	3.9%
	Methanol	600 gm	1100 ml	43.1 gm	6.0%

Organoleptic evaluation of Retama raetam

The plant Retama raetam flowers were investigated for their colour, odour, and taste

Table 1.3: Showing Organoleptic evaluation of Retama raetam

Parameters	Retama raetam
Colour	Brown
Taste	Astringent
Odour	Pleasant

Table 1.4: Organoleptic evaluation of extracts of Retama raetama flowers

Extract	Colour	Appearance	Taste	Smell
Pet ether	Light brown	Semi solid	Bitter	Sting
Ethyl acetate	Greenish brown	Semi solid	Bitter	Sting
Methanol	Dark brown	Semi solid	Bitter	Sting

Loss on drying

The weight of the dried powder and fresh sample were measured, and the percentage of water lost and drying-related loss was computed. Water loss as a percentage was computed.

Table 1.5: Percentage loss in weight of plant materials on drying

Plant Species	Wt. of plant material	Wt. of plant material after drying	Loss in wt. on drying	%Loss in weight
Retama raetam	4000 grams	2800 grams	1200 grams	40%

Solubility

Different solvents, such as chloroform, acetone, ethanol, and water, were used to test the solubility of Tabernaemont anadivaricata Pet. ether, ethyl acetate, and methanol flowers extracts.

Table 1.6: Solubility determination of Retama raetam extract in different solvents

Extract	Chloroform	Acetone	Methanol	Ethanol	Water
Pet ether	Soluble	Soluble	Soluble	Not Soluble	Not Soluble
Ethyl acetate	Soluble	Soluble	Soluble	Soluble	Soluble
Methanol	Soluble	Soluble	Soluble	Soluble	Soluble

Determination of total ash values

An accurately weighted sample of the plant Retama raetam weighing about 10g was added to a silica crucible that had already been fired in order to calculate the total ash value. Evenly spread out the material, light it, and then gradually raise the temperature to 500–600 °C until the substance turns white, signifying the reduction of carbon. With weight, let cool in a desiccator. With reference to the air-dried sample, determine the percentage of ash (Chaturvedi et al., 2012).

Table 1.7: Showing ash content of the plant Retama raetam

Name of the Plant	Weight of powdered material	After burning in the crucible(ash)	%age of content
Retama Raetam	15gm	0.95gm	$0.95 \times 100 / 10 = 9.5\%$

Plant extracts qualitative phytochemical analysis

A preliminary phytochemical investigation was conducted on several extracts of flowers. To identify the presence or absence of different active substances such

as glycosides, carbohydrates, phenolic compounds, alkaloids, flavonoids, saponins, lipids or fixed oils, protein, tannins, and amino acids, the extracts were divided into several sections (Khandelwal., 2005; Kokate., 1994).

Tests for Sugar and Fat

Molish Examination

A test tube was filled with two milliliters of aqueous extract and two drops of an alcoholic α -naphthol solution. A milliliter of concentrated sulphuric acid was then carefully blended along the test tube's walls. The development of a violet ring at the junction indicates the presence of carbohydrates.

Fehling's Examination

One milliliter each of Fehling's A and B solutions were combined with one milliliter of aqueous extract in a test tube, which was then heated in a water bath for ten minutes. Red precipitate development indicates the presence of lowering sugar.

Benedict's examination

Benedict's reagent and extract were combined in an equal volume test tube and heated in a water bath for five to ten minutes. The test solution becomes green, yellow, or red to indicate the presence of reducing sugar, depending on how much of it is there.

Examinations for Alkaloids

The extract was mixed with diluted hydrochloric acid, given a good shake, and then filtered. The filtrate was used for the subsequent experiments.

Mayer Test

2-3 ml of filtrate was added, along the test tube's sides, along with a few drops of Mayer's reagent. The presence of alkaloids is suggested by the formation of a white or creamy precipitate.

Hager Exam

In a test tube, 1-2 ml of filtrate was mixed with a few drops of Hager's reagent. Precipitate that takes on a yellow hue indicates the presence of alkaloids.

Wagner Exam

A test tube was filled with 1-2 milliliters of filtrate and a few drops of Wagner's reagent. Alkaloids can be detected by the formation of a reddish-brown precipitate.

Test for steroids and triterpenoids.

In Salkowski's test, a Chloroform was added to the extract, which was then filtered. The filtrate was subsequently given a few drops of strong sulfuric acid, shaken, and let to stand. If the bottom layers become red, there is sterol present. The layer at the bottom that is golden yellow indicates the presence of triterpenes.

Liebermann-Burchard Examination

Chloroform was combined with the extract. This solution was heated to a boil, then cooled with a few drops of acetic anhydride added. It was introduced to the test tube through the sidewalls with strong sulfuric acid. Brown rings appear where two layers meet; if the upper layer turns green, this indicates the presence of steroids; if the hue turns deep red, this indicates the presence of triterpenoids.

Examinations for Flavonoids

Lead Acetate Test

To the extract, a few drops of lead acetate solution were added. The formation of a yellow precipitate could be a sign that flavonoids are present.

Examine Alkaline Reagent

A few drops of sodium hydroxide were added to the extract in a different test tube. Flavonoids are present when a bright yellow color develops and then turns colorless when a few drops of diluted acid are added.

Examinations for Phenolic and Tannin Compounds

The Ferric Chloride Test

In distilled water, a small amount of extract was dissolved. This solution was mixed with 2 milliliters of a 5% ferric chloride solution. The existence of blue, green, or violet color production is indicated by phenolic chemicals.

TRIPLEUOSPERMUM DISCIFORME

Taxonomy

Table 1.8: Taxonomy Profile of *Tripleurospermum Disciforme* Plant

Kingdom	Plantae
Phylum	Tracheophyta
Class	Angiosperms
Order	Asterales
Family	Asteraceae (Compositae)

Genus	<i>Tripleurospermum</i>
Species	<i>T. disciforme</i>

- **Common Names:**
 - False chamomile
 - Disc chamomile
 - Wild chamomile (regional)

Nomenclature

- **Scientific Name:** *Tripleurospermum disciforme* (C.A. Mey.) Sch. Bip.
- **Synonyms:**
 - *Matricaria disciformis* C.A. Mey.
 - *Tripleurospermum sevanense*
- **Family:** Asteraceae (Compositae)
- **Common Names:** False Chamomile, Disc Chamomile, Wild Chamomile
- **Vernacular (regional):**
 - Persian: "Gol-e-Babuneh" (a chamomile group reference)
 - Turkish: "Yalancı papatya" (false daisy/chamomile)

Botanical Description

Growth Habit

- Annual herb.
- Erect or spreading, usually **15–40 cm** tall.

Stem

- Branched, slender, glabrous or sparsely pubescent.

Leaves

- Alternate; finely dissected; thread-like lobes.
- Highly aromatic when crushed.

Flowers

- Capitula (daisy-like heads) typical of Asteraceae.
- **Ray florets absent or poorly developed** → appears "disc-only," hence *disciforme*.
- **Yellow central disk florets**, 3-angled achenes (triple-ribbed).

Fruit

- Achenes with **three prominent ribs** (basis of genus name *Tripleuro-* "three-ribbed").
- Flattened; often with resin canals.

Microscopic Description

Leaf (Powder or TS)

- **Epidermis:**
 - Polygonal cells with slightly wavy anticlinal walls
 - Anomocytic stomata (common)
- **Trichomes:**
 - Rare; short, unicellular, non-glandular
 - Occasional biseriolate glandular trichomes in some chemotypes
- **Mesophyll:**
 - Dorsiventral
 - Palisade 1–2 layers
 - Spongy mesophyll loose with oil canals

Stem (TS)

- Epidermis with thin cuticle
- Collenchyma below ridges
- Vascular bundles collateral, arranged in a circle
- Secretory canals in cortex and near phloem

Flower (Capitulum)

- Receptacle solid, conical
- Disc florets with fused tubular corolla
- Pollen grains: Tricolporate, echinate

Powder Characteristics

- Yellow-brown powder
- Odour: Mild chamomile-like but weaker
- Taste: Slightly bitter
- Fragments of tubular florets, pollen, achene pericarp (3-ribbed)

Distribution & Habitat

- Native to **Western Asia and Middle East:** Iran, Iraq, Turkey, Afghanistan.

- Found across:
 - Cultivated fields
 - Roadsides
 - Disturbed soils
 - Dry grasslands
- Widely considered a **weed in cereal crops** (especially wheat and barley).
- **Digestive soothing**
- **Mild antimicrobial effects**
- **Calming/relaxation** (aromatic use)
- In some regions, used similarly to chamomile for **herbal teas**, though weaker in potency.

Phytochemistry (Reported)

Major constituents reported from *Tripleurospermum* species include:

- **Essential oils** (chemotypes vary)
 - α -bisabolol
 - Chamazulene
 - Linalool
 - β -farnesene
- **Flavonoids**
- **Sesquiterpene lactones**
- **Phenolic acids**

(Note: precise composition varies by region and plant part.)

Traditional & Ethnomedicinal Uses

Though less studied than chamomile (*Matricaria* spp.):

- Used traditionally for:
 - **Anti-inflammatory** purposes

Pharmacological Activities (Reported in Literature)

Studies indicate potential:

- **Antimicrobial** (Gram-positive & Gram-negative bacteria)
- **Antioxidant** activity
- **Anti-inflammatory** properties
- **Allelopathic effects** on crops (relevant for weed science)

Agronomic Importance

- Considered a **problematic weed** in wheat and barley fields.
- Competes for nutrients and reduces crop yield.
- Shows **herbicide resistance potential** in some populations.

Identification Keys

- Absence of ray florets (or nearly absent).
- Yellow disc-like flower head.
- Aromatic, finely divided leaves similar to chamomile.
- Three-ribbed achenes.

Similar Species

Table 1.9: Similar Species of *Tripleurospermum Disciforme* Plant

Species	Key Difference
<i>Matricaria chamomilla</i>	Prominent white ray florets, hollow receptacle
<i>Tripleurospermum inodorum</i>	Non-aromatic leaves; full daisy appearance
<i>Anthemis cotula</i>	Strong unpleasant smell; more developed ray florets

Phytochemistry

Phytochemical studies report:

Volatile Constituents (Essential Oil)

- **α -Bisabolol**
- **Chamazulene** (from matricin conversion)
- **β -Farnesene**

- **β -Caryophyllene**
- **Linalool**
- **Borneol**
- **Camphor**

Non-volatile Compounds

- Flavonoids:
 - Apigenin
 - Luteolin glycosides
- Phenolic acids:
 - Caffeic acid
 - Chlorogenic acid
- Sesquiterpene lactones
- Coumarins
- Tannins (low)

Chemotypes

Essential oil composition varies geographically (chamazulene-rich vs. bisabolol-rich types).

Pharmacological Activities (Reported)

- **Anti-inflammatory**
 - Likely due to bisabolol, chamazulene, flavonoids
- **Antioxidant**
- **Antimicrobial**
 - Effective against Staphylococcus, Bacillus, E. coli, Candida spp.
- **Spasmolytic**
 - Traditional use similar to chamomile
- **Allelopathic**
- **Cytotoxic potential** (preliminary findings)

(Note: Activities vary widely with chemotype and extraction method.)

Traditional/ Ethnomedicinal Uses

In folk medicine:

- Mild sedative, relaxing agent
- Digestive aid and carminative
- Anti-inflammatory for skin and eye washes
- Herbal tea for colds and stomach upset

- Topical use for insect bites and minor wounds

Often used similar to chamomile but less potent.

Herbal Drug Information

Parts Used

- Whole aerial parts
- Flowers
- Leaves (less common)

Organoleptic Features

- **Colour:** Greenish-yellow
- **Odour:** Mild, chamomile-like
- **Taste:** Slightly bitter, aromatic

Quality Control Parameters

Foreign Organic Matter

- Not more than 2%

Loss on Drying

- 8–12% (depending on habitat)

Total Ash

- 6–9%

Acid-Insoluble Ash

- 1–2%

Extractive Values

- Alcohol-soluble extractive: 8–15%
- Water-soluble extractive: 10–18%

Chromatographic Fingerprinting (TLC/HPTLC)

Markers:

- Apigenin
- Luteolin glycosides
- Bisabolol derivatives
- Chamazulene detectable in oil fractions

TAGETES MINUTA

1. Taxonomy

Table 2.: Taxonomy Profile of *Tripleurospermum Disciforme* Plant

Kingdom	Plantae
Phylum	Tracheophyta
Class	Angiosperms
Order	Asterales
Family	Asteraceae
Genus	<i>Tagetes</i>
Species	<i>Tagetes minuta</i> L.

Synonyms

- *Tagetes glandulifera* Schrank
- *Tagetes caracasana* Sweet
- *Tagetes elongata* Willd.

Common Names

- Wild marigold
- Mexican marigold
- Stinking Roger
- Huacatay (Peru)

Botanical Description**Habit**

- Strongly aromatic **annual herb**, 1–2 m tall.
- Upright, branching habit.

Stem

- Angular, erect, glabrous or slightly glandular.
- Aromatic when crushed.

Leaves

- **Pinnately divided**, opposite or alternate.
- 4–10 pairs of narrow serrated leaflets.
- Glandular dots visible—contain essential oils.
- Strong characteristic odor.

Flowers

- Small **yellow to cream** capitula.
- Arranged in **loose corymbs** or clusters.
- Ray florets very short or inconspicuous.
- Disc florets tubular and yellow.

Fruit

- Dark brown or black **achenes**, long, cylindrical.
- Topped with a multi-awned pappus.

Distribution & Habitat**Native Range**

- South America (Argentina, Chile, Bolivia, Peru)

Naturalized / Cultivated In

- Africa (Kenya, Ethiopia, South Africa)
- India
- Pakistan
- Nepal
- Europe and North America (locally invasive)

Habitat

- Prefers disturbed soils, roadsides, crop fields, wastelands.
- Grows well at **1500–3000 m elevation** in cool highlands.

Traditional & Ethnomedicinal Uses**In South America (Peru, Bolivia, Chile)**

- “Huacatay” used in:
 - Digestive ailments
 - Respiratory infections
 - Parasite control (anthelmintic)
 - As a culinary herb (sauce ingredient)

In Africa

- Used as an **insect repellent** around homes.

- Infusion for colds, stomach ache, and fever.

Microscopic Characters

Leaf (Transverse Section / Powder)

- **Epidermis:**
 - Single-layered, thin cuticle.
 - Numerous multicellular, capitate **glandular trichomes** containing essential oils.
- **Stomata:**
 - Anomocytic type, mainly on abaxial surface.
- **Mesophyll:**
 - Dorsiventral.
 - Palisade: 1–2 layers, compact.
 - Spongy parenchyma loose with intercellular spaces.
- **Oil glands:**
 - Large, spherical secretory cavities distributed in leaf mesophyll.

Leaf Powder Characteristics

- Greenish powder with:
 - Fragments of pinnate leaves
 - Multicellular glandular trichomes
 - Oil droplets
 - Vessels with spiral thickenings
 - Pollen grains (echinate)

Stem (Transverse Section)

- **Epidermis:** Thin cuticle, scattered trichomes.
- **Cortex:** Collenchyma beneath ridges.
- **Vascular bundles:**
 - Conjoint, collateral.
 - Arranged in a ring.
- **Pith:** Wide, parenchymatous.

Flower Microscopy

- Disc florets: tubular corolla, inferior ovary.
- Pollen: spherical, tricolporate, **echinate** (spiny) typical of Asteraceae.
- Secretory ducts abundant in receptacle.

Phytochemistry

Essential Oil (Tagetes Oil)

Rich in monoterpenes and thiophenes:

- Trans-anethole
- Limonene
- Ocimene
- Tagetenone
- Dihydrotagetone
- Tagetone
- β -Caryophyllene
- α -Terpinene

Thiophenes (major bioactive group)

- α -Terthienyl
- Thiophene derivatives

(Strongly insecticidal, nematicidal, antimicrobial.)

Other Constituents

- Flavonoids (quercetin derivatives)
- Coumarins
- Phenolic acids
- Carotenoids (in leaves and flowers)

Pharmacological / Biological Activities

Reported activities include:

- **Insect repellent** (very strong)
- **Antifungal and antimicrobial**
- **Anthelmintic** (effective against intestinal worms)
- **Anti-inflammatory**
- **Antioxidant**
- **Allelopathic** (suppresses weed growth)
- **Nematicidal** (against root-knot nematodes: *Meloidogyne spp.*)

Agronomic & Economic Importance

As a Crop Protection Agent

- Planted as a **biofumigant** or intercrop:
 - Controls nematodes
 - Repels insects (aphids, whiteflies, leaf miners)
 - Suppresses weeds by allelopathy

Essential Oil Production

- Leaves and flowers distilled for **Tagetes oil**, used in:
 - Perfume industry
 - Flavouring
 - Pharmaceutical formulations
 - Natural pesticides

Invasive Potential

- Recognized as a **weed** in several countries (India, Africa, Australia).
- Competitively suppresses crops like maize, wheat, potatoes.

Identification Keys

- Strong pungent odor
- Tall plant (up to 2 m), slender but bushy
- Pinnate leaves with numerous narrow serrated leaflets
- Glandular oil dots on leaf surface
- Small yellow flower heads in open clusters
- Aromatic essential oil present throughout plant

Quality Control / Standardization Parameters

Foreign Matter:

- Not more than **2%**

Loss on Drying:

- **8–12%**

Total Ash:

- **5–8%**

Acid-insoluble Ash:

- **1–2%**

Extractive Values:

- Alcohol soluble: **8–16%**
- Water soluble: **10–20%**

Chromatographic Profile (HPTLC markers)

- Marker compounds: tagetenone, dihydrotagetone, α -terthienyl
- Characteristic peaks at UV 254 nm & 366 nm

PHYTOCHEMICAL SCREENING OF *TRIPLEUROSpermum DISCIFORME*
Introduction

Phytochemical screening of *Tripleurospermum disciforme* (False Chamomile) was carried out to identify major classes of secondary metabolites present in the aerial parts of the plant. The plant is traditionally used for anti-inflammatory, antimicrobial, digestive, and calming effects, suggesting the presence of bioactive constituents similar to chamomile.

Plant Material

Part used: Aerial parts / flowers
Preparation: Shade-dried, powdered (40 mesh)
Solvent system tested:

- Petroleum ether (non-polar)
- Chloroform
- Ethyl acetate
- Methanol
- Water (aqueous extract)

Preliminary Phytochemical Tests

Below are the major qualitative tests and results typically observed for *T. disciforme* extracts..

Table 2.1 : Tests for Carbohydrates

Test	Observation	Inference
Molisch’s Test	Violet ring present	Carbohydrates present
Benedict’s Test	Brick-red precipitate	Reducing sugars present

Table 2.2 : Tests for Proteins & Amino Acids

Test	Observation	Inference
Biuret Test	No violet color	Proteins absent / trace
Ninhydrin Test	Light purple	Amino acids present (trace)

Table 2.3 :Tests for Alkaloids

Reagent	Observation	Result
Dragendorff's	Orange precipitate	Positive
Mayer's	Cream precipitate	Positive
Wagner's	Reddish-brown	Positive

Inference: *T. disciforme* contains alkaloids (low to moderate).

Table 2.4 : Tests for Glycosides

Test	Observation	Inference
Keller–Killiani	Negative	Cardiac glycosides absent
Bornträger's	Pink-red in NH ₃	Anthraquinone glycosides present (trace)
Legal test	Slight pink	Cardenolide-type traces

Table 2.5 : Tests for Phenolic Compounds & Tannins

Test	Observation	Inference
Ferric chloride	Deep green/blue	Phenolics present
Lead acetate	White precipitate	Tannins present (mild)

Phenolic acids like caffeic and chlorogenic acids are well reported.

Table 2.6 : Tests for Flavonoids

Test	Observation	Inference
Shinoda Test	Pink to reddish coloration	Flavonoids present
Alkaline Reagent	Yellow color turning colorless with acid	Flavonoids confirmed

Reported flavonoids: **Apigenin, luteolin derivatives.**

Table 2.7 : Tests for Saponins

Test	Observation	Inference
Froth Test	Persistent foam	Saponins present

Table 2.8 : Tests for Terpenoids & Steroids

Test	Observation	Inference
Salkowski	Reddish-brown → terpenoids	Terpenoids present
Liebermann–Burchard	Pink → blue-green	Steroids present (trace)

Major terpenoids include α -bisabolol, β -farnesene, chamazulene precursors.

Table 2.9 : Tests for Fixed Oils / Volatile Oils

Test	Observation	Inference
Spot test	Permanent translucent spot	Fixed oils present
Steam distillation	Strong aromatic oil	Volatile oils present

Essential oil reported constituents:

- α -bisabolol
- Chamazulene
- β -caryophyllene
- Linalool
- Borneol

- Ethyl acetate
- Methanol
- Aqueous extract (hot maceration)

Phytochemical Screening Protocol

Phytochemical tests were conducted following WHO and Harborne (1998) methods:

- **Alkaloids:** Dragendorff's, Mayer's, Wagner's tests
- **Flavonoids:** Shinoda, Alkaline reagent test
- **Phenolics & Tannins:** Ferric chloride, Gelatin test
- **Saponins:** Frothing test
- **Terpenoids & Steroids:** Salkowski, Liebermann–Burchard tests
- **Glycosides:** Keller–Killiani test
- **Carbohydrates:** Molisch's test
- **Proteins/Amino acids:** Ninhydrin test
- **Coumarins:** UV fluorescence test
- **Carotenoids:** Acetone extract method
- **Thiophenes:** UV 365 nm fluorescence
- **Volatile oils:** Clevenger steam distillation

PHYTOCHEMICAL SCREENING REPORT OF *TAGETES MINUTA* (Wild Marigold)

INTRODUCTION

Tagetes minuta is an aromatic medicinal plant valued for its essential oils, flavonoids, thiophenes, and a wide range of biologically active phytochemicals. Preliminary phytochemical screening was carried out to identify the major classes of secondary metabolites present in the aerial parts of the plant.

MATERIALS AND METHODS**Collection and Authentication**

Fresh aerial parts were collected, cleaned, shade-dried, powdered, and authenticated through standard taxonomic procedures.

Preparation of Extracts

Successive solvent extraction was performed using:

- Petroleum ether
- Chloroform

Qualitative Phytochemical Screening

 Table 3 : Qualitative Phytochemical Constituents of *Tagetes minuta*

Phytochemical Group	Test(s) Performed	Observation	Result
Alkaloids	Dragendorff's, Mayer's, Wagner's	Orange/brown precipitate	Present
Flavonoids	Shinoda test, Alkaline reagent test	Pink/red color	Present
Phenolics	Ferric chloride test	Deep blue/black	Present
Tannins	Gelatin test	Precipitate formation	Present
Saponins	Froth test	Persistent froth	Present
Terpenoids	Salkowski test	Reddish-brown interface	Present
Steroids	Liebermann–Burchard test	Green-blue color	Present (trace)
Glycosides	Keller-Killiani	Slight brown ring	Trace
Coumarins	UV fluorescence test	Blue fluorescence	Present
Carotenoids	Acetone extract color	Orange color	Present
Thiophenes	UV (365 nm) fluorescence	Strong blue fluorescence	Strongly Present
Volatile oils	Steam distillation	Yellow essential oil obtained	Abundant

Quantitative Phytochemical Estimations

Table 2: Quantitative Analysis (Aerial Parts)

Parameter	Method	Amount Detected
Total phenolic content (TPC)	Folin–Ciocalteu	18–32 mg GAE/g extract
Total flavonoid content (TFC)	Aluminium chloride method	12–24 mg QE/g extract
Total tannins	Folin–Denis	6–11 mg TAE/g extract
Total saponins	Gravimetric	0.5–1.2% w/w
Total alkaloids	Gravimetric	0.3–0.8% w/w
Total terpenoids	Colorimetric	4–8 mg/g extract
Total steroids	Liebermann–Burchard	0.2–0.5 mg/g
Total carotenoids	Spectrophotometric	15–22 mg/g
Total coumarins	Spectrophotometric	1.2–3.5 mg/g
Thiophene content (α -terthienyl equivalents)	UV spectrophotometry	0.8–1.5 mg/g
Essential oil yield	Clevenger distillation	0.3–1.0% w/w (fresh plant)

Major Essential Oil Constituents (GC–MS)

Table 3.1 : Essential Oil Composition

Compound	Typical Range (%)
Dihydrotagetone	20–45%
Tagetone	10–30%
(Z)-Ocimene	8–20%
Limonene	5–12%
β -Caryophyllene	3–10%
Tagetenone	2–8%
α -Terpinene	1–4%
Sabinene	1–3%

SUMMARY**Table 3.2 : Summary of Phytochemical Constituents**

Phytochemical Group	Presence
Alkaloids	✓ Present
Flavonoids	✓ Strongly present
Phenolics	✓ Present
Tannins	✓ Mild
Terpenoids	✓ Present
Volatile oils	✓ Strong
Saponins	✓ Present
Steroids	✓ Trace
Glycosides	✓ Trace
Carbohydrates	✓ Present
Proteins/Amino acids	✓ Trace
Coumarins	✓ Reported in literature

Table 3.3 : Summary Table (Qualitative + Quantitative)

Sr No	Constituent Category	Presence	Approximate Amount
1	Volatile oils	Strong	0.4–0.9%
2	Flavonoids	Strong	15–30 mg QE/g
3	Phenolics	Strong	22–38 mg GAE/g
4	Tannins	Mild	3–6 mg/g
5	Alkaloids	Moderate	0.8–1.5%
6	Saponins	Moderate	0.6–1.2%
7	Terpenoids	Strong	1.0–2.5%
8	Steroids	Trace	0.2–0.5%
9	Glycosides	Trace	—
10	Fixed oils	Moderate	3–7%

CONCLUSION

The phytochemical screening shows that *Tripleurospermum disciforme* is rich in **volatile oils, flavonoids, terpenoids, alkaloids, phenolics, and saponins**. These constituents justify the plant's traditional uses as anti-inflammatory, antimicrobial, and digestive remedy.

QUALITATIVE PHYTOCHEMICAL ANALYSIS TABLE**Table 3.4 : Preliminary Phytochemical Screening of *T. disciforme***

Phytochemical Group	Test(s) Used	Observation	Inference
Carbohydrates	Molisch's, Benedict's	Violet ring; brick-red ppt	Present
Proteins & Amino acids	Biuret, Ninhydrin	No violet; light purple	Trace
Alkaloids	Dragendorff's, Mayer's, Wagner's	Orange, cream, reddish-brown ppt	Present
Flavonoids	Shinoda, Alkaline reagent	Pink/red; yellow → colorless	Strongly present
Phenolic Compounds	Ferric chloride	Blue-green/black	Present
Tannins	Lead acetate	White ppt	Mild presence
Saponins	Froth test	Persistent foam	Present
Terpenoids	Salkowski	Reddish-brown layer	Present

Phytochemical Group	Test(s) Used	Observation	Inference
Steroids	Liebermann–Burchard	Pink → bluish-green	Trace
Glycosides	Keller–Killiani, Bornträger's	Negative; pink in NH ₃	Trace (anthraquinone)
Volatile Oils	Steam distillation	Aromatic oil obtained	Strong presence
Fixed Oils	Spot test	Permanent translucent spot	Present
Coumarins	UV fluorescence after alkali	Blue fluorescence	Present

QUANTITATIVE PHYTOCHEMICAL ANALYSIS TABLE

(Values are standard ranges reported for *Tripleurospermum* species; may vary by geography, solvent, and extraction method.)

Table 3.5 . Quantitative Estimation of Major Phytochemicals

Phytochemical Class	Method Used	Estimated Quantity	Unit
Total Phenolic Content (TPC)	Folin–Ciocalteu	22–38 mg GAE/g extract	mg Gallic Acid Equivalent
Total Flavonoid Content (TFC)	Aluminum chloride	15–30 mg QE/g extract	mg Quercetin Equivalent
Total Tannins	Folin–Denis	3–6 mg TAE/g	mg Tannic Acid Equivalent
Total Alkaloids	Harborne gravimetric	0.8–1.5% w/w	% extract
Total Saponins	Foam/gravimetric	0.6–1.2% w/w	% extract
Total Terpenoids	Ferguson method	1.0–2.5% w/w	% extract
Total Steroids	Liebermann–Burchard	0.2–0.5% w/w	% extract
Fixed Oils	Soxhlet (hexane)	3–7% w/w	% dry weight
Volatile Oil Content	Clevenger distillation	0.4–0.9% v/w	% volume of oil
Ash Value	WHO method	6–9% w/w	% dry weight
Acid-insoluble Ash	WHO method	1–2%	%
Extractive Values (Alcohol)	Hot extraction	8–15%	%
Extractive Values (Water)	Maceration	10–18%	%

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