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Pharmacognostical and phytochemical studies on leaves of *Tagetes erecta* Linn.

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ABSTRACT

Background: *Tagetes erecta* Linn. (Asteraceae) is a well-known plant for its antihypertensive, antioxidant, antidiabetic, aphrodisiac, and hepatoprotective properties. To investigate the pharmacognostic, physicochemical, and phytochemical determinations of this plant's leaves. **Materials and Methods:** The macroscopic characteristics of the leaves, such as size, colour, surface characteristics, texture, fracture characteristics, and odour, were studied pharmacognostically. The cellular characteristics of the drug were studied using a microscope on both the intact leaves and the powdered drug. According to WHO guidelines, extractive values, loss on drying (LOD), total ash, water-soluble and acid-insoluble ash, and moisture content of *Tagetes erecta* leaves powder were determined. For the various phytoconstituents, preliminary phytochemical screening and qualitative chemical examination studies have been conducted. **Results:** TLC analysis revealed the presence of alkaloids, glycosides, flavonoids, steroids, saponins, and tannins. Microscopic examination revealed the presence of xylem vessels, vascular bundles, and phloem fibres. **Conclusion:** To authenticate, standardise, and avoid adulteration in the raw material, pharmacognostical and preliminary phytochemical screening of *Tagetes erecta* leaves will be beneficial. The diagnostic microscopic characteristics and physicochemical data will aid in the creation of a monograph. The chromatographic fingerprinting profile can be used to standardise *Tagetes erecta* leaf extracts and formulations.

Key words: *Tagetes erecta; macroscopy; microscopy; thin layer chromatography.*

INTRODUCTION

Natural plant products have been used for a variety of reasons throughout human history. Many of these natural compounds exhibit biological activity that could be useful in medication development. To cure different disorders, including cancer, the Indian school of medicine known as "*Ayurveda*" uses mostly plant-based medications or formulations. In the worldwide market, herbal medications have a lot of room for expansion. Herbal pharmaceuticals have been the subject of research in the areas of natural product chemistry, pharmacognosy, pharmaceutics, pharmacology, and clinical therapies, and most of the major pharmaceutical companies have updated their strategy to favour natural products. Many herbal cures have been advised for the treatment of various ailments, either separately or in combination, in various medical treatises.[1] Traditional civilizations all throughout the world use medicinal plants and derived medicine, and they are becoming increasingly popular in modern society as natural alternatives to manufactured chemicals.[2] The plant *Tagetes erecta,* also known as Marigold, is a member of the Asteraceae family (Compositae). It is a sturdy, branching herb that is native to Mexico and other warmer portions of America, as well as naturalised in the tropics and subtropics, such as Bangladesh and India. It's a popular garden plant that produces a highly scented essential oil (Tagetes oil), which is mostly utilised in the formulation of high-end perfumes. Various portions of

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plant sources have been employed as nutraceuticals, food supplements, ancient remedies, significant constituents in contemporary medications, and *Ayurveda* since the *Rig-Veda*. The therapeutic properties of bioactive elements in plants, such as alkaloids, tannins, flavonoids, and phenolic compounds, have a clear physiological effect on the human body.[3]

Although studies showing the full phytochemical screening of *Tagetes erecta* have been published, several secondary metabolites in the plant have been found to be present. Thus, pharmacognostic research and proximate analysis of *Tagetes erecta* leaves have been attempted in the current work. The extraction of the leaves into various extracts, followed by chemical tests and TLC analyses, was another method used for the initial phytochemical screening of the leaves.[4]

Materials and Methods

Collection and authentication of plants

The leaves of *Tagetes erecta* Linn. Were collected from Amravati (Maharashtra). The plant was identified and authenticated by Dr. Indrapratap S. Thakare, Department of Agriculture Botany, P. R. Pote Patil College of Agriculture, Amravati and dried in the shade at room temperature. Dried leaves were powdered in grinder and powder material was kept in air tight container for further study.

Macroscopic evaluation

According to WHO guidelines, the size, colour, surface characteristics, texture, fracture characteristics, and odour of the leaves were investigated.

Microscopic evaluation

The cellular characteristics of the drug were studied under the microscope on both the intact leaves and the powdered drug.

Study of Transverse section

The leaves were placed in a test tube, and 5% potassium hydroxide in methanol was added to keep the sample submerged. For a few minutes, the samples were boiled. Transverse sections of the drugs were placed in a watch glass filled with water using a brush. The sections were then transferred to a watch glass containing a 1:1 solution of Phloroglucinol-Hydrochloric acid and stained for 2-3 minutes.

The sections were then transferred to water-filled watch glasses, where the excess stain was washed away. With the help of a brush, the sections were then placed on clean glass micro-slides. A few drops of water were added, and the slide was covered with a clean cover-slip. The slides were prepared for examination under the microscope. T.S. is depicted of leaves with various cellular characteristics without staining, phloroglucinol and Sudan red were used to identify starch, mucilage, fats, and fixed oils in transverse sections.[5]

Physicochemical evaluation

1. Determination of Loss on drying

The loss on drying is the weight loss in percent w/w caused by the loss of water and any volatile matter that can be driven off under specific conditions.

Procedure

In a silica crucible, 2 gm of air dried drug reduced to powder was placed. Initially, the crucible was cleaned and dried, and the weight of an empty dried crucible was determined. The powder was applied in a thin, even layer. The crucible was then placed in a 1050°C oven. The powder was dried for 4 hours and cooled to room temperature in desiccators, and the weight of the cooled crucible with powder was recorded.

Weight of empty crucible = x g

Weight of dried leaf powder = y g.

Weight of Crucible + leaf powder = x + y g.

Weight of Crucible +leaf powder after drying at 105 = z

Loss in weight due to removal of moisture L = (x + y) - z

% LOD = final weight/Initial weight X 100

% LOD = L/y X 100^6

2. Determination of Total Ash value

Weighing and lighting a crucible

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2 gm of mucilage powder were weighed and placed in a crucible.

In the Muffle furnace, incinerate the crucible at a temperature not exceeding 450°C.

The desiccator was used to cool the crucible.

The procedure was repeated until it became white and reached a constant weight.

The percentage of ash was calculated using air dried drug.

Total Ash = 100/Y × (y) where: Y- Weight of powder taken in gm

y - Weight of ash in gm[7]

3. Determination of Acid- insoluble Ash

Total ash obtained in the previous step boiled for 5 minutes in a 100 mL beaker with 25 mL dil. HCl.

The insoluble matter was collected and washed with hot water.

Ignited to a fixed weight.

Insoluble in acid 100/Y = Ash (y)

Where: Y is the weight of insoluble matter in grammes, and y is the weight of ash in gm.[7]

4. Determination of Water Soluble Ash

Total ash obtained in the previous step boiled for 5 minutes in a 100 mL beaker with 25 mL water.

The insoluble matter was collected and washed with hot water. Ignited to a fixed weight.

The percentage of water soluble ash was calculated using air dried drug.[7]

5. Determination of Moisture content

A 2gm sample was placed on a tarred Petri dish and baked.

Drying of the sample was done at 105°C till the weight of the sample remained constant.

% moisture content = 100/Y× (y) where: Y - Weight of powder taken in gm y - Weight of powder after constant drying in gm.[8]

6. Determination of petroleum ether, chloroform, methanol and water-soluble extractive value

In a closed flask, 20 g of air dried, coarsely powdered *Tagetes erecta* leaves were macerated with 100 ml of petroleum ether for 24 hours, shaking frequently during the first 6 hours, and allowed to stand for 18 hours. The mixture was quickly filtered, and precautions were taken to prevent the loss of petroleum ether. In a Petri dish, 25 ml of the filtrate was evaporated to dryness, dried at 105°C, and weighed. The percentages of petroleum ether soluble extracts were calculated using the air dried sample as a reference. The procedure was repeated, but instead of petroleum ether, chloroform, methanol, and water were used.[9]

Preparation of extracts

Collected plant material was dried under shade and grounded in to coarse powder. Powder so obtained was subjected to soxhlet extraction in order to prepare whole extract and also successive solvent extracts.[10]

Preparation of successive extracts of leaves and flowers

The solvents were extracted one after the other in decreasing sequence of polarity, as shown below: Petroleum ether > Chloroform > Ethyl acetate > Methanol > Ethanol > Water.

100g of dried coarse leaf powder was weighed and packed loosely in a thimble of soxhlet, with a thin layer of cotton at the bottom to ensure the powder did not enter the distillation route. To avoid bumping the solvent, porcelain chips were placed in the round bottomed flask, and the thimble was inserted into RBF's mouth. Three syphons of petether were built to pass through the powder, and the soxhlet was supplied with a condenser. The solvent was heated to reflux between 60 and 800 degrees Celsius, which is the boiling point of petether. The solvent vapour rises up a distillation arm and into the chamber containing the solid thimble. The condenser makes sure that any solvent vapour cools and drips back into the solid material chamber. Warm solvent progressively fills the chamber housing the solid substance. In the heated

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solvent, some of the desired chemical will dissolve. When the Soxhlet chamber is nearly full, a syihon side arm automatically empties the chamber, returning the solvent to the distillation flask. This cycle can be repeated as many times as desired for a total of 5 hours. The medication was taken out of the thimble and set aside to dry. To obtain dry extract, the solvent was collected and placed on a heating mantle for evaporation. Other leaf extracts were made using the same process, utilising successive solvents in the order listed above.[10]

Fig. 1: Soxhlet Extraction

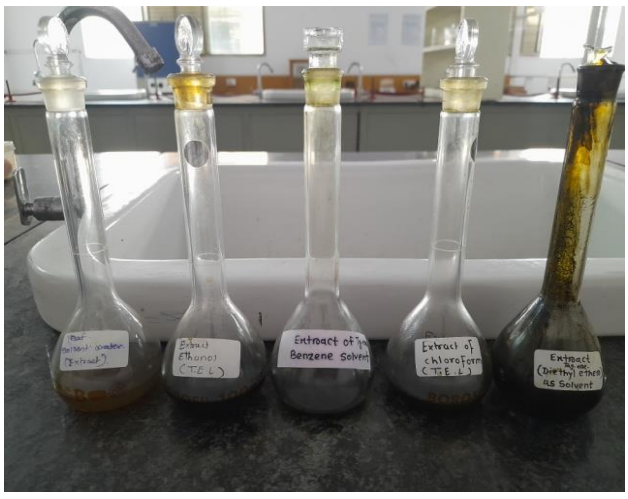

Fig. 2: Extractive Samples

Preliminary Phytochemical Screening[11]

Test for Carbohydrates

Molisch's test (General test): To 2-3ml aqueous extract, add few drops of alpha-naphthol solution in alcohol, shake and add conc. H_2SO4 from sides of the test tube. Violet ring is formed at the junction of two liquids.

Test for Alkaloids

Evaporate the aqueous, alcoholic and chloroform extracts separately. To residue, add dilute Shake well and filter. With filtrate, perform following tests: Wagner's test: 2-3ml filtrate with few drops Wagner's reagent gives reddish brown ppt.

Test for Cardiac Glycosides

Keller-Killiani test

To 2ml extract, add glacial acetic acid, one drop 5% FeCl3 and conc. H2SO4. Reddish brown colour appears at junction of the two liquid layers and upper layer appears bluish green.

Test for Flavonoids

To small quantity of residue, add lead acetate solution. Yellow coloured precipitate formed.

Test for Tannin

To 2-3ml of aqueous or alcoholic extract, add few drops of following reagents:

Dilute iodine solution: transient red colour.

Test for Steroids

Salkowski reaction: To 2ml of extract, add 2ml chloroform and 2ml conc. H_2SO4. Shake well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.

Test for Fat and Oil

Press powder of crude drugs between two filter paper. Filter paper gets permanently stained due to oil.

Test for Saponins

Foam test: Shake the drug extract or dry powder vigorously with water. Persistent stable foam observed.

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Test for Protein

Biuret test: To 3ml test solution add 4% NaOH and few drops of 1 % $CuSO_4$ solution. Violet or pink colour appears.

Qualitative analysis for different chemical constituents[12]

Chromatography techniques[13]

Chromatography is a method of separating molecules based on their size, shape, and charge. During chromatography, analytes are dissolved in solvent and then passed through a solid phase that serves as a sieve medium. The molecule is divided as it passes through the molecular sieve. Paper and thin layer chromatography are chromatographic procedures that easily provide qualitative information while also allowing quantitative data to be obtained.

Thin Layer Chromatography (TLC)[14]

TLC has a number of advantages over paper chromatography, including adaptability, speed, and sensitivity. TLC is an adsorption chromatography technique in which materials are separated by the interaction of thin layers of adsorbent on a plate. The approach is mostly used to separate low molecular weight molecules. The Rf value was calculated using Thin Layer Chromatography developed in a Twin through chamber with a silica gel 60 F254 pre coated aluminium plate of 0.2 mm thickness and an ethyl acetate: methanol (1:1) developing solvent system.

The qualitative evaluation by TLC studies of the crude hydroalcoholic extract of *Tagetes erecta* leaves also showed presence of similar constituents as shown in preliminary phytochemical screening. The mobile phase and detecting reagents of various classes of compound are shown in table 1.[15]

Table 1: TLC profile for hydro-alcoholic extract of *Tagetes erecta Linn.* Leaves[15]

SN	Groups	Mobile Phase	Detection
1.	Carbohydrate	Ethyl acetate:Propanol:Water (4:1:2)	Stal's indicator
2.	Alkaloids	Methnol:Water:Ammonia (70:20:10)	Dragendroff reagent
3.	Cardiac glycosides	Chloroform:Acetone (7:3)	UV 365nm
4.	Flavonoids	Ethyl acetate:Formic acid:Glacial acetic acid:Water (100:11:11:26)	Aluminium chloride reagent
5.	Tannins	Chloroform:Methanol: Water (65:35:10)	UV 365nm
6.	Steroids	Cyclohexane:Diethyl ether:Ethyl acetate (4:3:2:5)	Anisaldehyde Sulphuric acid
7.	Fat and Oil	Toluene:Ethyl acetate (93:7)	Vanillin Sulphuric acid reagent
8.	Saponin	Chloroform:Glacial acetic acid:Methanol:Water (64:32:12:8)	Vanillin Sulphuric acid reagent
9.	Protein	Butanol:Acetic acid:Water (4:1:1)	UV 365nm

Results and Discussion

Macroscopic Evaluation

Morphological studies revealed the shape of *Tagetes erecta* Linn. leaves. Leaves occur in their entirety. The leaves are lanceolate in shape. The leaves are a dark green colour. Table 2 summarises all of the organoleptic features investigated.

Table 2: Organoleptic features of *Tagetes erecta Linn.* leaves

SN	Features	Observations
1.	Shape	Lanceolate
2.	Width	1-2cm
3.	Length	5 -7cm

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4.	Color	Dark green
5.	Odor	Pungent
6.	Taste	Bitter

Microscopic Evaluation

The leaf's upper and lower epidermis were made up of a single layer of ovate and oblong ovate ordinary cells with mean thicknesses of 16.32 m adaxially and 17.68 m abaxially. Mesophyll was not homogeneous, with palisade 1-2 rows of 108.8 m adaxially and spongy 5-7 rows of 115.5 m abaxially. A single circular elliptic collateral vascular bundle with 7-9 rows of tracheary elements makes up the midrib.

Table 3: Micro-chemical tests performed on transverse section of *Tagetes erecta* Linn. leaves

SN	Test	Observation	Inference
1.	TS + Phloroglucinol + Conc. HCl	Red / Pink	Xylem, Vascular bundles
2.	Sudan red III	Pink	Cuticle
3.	Without Staining	Identified Cells	Epidermal Cells

Physicochemical Determinations

Physicochemical parameter like Extractive values, LOD (Loss on drying), Ash value, acid insoluble ash, water soluble, moisture content of *Tagetes erecta* leaves powder were determined as per WHO guideline.

The results are as follows:

Table 4: Physicochemical parameters

SN	Physicochemical parameters	Physicochemical parameters
1.	Loss on drying	7.46 %
2.	Total ash value	11.98%
3.	Acid insoluble ash	3.16%
4.	Water soluble ash	3.53%
5.	Moisture content	14.6%

Extraction of Plant Material

Successive solvent extraction values in various organic solvents were observed follows.

Table 5: Percentage yield of successive extraction

SN	Solvent	Percentage
1.	Diethy Ether	0.9%
2.	Benzene	1.9%
3.	Chloroform	2.18%
4.	Ethanol	7.1%
5.	Water	10.7%

Preliminary Phytochemical Screening

Chemical tests on all of the extracts obtained after successive extraction revealed the presence of alkaloids, glycosides, flavonoids, carbohydrates, tannins, and steroids. The results are summarizing in table 6.

Table 6: Observation of Preliminary Phytochemical Screening of extract

Chemical constituents	Diethyl ether extract	Benzene extract	Chloroform extract	Ethanol extract	Water extract
Carbohydrates	-	+	+	+	+
Alkaloids	+	+	+	+	+
Glycosides	+	+	+	+	-
Flavanoids	-	-	-	+	+
Tannins	+	+	+	-	+
Steroids	+	+	+	+	-
Fat and oil	+	+	+	-	-
Saponin	+	+	-	-	-
Proteins	-	-	-	-	-

'+' = present and significant; '-' = absent.

Table 7: Results of Preliminary Phytochemical screening of successive extracts

SN	Extracts	Results
1.	Diethyl ether	Alkaloids, Glycosides, Tannins, Fat & oils, Steroids
2.	Benzene	Carbohydrates, Alkaloids, Glycosides,Tannins, Saponins & Steroids

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3.	Chloroform	Carbohydrate, Glycosides, Alkaloids, Tannins, Steroids, Fat & oils
4.	Ethanol	Carbohydrate, Glycosides, Flavonoids, Alkaloids & Steroids
5.	Water	Carbohydrates, Alkaloids, Flavonoids & Tannins

Qualitative evaluation by Thin Layer Chromatography

Solvent extraction was performed on a dried powdered leaves sample of *Tagetes erecta* Linn. Thin layer chromatography on silica was performed on approximately 20g of the extract. The systematic order of solvent selection demonstrates the effect of polarity on the extraction and the extracted phytochemicals.

During the thin layer chromatography procedure, the fractions with different Rf values were separated. Table 8 shows results of observation of phytochemical analysis of *Tagetes erecta* L. by observing the spots on TLC plates.

A convenient way for chemists to report the results of a TLC plate in lab is through a "retention factor" or Rf value which quantitates a compounds movement. To measure how far a compound travelled, the distance is measured from the compounds original location to the compounds location after elution in figure.

Table 8: Rf value of isolated compounds of *Tagetes erecta* Linn. extract

Chemical constituents	**Retention factors of different extract**				
	Diethyl ether extract	**Benzene extract**	**Chloroform extract**	**Ethanol extract**	**Water extract**
Carbohydrates	Absent	0.8	0.75	0.66	Absent
Alkaloids	0.75	0.76	0.5	0.6	0.6
Glycosides	0.8	0.74	0.5	0.54	Absent
Flavanoids	Absent	Absent	Absent	0.65	0.73
Tannins	0.8	0.58	0.45	Absent	0.75
Steroids	0.5	0.6	0.8	0.25	Absent
Fat and oil	0.8	0.8	0.75	Absent	Absent
Saponin	0.40	0.25	Absent	Absent	Absent
Proteins	Absent	Absent	Absent	Absent	Absent

CONCLUSION

The current study provides information on the preliminary phytochemical and pharmacognostic screening of *Tagetes erecta* leaves, which may be helpful in order to standardise, authenticate, and prevent any adulteration. The construction of a monograph will benefit from the diagnostic microscopical characteristics and physicochemical information presented in this work. The current phytochemical screening of *Tagetes erecta* revealed the presence of bioactive compounds such as flavonoid, steroids, alkaloids, glycosides, and tannin that have medicinal value and a distinct physiological action on the human body. Additionally, *Tagetes erecta* leaf extracts can be subjected to pharmacological screening due to the presence of several phytochemicals that may have therapeutic activity.

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