A Comprehensive Review on Phytochemistry, Analytical and Pharmacological profile of *Tagetes* species

ABSTRACT

The demands of both individuals and their communities in terms of health care have been greatly impacted by medicinal plants. In developing nations, the use of herbal remedies derived from medicinal plants is a common practice. Since ancient times, people have recognized the therapeutic benefits of traditional herbal medicines.In the plant kingdom, *Tagetes* species, which are most widespread in the Asteraceae family, are utilized in a variety of contexts, including medicine, cosmetic preparation, and residential design. It comes in a variety of colors and fragrances. A number of extraction procedures and phytochemical screening methods and analytical methods for the quantification of its constituents were available in the scientific reports. The phytoconstituents present in Tagetes species are responsible for their pharmacological activities, such as antibacterial, antioxidant, hepatoprotective activities, etc.This review provides valuable insights on phytochemistry, analytical and pharmacological profiles of various types of *Tagetes* species.

Keywords: Tagetes Erecta, Tagetes Patula, Tagetes Minuta, Hydro- distillation.

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1. INTRODUCTION

Traditional civilizations worldwide utilize medicinal plants and their derivatives extensively, and they are also gaining popularity in contemporary society as natural substitutes for artificial chemicals[1]. The field of herbal medicine has grown exponentially in the last several decades. According to estimates from the World Health Organization, almost 80% of the world's population receives their medical care mostly from traditional practitioners using mostly plant-based medications. Currently, 30% of medications are derived from natural sources[2]. Many plant species and natural compounds made from plants are used to treat infectious diseases in the indigenous health care delivery system[3]. The screening of medicinal plants for antibacterial activity and phytochemicals as possible novel therapies is of tremendous interest to clinical microbiologists. Secondary metabolites are the active ingredients in many medications that are found in plants[4,5]. The combinations of secondary metabolites found in plants, such as alkaloids, steroids, tannins and phenolic compounds, flavonoids, resins, fatty acids, and gums, which can have specific physiological effects on the body, are usually responsible for the positive medicinal effects of plant materials. Although *Tagetes*, or marigolds, are native to America, they are now cultivated in several African, Asian, and European nations. Numerous species of this genus, including *T. erecta,T. patulaand T. minuta*(Figure 1) are grown for their aesthetic qualities and are being investigated for their potential therapeutic benefits due to their traditional medicinal applications[6]. *Tagetes* is a genus containing 50 species of herbaceous, usually annual or perennial plants that are decorative and therapeutic in nature. They are members of the Asteraceae family. Nematicide, cosmetic, antibacterial, diuretic, depurative and insect repellent qualities are only a few of its many uses. Antioxidants are present in the flower's

essential oil[7]. Marigolds are available in a variety of colors, the most popular being yellow and orange. The majority of *Tagetes* species are very valuable in cosmetic treatments and have a strong, unpleasant odour. *Tagetes* species are accessible nowadays in a wide variety of variations.

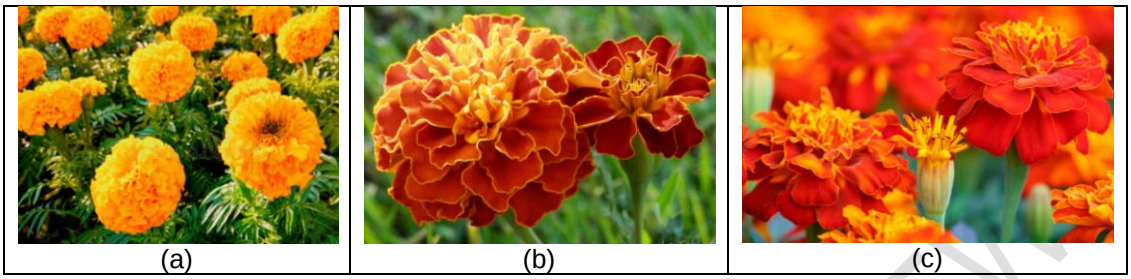

Figure 1. (a) *Tagetes erecta*, (b) *Tagetes patula*, (c) *Tagetes minuta*

2. SOME OF THE MAJOR *TAGETES* VARIETIES

2.1 Tagetes Erecta

Alternatively known as American or African marigold. These are fast-growing annual flowering plants that range in height from dwarfs (6–8 inches) to medium–tall, erect plants (10–13 feet). They have a shorter flowering period, blooming from midsummer until frost, and produce big, up to 5-inch-wide double flowers. Marigolds, which are scarlet in color, are not among these yellow to orange blooms.

Botanical Classification[8]

Kingdom	Plantae
Order	Asterales
Family	Asteraceae
Genus	*Tagetes*
Species	*Tagetes Erecta*

Phytochemical Constituents

Numerous chemical components, including thiophenes, flavonoids, carotenoids, and triterpenoids, have been isolated as a consequence of studies conducted on its various portions. Quercetagetin, a glucoside of quercetagetin, phenolics, syringic acid, methyl-3,5-dihydroxy-4-methoxy benzoate, quercetin, thienyl and ethyl gallate have all been shown to be present in *Tagetes Erecta* plants. As an oxy-carotenoid, or xanthophyll, lutein has two cyclic end groups-one beta and one alpha-ionone ring-as well as the fundamental C–40 isoprenoid structure that characterizes all carotenoids[9].

Uses

- Folk medicines use several components of this plant, particularly the bloom, to treat a variety of illnesses.
- The leaves can help with earaches, piles, kidney problems, muscle soreness, ulcers, and wounds.

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- The flower has been used to treat fevers, epileptic fits, stomachic ailments, scabies, liver complaints, astringent, carminative, and eye diseases[10].

2.2 Tagetes patula

Tagetes patula is also known as French Marigolds. This set of marigold cultivars ranges in height from 5 to 18 inches. The hues of flowers include orange, yellow, and red. Bicolor patterns in red and orange are also present. The flowers measure two inches in diameter. French marigolds are great in large plantings and as a border for flowerbeds. Additionally, they thrive in window boxes and containers[8].

Botanical Classification[11]

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Asterales
Family	Asteraceae
Genus	Tagetes L
Species	*Tagetes patula*

It has been discovered that *Tagetes patula* contains phytochemicals, such as flavonoids, thiophenes, and terpenoids, or essential oils, in its roots, leaves, and flowers[12]. Nearly twenty-one active compounds are present in *Tagetes patula* essential oil, with α-terthinyl, Pentatriacontane, and 2-ethyl-dodecanol being important ones. Piperitone, piperitenone, (E)-β-ocimene, limonene, α-terpinolene, and β-caryophyllene are also present in it[13].Numerous flavonoids, including quercetin, quercetagetin, patulin, quercetin -3-glucoside, quercetin-7-glucoside, quercetin-3,7-Di glucoside, and lutein, are found in *Tagetes patula*. Four different thiophene forms, such as 5-(4-hydroxy-1-butenyl), 2,2'-bithienyl, and 5-(4-acetoxy-1-butenyl), are found in the roots, shoots, and flowers[14].

Uses

Tagetes is a versatile plant that can be used for food flavouring, anthelmintic, insecticidal, ritual, medicinal, and decorative purposes.

- *Tagetes Patula* flowers and whole plant are used to make eyewash, eye cures, eyewash for rheumatism, stomach and intestinal issues, renal and hepatic illnesses, fever, pneumonia, and pleasant drinks.
- Flowers have sedative, diuretic, digestive, aromatic, and carminative properties.
- Flowers are distilled and used to cure diarrhoea, severe constipation, colic, coughs, and flatulence.
- *Tagetes patula* flower methanolic extract exhibits anti-oxidant and anti-acute and chronic inflammation-fighting properties.

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- *Tagetes patula* leaves are applied to boils and carbuncles and used as a remedy for piles, muscle aches, and renal problems. Their juice is recommended for ophthalmia and earaches.
- Apply leaf paste as soon as possible after preparation to cuts and wounds and leaf application for external haemorrhage as well.
- The leaves are utilized to make natural repellent since they have insecticidal properties.
- Dried flowers are used to dye meals a yellow hue, adulterate saffron, and colour textiles[15].

2.3 Tagetes minuta

Tagetes minuta L. plants are aromatic annual herbs in the sunflower family (Asteraceae), one of the most abundant taxonomic groups of plants, with about 23,000 species and roughly 1,000 genera. *Tagetes Minuta* gets its special name from the Latin word "minutes," which means "small," in reference to its diminutive capitula. Numerous kinds of *Tagetes* are farmed economically as a versatile new crop in their particular agro-ecological zones throughout several nations. This marigold species has the potential to reach heights of 0.6–2 meters.

Botanical Classification

Kingdom	Plantae
Order	Asterales
Family	Asteraceae
Genus	*Tagetes*
Species	*Tagetes Minuta*

Chemical constituents

Secondary metabolites found in *T. minuta* include flavonoids, terpenoids, saponins, thiophenes, and monocyclic, bicyclic, and acyclic monoterpenes. The quantity of these metabolites varies. According to earlier research, the main constituents of *T. minuta* essential oil include rosefuran (11.94%), betaocimene (16.83%), and cis-ocimene (28.5%). The orange-yellow carotenoid present in the oil of *Tagetes Minuta* (petals) florets and other species of marigolds is abundant in Tagetes powders and extracts [16,17].

Uses

- Used in a variety of foods, including pasta, sauces, vegetable oils, margarine, mayonnaise, salad dressing, baked goods, candies, dairy products, ice cream, yogurt, citrus juice, mustard, and so on, as a food colouring and flavouring.
- Additionally, it has a number of medicinal uses, including treating colds, respiratory infections, gastrointestinal issues, and acting as an insecticide, sedative, antispasmodic, and anti-parasitic[18].

3. EXTRACTION METHODS

Several extraction methods were available in literature, a few of which were described below.

3.1 Cold extraction

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A 500 mL conical flask containing 50 grams of flower powder was filled with 200 mL of distilled water, sealed with a rubber cork, and incubated for a full day. A sterile Whatman no. 1 filter paper was used to filter this soaked material before being placed into a sterile conical flask. After that, the filtrate was dried by water-bath evaporation at 100°C. Until they were needed, the standard hot aqueous extracts were kept in a refrigerator at 4°C.

3.2 Hot Extraction

In a conical flask, 50 grams of the dried flower powder were steeped in 200 mL of water and brought to a boil for 30 minutes. After a full day of inactivity, the contents of the flask were filtered through sterile filter paper and dried at 100 °C. The resulting standard extracts were kept in a refrigerator at 4°C until they were needed for use[19].

3.3 Maceration

After giving the leaves a thorough water wash, they are dried in a hot air oven set at 40°C.After drying, the leaves were crushed. The powdered leaves were steeped in 96% ethanol and shaken at 150 rpm for 24 hours. Repeat the maceration process until you have an almost clear filtrate. To get a thick extract, the solvent was first evaporated using a rotary evaporator and then again in a water bath[20].

In an extraction procedure, maceration was applied to the powdered plant components for four days.The powdered substance was immersed in 90% ethanol. Twice a day, the mixture was stirred. The combination was filtered and the marc was pressed after the fourth day. There were three iterations of this process. After mixing the whole alcoholic fraction, the ethanol was allowed to evaporate. The material that had a syrupy consistency was boiled in a water bath until a dry extract was produced. As a result, the *Tagetes erecta* flower ethanolic extract was labeled and kept in a desiccator until needed later[21].

3.4 Soxhlation

The plant material used in this extraction procedure was dried in the shade and cleaned with water before being ground into a powder using a mixer grinder. 100 grams of powder were extracted using methanol in a Soxhlet extractor at 40 °C. The extraction process was kept going until the thimble's solution was clear. Desiccator was used to store this extract[22].

In second procedure, 5 g of the dehydrated plant material was extracted by maceration in 75 ml of a methanol: water mixture at a 4:1 ratio while the mixture was kept dark and at room temperature for a full day. For 72 hours, this phase was carried out three times. Using a rotary evaporator, the extracts from each step were collected, filtered, and concentrated at 40 °C to produce a final dry weight of 300 mg. Prior to antimicrobial testing, the extracts were resuspended with 1 ml of 80% methanol and kept at -86 °C[23].

In a third procedure, 250 mL of methanol was used as the solvent while 50 g of dried and powdered plant material was extracted using the Soxhlet method for eight hours. A rotating vacuum pump was used to concentrate the resulting yellowish-brown fluid. Five grams of brownish-yellow oil were extracted. The methanol extract was collected by repeating the procedure in multiple batches. For additional research, the extract was kept in a freezer at 4° C [24].

3.5 Hydro-distillation (extraction of essential oil)

With a Clevenger apparatus, a known quantity of each marigold (*Tagetes erecta L*.) sample was hydro-distilated for four hours. Phytochemical screening was applied to the extracted oil[25].

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3.6 Cold Maceration method

Plant samples were gathered, thoroughly cleaned, rinsed, and dried. Plant material in powder form was extracted using a hydro-alcoholic solvent (30:70) and allowed to stand for four to five days at a time. All unextractable material, such as cellular components and other components that are insoluble in the extraction solvent, was filtered out of the extract using filter paper. The extract was placed to a beaker and allowed to evaporate, discarding any surplus moisture before gathering it into an airtight container. To determine whether distinct phytoconstituents were present, a qualitative examination of extracts made in various solvents was done[26].

4. PRELIMINARY QUALITATIVE PHYTOCHEMICAL ANALYSIS

Based on established procedures, the extracts of *Tagetes patula, Tagetes erecta, Tagetes minuta* were qualitatively examined for several phytoconstituents such as phenols, tannins, coumarins, proteins, carbohydrates, flavonoids, and saponins.

4.1 Alkaloids[27]

Dragendroff's test: Hydrochloric acid (2 M) was added to 5 mL of distilled water containing a few milligrams of extract until an acid reaction was observed. One milliliter of Dragendroff's reagent was added to this. Alkaloids will be identified, when an orange or orange red precipitate forms.

Hager's test: A few drops of Hager's reagent were applied to 1 milliliter of extract. The presence of alkaloids is confirmed by the formation of yellow precipitate.

Wagner's test: Hydrochloric acid (1.5 mL v/v) was added to 1 mL of extract. A few drops of Wagner reagentwas added to it. The presence of alkaloids is indicated by a yellow or brown precipitate.

Mayer's test: One millilitre of extract was mixed with a few drops of the Mayer's reagent. Pale-yellow colour indicates presence of Alkaloids.

4.2 Carbohydrates[28]

Molisch's test: A few drops of a recently made 20% alcoholic alpha naphthol solution were added to 2 milliliters of extract. Concentrated sulfuric acid (2 mL) was poured through the test tube's walls. Carbohydrates are indicated by the appearance of a reddish-violet ring, which vanishes when too much alkali is added.

Benedict's test: Benedict's solution (5 mL) was boiled and added to 0.5 mL of extract. Brick red indicates presence of carbohydrates.

Fehling test: Each of Fehling solutions A and B (1 mL) was boiled and added to 2 mL of extract. The presence of lowering sugar is indicated by the formation of red color.

4.3 Flavonoids[29]

Shinoda test: Diluted hydrochloric acid (10 drops) and a little piece of magnesium were melted and added to 0.5 mL of extract. The presence of flavonoids is indicated by the formation of pink or reddish-brown color.

Alkaline reagent test:

The sample (0.5 mL) and 1 mL of 2N sodium hydroxide were added. The presence of flavonoids was indicated by the yellow color.

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4.4 Steroids[29]

Liebermann- Burchard's test:

Only a few milligrams of extract were found in acetic anhydride. One milliliter of concentrated sulfuric acid was heated, cooled, and applied to the test tube's sidewalls. Steroid presence is shown by the formation of green hue.

Salkowski reaction: The drug's chloroform extract was poured over the test tube's sides with 1 milliliter of concentrated sulfuric acid. Steroids are present when the chloroform layer begins to turn red.

4.5 Triterpenoids[28]

Liebermann-Buchard's test:

The extract dissolved in acetic anhydride in a few milligrams. One milliliter of heated and cooled concentrated sulfuric acid was applied to the test tube's sidewalls. Triterpenoids are present when green coloration forms.

ChloroformTest

Carefully add 2 mL of chloroform and concentrated sulfuric acid to 0.5 mL of sample. The presence of terpenoids was revealed by the formation of a reddish brown color at the interface.

4.6 Proteins[28]

Biuret's test: Copper sulfate solution (5-8 drops, 10% w/v) was added to 1 mL of extract and heated. The presence of proteins is shown by the formation of a violet red hue. **Million's test**: Million's reagent (5–6 drops) were applied to 1 milliliter of extract. The presence of proteins is shown by the formation of a white precipitate that turns red when heated.

4.7 Saponins[27]

A drop of sodium bicarbonate was added to a test tube holding five milliliters of extract. After three minutes of rigorous shaking, the test tube was left. Saponins are present when froth that resembles honeycomb forms.

4.8 Tannins[27]

Lead Acetate solution was added to 2 mL of extract. Formation of white ppt indicates Presence of tannins.

4.9 Phenols[28]

Two milliliters of distilled water and a few drops of 10% ferric chloride were added to one milliliter of the sample. Phenols were present when a blue or green color formed.

4.10Coumarins

NaOH (10%) was applied at a volume of 0.5 mL of sample. The development of a yellow hue suggested the presence of coumarins[22, 25].

5. ANALYTICAL METHODS FOR QUANTIFICATION

5.1 UV-Visible Spectrophotometer

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A spectrophotometric technique in the UV visible range was devised to quantify quercetin in *Tagetes erecta*extract[30]. Quercetin's validated characteristics show a strong linear relationship between concentration and absorption, with a correlation coefficient (R^2) value of 0.999. The percent relative standard deviation, for quercetin was determined to be 0.38±0.020 for intra-day accuracy and 0.33±0.015 for inter-day accuracy. The data clearly showed that the UV Spectrophotometric approach was found to be efficient, accurate, responsive, dependable, speedy, and cost-effective for routinely estimating quercetin in *Tagetes erecta* extract.

5.2 High performance liquid chromatography

Lutein content in Marigold Flower (*Tagetes erecta L.)*concentrates used for food supplement production was determined using an HPLC technique. Using model solutions and concentrates spiked with lutein standards (concentrations ranging from 0.5 µg/g to 3 µg/g), the accuracy, precision, and recovery were assessed (n = 6–10). 0.7 µg/g was the limit of quantification (LOQ, S/N = 10) and 0.22 µg/g was the limit of detection (LOD, S/N = 3).
With a correlation coefficient of 0.9982, the calibration curve was linear within a suitable concentration range. Repeatability was assessed for genuine plant concentrates (n = 6) spiked with the same analyte concentrations as well as for standard solutions at 0.5, 1, 2, and 3 µg/g. RSD for standard solutions was 1.4%, while recoveries ranged from 99.0 to 101%. RSD for spiked concentrates was 2.5%, and recoveries ranged from 97-92%. Regarding intraday variance, recovery for conventional solutions ranged from 98.8 to 101%, with an RSD of 2.6%. The recovery for spiking lutein concentrations was 98.3–101%[26,] with an RSD of 2.8%[31].

5.3 Liquid Chromatography-Tandem Mass Spectrometry

Tageteserecta L. marigold flowers were used to quantify lutein using liquid chromatography-tandem mass spectrometry. Lutein was separated chromatographically at 35 °C using an ACQUITY UPLC C18 BEH 130 Å, 1.7 µm, 2.1×100 mm column. Multiple reactions monitoring mode (MRM) and positive electrospray ionization were employed for detection. The results showed excellent linearity, with a correlation coefficient R greater than 0.999. Limits of quantitation (LOQ) and detection (LOD) were 0.0240 and 0.0078 ng/mL, respectively. At three different quality control concentration levels, intraday fluctuations were within 0.31-1.75 percent, and intraday accuracy ranged from 100.4 to 102.7 percent[32].

5.4 Gas Chromatography-Mass Spectroscopy

A Gas Chromatography-Mass Spectroscopy was created to assess and screen *Tagetes erecta'*sbioactive constituents. Leaf extract samples yielded the identification of roughly 19 different biocompounds. The bioactive chemicals in the leaf sample had varying retention times, ranging from 16.015 to 27.349, and varying area percentages, from 0.72 to 16.45. From a *Tagetes erecta* methanol extract sample, about 31 phytochemicals were found. Thirty-one phytochemicals had retention times ranging from 15.121 to 29.232 and area percentages ranging from 0.44 to 14.44[33].

6. PHARMACOLOGICAL ACTIVITIES

Based on the established review literature *Tagetes* species such as *Tagetes erecta, Tagetes patula, and Tagetes minuta*exhibits various pharmacological activities such asanti- oxidant, anti-microbial, hepatoprotective and larvicidal activities. The details of the same were provided in Table 1.

Table 1. Pharmacological activities of various Tagetes species

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Plant Species	Extract	Phytoconstituents	Activity	Reference
Tagetes Erecta	Alcoholic	Patulitrin	Anti-Bacterial	3
Tagetes. Erecta	Petroleum Ether, Chloroform, Ethyl acetate, Methanol	Any Chemical Constituent	Antioxidant	4
Tagetes. Erecta	Ethanolic Extract	All Chemicals	Hepatoprotective	5
Tagetes.Erecta	Light Petroleum Ether Extract (Essential Oil Extract)	18 Compounds	Antioxidant	7
TagetesMinuta	Methanolic	7 Compounds	Anti-Microbial	10
Tagetes.Patula	Hydro-Distillation	Essential Oil	Larvicidal	15
Tagetes.ErectaAnd Tagetes Patula	Cold And Hot Aqueous extract Methanolic Extract	Thiophene, Quercetin, Terpene, Carotenoid. Piper tine, Terpinolene	Anti-Microbial	18
Tagetes Patula	Hydroalcoholic	Flavonoids, Thiophene, Steroid	Antioxidant	25

CONCLUSION

The phytoconstituents present in Tagetes species are responsible for their pharmacological activities, such as antibacterial, antioxidant, hepatoprotective activities, etc. This review provides valuable insights on phytochemistry, analytical and pharmacological profiles of various types of Tagetes species.

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