WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 5, 613-627. Research Article ISSN 2277–7105

# PHYTOCHEMICAL SCREENING OF LEAF AND FLOWER EXTRACT OF TAGETES ERECTA L. AND THEIR ANTIMICROBIAL EFFICIENCY AGAINST SOME MICROBIAL STRAINS

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Article Received on
07 January 2025,

Revised on 27 Jan. 2025,
Accepted on 17 Feb. 2025

DOI: 10.20959/wjpr20255-35149

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## ABSTRACT

**Objective:** The aim of this study was to examine the phytochemical screening of *tagetes erecta* and find out the antioxidant activity of marigold against gram's positive and negative bacterial strains. **Methodology:** The fresh leaf and flower of marigold extracted in 70% ethanol, methanol or acetone. The proximate investigation, as well as the minerals content of leaf and flowers, were carried out using the wet acid digestion method for multiple nutrient determination. Preliminary phytochemical screening for alkaloids, flavonoids, terapanoids, Steroids, quinones, glycosides, phenols, coumarins, carbohydrates, tanin and saponins were conducted by following the standard procedures. Antioxidant activity was also carried out by using the standard protocol (aneja). **Result:** The qualitative and quantitative screening of fresh leaf and flowers of marigold exhibited the considerable amount of terponoids, steroids, flavonoids, alkaloids, quinones, coumarins and carbohydrates in the extract. In vitro, activity of *tagetes erecta* clearly demonstrated that both the fresh leaf and flowers have phytochemical attributes & antioxidant activity. **Conclusion:** *Tagetes Erecta* possess significant antioxidant and metabolic activity. It also contains considerable amount of primary & secondary metabolites, minerals and nutrients. This suggested that dietary uptake of leaf and flower of marigold could be potentially protective against diverse diseases like skin problem, body pain, wound, swelling, diabetes etc. It's also showing anticancerous

activity against colon cancer due to the presence of flavonoids in it. hence, it has further scope, in health care industries.

**KEYWORDS:** Antioxidant activity, Bacterial Strains, Extraction, Marigold, Phytochemical screening.

## 1. INTRODUCTION

Medicinal plant products have been used in human history for various motives. These medicinal plants also have metabolic activity that can involve in drugs designing. In the traditional system of medicine known as "Ayurveda" uses to treated various diseases including cancer, diabetes, blood pressure etc. Those drugs which made by these medicinal plants, have great network in the global market (Singh Y., *et al* 2020). The origin of various species of marigold like Ganda, Caltha, and gold bloom etc has been form the early 20 centuries (Chiou., *et al* 2007).

Marigold belongs to Asteraceae family containing 45-50 species of herbaceous plant. The medicinal value of this plant is very high due to the presence of variousalkaloids, tannins, flavonoids and glycosides compounds. Hence it has a definite physiological action on the human body. Marigold are not only a major resources base for the traditional medicine & herbal industry but also provide health security to large population of the world (Sandeep *et al.*, 2017).

Marigold (*Tegetes Erecta L.)* is one of the oldest medicinal plant. It is the world 's largest cultivated crop because of its high medical value & it also used in cosmetics. It is cultivated in tropics and temperate zones throughout the world. The largest commercial producers of *Tageteserecta* inIndia, China and Mexico. Marigold plants is important in nutritional, medicinal and industrial uses. It has been documented for various medicinal properties like antioxidant, antibacterial, anti-inflammatory, wound healing properties, diuretic activities etc. The phenolic compounds in fresh leaf and flowers of marigold plant may also be beneficial in the prevention of coronary heart disease, skin problem, diabetes, swellings and wounds. They also play a predictable role in the prevention of various diseases associated with oxidative stress, such as cancer, cardiovascular, type-2 diabetes, body pain and neurodegenerative diseases (Raja W., *et al* 2020). Antioxidants are phytochemicals, metabolites, enzymes, protein and other nutrients that protect our body cells from damage caused by free radicals (Emmanuel *et al.*, 2020).

Since ancient times, scientist have been survey nature to discover new drugs. Beneficial products can be taken from any part of the medicinal plant such as leafs, flowers and fruits etc. Herbal products also have been part of phytomedicines. Medicinal properties of marigold have been utilized for primary healthcare. Almost 79.9% of world's population depend on traditional medicines. In Ayurveda 90% prescription were based on drugs obtained from plants. Medicinal plants easily available, less cost, safe and rarely have side effects. Chemical constituents of the metabolites (primary &secondary) in plants is desirable (M. Patil *et al.,* 2015).

*Tagetes erecta L.* contain many phytochemically important compounds like flavonoids, alkaloids, tannins, saponins, steroids, terpenoids and minerals like calcium, iron, magnesium, manganese, copper and zinc. These compounds impart many of the pharmacological activities like antioxidant, antibacterial, antiinflammatory and analgesic to marigold (Anilkumar *et al*., 2010). In ethnomedicine marigold is used in to treat other skin problems.

Medicinal plants are highly reliable source of antimicrobial agents that may inhibit or kill to pathogens. In India, several medicinal plants used for their therapeutic value from ancient times to cure different types of diseases. The Phytochemical screening of any medicinal plant can help to determined its antimicrobial activities. Flower extract of marigold plant contain flavonoids which possess antimicrobial activity against cancer. Flavonoids of marigold flower proved that it's highly antiviral and antibacterial against micro-organisms (R. Devika and Y. Justin Koilpillaij).

The antibacterial activity of different extract of *tagetes erceta* like flowers and leaf against *Bacillus subtils, E.coli, S. aureus, Spore forming Bacilli Streptobacilli* and *Pseudomonas.* The extractpossesses antimicrobial activity against gram positive or negative bacterial strains & shows a maximum zone of inhibition.

The aim and objectives of the present study were to extract the leaf and flower of marigold plant with suitable solvent. Then examine the Phytochemicals analysis and find out Antibacterial efficiency against some gram positive and negative bacterial strains to treat various diseases.

## 2. MATERIALS AND METHODS

### 2.1. Media and Chemical used

All the chemicals and media (NAM) used in the present investigation was obtained from Hi – Media Laboratories Ltd. India.

### 2.2.Sample collection

Sample (fresh flower and leaf) were collected at full bloom stage from the Biosciences department, College of Applied Education and Health Sciences, Meerut. All fresh samples (flower and leaf) washed with Double distilled water and then 70 % ethanol for phytochemical analysis.

### 2.3.Extract preparation

Dried powered of leaf & flower of marigold (10 gm) were extracted by continue mixing in 100 ml (50%) methanol, 24 h at room temperature. After the filtrations process, methanol was evaporated until only water remained through evaporation on water bath at 60 – 70 ℃ temperature. The final extract of leaf and flower was kept in air tight box.

### 2.4.Phytochemical Analysis of Leaf & Flower Extraction

The samples were tested for several phytochemicals using standard procedures as quoted by Mostafavi and Pezhhanfar (2015) to identify the phytochemical attributes.

#### 2.4.1.Test for terpenoids (Chloroform test / Salkowaski test)

0.5 ml of each extract was mixed in 2 ml of chloroform, and concentrated Sulphuric acid (2 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results of the terpenoids.

#### 2.4.2.Test for protein (Biuret test)

Small quantity of the extract was dissolved in 5 ml of water and subjected to Xantho protein test. To 3 ml of the extract, 1ml of concentrate Nitric acid was added. A white precipitate was obtained. The solution was heated for 1minute and cooled under tap water. It was made alkaline by excess of 40% NaOH. Appearance of orange precipitate indicates the presence of protein.

**2.4.3.Test for steroids**

0.5ml of oil sample equal volume of chloroform was added and subjected with few drops of concentrated sulphuric acid where the appearance of brown ring indicated the presence of steroids.

**2.4.4.Test for flavonoids (Alkaline reagent test)**

The extract was treated with concentrated Sulphuric acid. Appearance of yellowish orange show the presence of anthocyanins, yellow to orange colour show the presence of flavones, and orange to crimson show the presence of flavonones.

**2.4.5.Test for alkaloids**

A small portion of the extract was stirred separately with 1 ml of dilute Hydrochloric acid and filtered. The filtrate was treated with Dragandroff's reagent. Appearance of organic precipitate shows the presence of alkaloids.

**2.4.6.Test for quinones**

Taken 0.5ml of sample, 0.5ml of concentrated sulphuric acid was added. Formation of red colour indicated the presence of quinines.

**2.4.7.Test for glycosides**

Small quantity of the extract o was hydrolysed with 5ml Hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Fehling's test. To 2ml of Fehling's solution (1ml of Fehling's A and 1 ml of Fehling's B solution), 2ml of extract was added, mixed well and boiled. Appearance of yellow or red colour precipitate indicates the presence of reducing sugars.

**2.4.8.Test for phenols**

Take 1ml of the sample, 2ml of distilled water followed by few drops of 10% ferric chloride was added. Formation of blue or green colour indicated the presence of phenols.

**2.4.9.Test for triterpenoids**

Take 0.5ml of sample, 0.5ml of Libemann – Buchard Reagent (aectic anhydride + concentrated sulphuric acid) was added. Formation of blue green colour indicated the presence of triterpenoids.

### 2.4.10. Test for coumarins

To 0.5 ml of sample, 0.5ml of 10% NaOH was added. Formation of yellow colour indicated the presence of coumarins.

### 2.4.11. Test for carbohydrates

To 0.5ml of sample, 1ml of Molisch's reagent and few drops of concentrated sulphuric acid were added. Presence of purple or reddish colour indicated the presence of Carbohydrates.

### 2.4.12. Test for tannins

About 0.5 ml of the sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

### 2.4.13. Test for saponins (Froth test)

About 1 ml of the sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

### 2.5.Antibiotics sensitivity test

The test microorganisms were also tested for their sensitivity against the antibiotics (extract of leaf & flower). Using sterile cotton swabs, the fresh broth of different cultures spread on different agar plates. After that used sterilized forceps, the antibiotics disc was aseptically placed over the agar plates. The plates were incubated at 37℃ for 24 hours and the diameter of inhibition zones were measured in mm.

## 3. RESULT

### 3.1. Sample collection

Plant sample collected in sterilize stone cup and transferred into microbiology laboratory for the process of sterilization.

**Figure 1: Plant of marigold.**

**Figure 2A: Leaf of Marigold.**

**Figure 2B: Flower of Marigold.**

### 3.2. Preparation of extraction

Leaf and flower of marigold crushed with the help of sterilized mortal pistolby using normal saline as solvent agent, after that the extraction filter through whatsman filter paper.

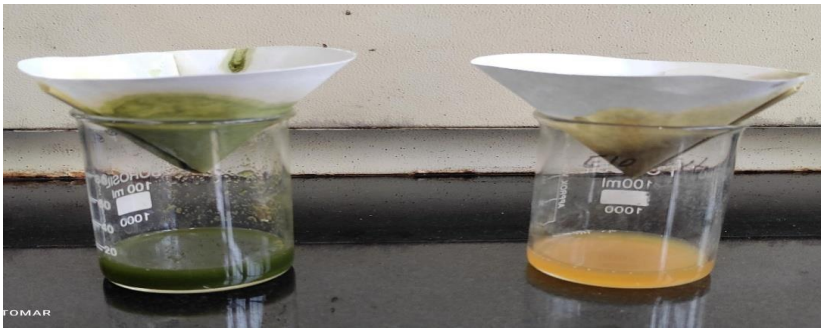

**Figure 3: Plant Extraction. (A) Leaf Extract, (B) Flower Extract.**

### 3.3. Phytochemicals analysis

The plant extract was screened for the presence of major secondary metabolites such as Alkaloids, Flavonoids, Saponin, Terpenoids, Tannin, Glycosides, Steroids, Proteins, Quinones, Phenol, Carbohydrates and Coumarins according to common phytochemical methods. The tests were based on visual observation of the change in the colour or formation of precipitate after the addition of specific reagent. The result of phytochemical analysis

shown in table 1. The present study exhibited the presence and absence of phytochemical compounds in the extract. In this screening, Chloroform test, steroids, Flavonoids, Alkaloids, Quinones, Coumarins, Carbohydrates and Tannin test show positive results but protein, phenol, Saponin given negative result.

**Table 1: Result of Phytochemical test in fresh leaf and flowers of marigold.**

| S. No. | Phytochemical test | Fresh leaf | Flower |
|---|---|---|---|
| 1. | Terpenoids test (Chloroform test) | Positive | Positive |
| 2. | Protein test (Biruet test) | Negative | Negative |
| 3. | Steroids test | Positive | Negative |
| 4. | Flavonoids test (Alkaline reagent test) | Positive | Positive |
| 5. | Alkaloids test | Positive | Positive |
| 6. | Quinones test | Positive | Positive |
| 7. | Glycosides test | Positive | Negative |
| 8. | Test for phenol | Negative | Negative |
| 9. | Coumarins test | Positive | Positive |
| 10. | Carbohydrates test | Positive | Positive |
| 11. | Tanin test | Positive | Positive |
| 12. | Saponins test (Forthest test) | Negative | Positive |

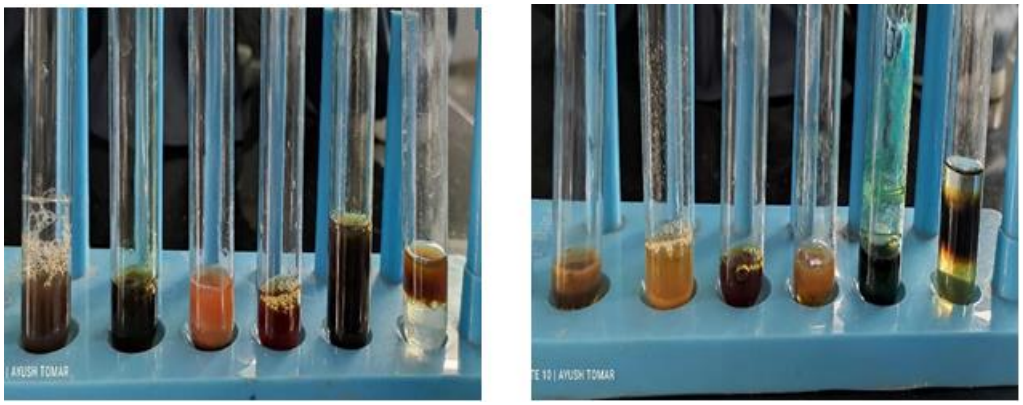

**Figure 4: Phytochemicals analysis of leaf extractof marigold.**

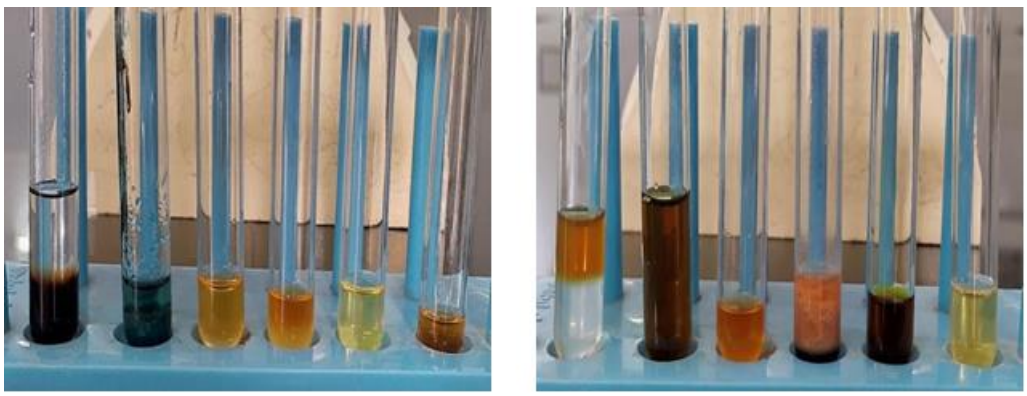

**Figure 5: Phytochemical analysis of flower extract of marigold.**

### 3.4 Effect of Leaf and Flower extract against Gram's positive & negative bacterial strains

The examined leaf and flower extract shown positive results against all strains of gram positive and negative. Leaf and flower extract work against *B. subtils, E. coli, S. aureus, Pseudomonas, Spore forming bacilli* & *Streptobacilli.* Leaf extract from marigold shown less zone inhibition when compared to flower extract. The highest zone of inhibition was recorded by *S. aureus* (figure.) (8mm & 12mm), respectively followed by five other organism like *B.Subtils, E.coli, Pseudomonas, spore forming bacilli and Streptobacilli shown different concentrations against extract of leaf and flower of Tagetes Erecta.*

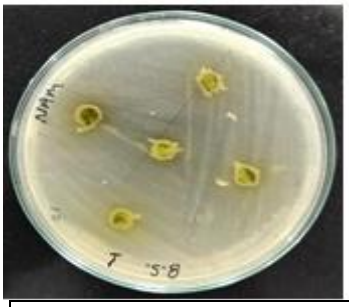

**Figure 6A: Inhibition zone of extract against *B. Subtils*.**

**Figure 6B: Inhibition zone of extract against *S. aureus*.**

**Figure 6C: Inhibition zone of extract against *E. Coli*.**

**Figure 6D: Inhibition zone of extract against *Pseudomonas*.**

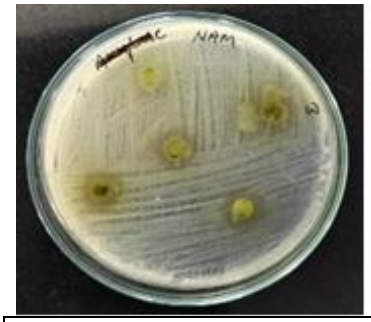

**Figure 6E: Inhibition zone of extract against *Spore forming bacilli*.**

**Figure 6F: Inhibition zone of extract against *Streptobacilli*.**

**Figure 6: Zone of Inhibition of Flower and Leaf Extract against different bacterial strains.**

**Table 2: Antibiotics sensitivity test against some bacterial strains.**

| S. No. | Bacteria | Leaf result | Flower result | Dimeter of leaf (mm) |
|---|---|---|---|---|
| 1. | *Bacillus subtils* | + | + | 3 |
| 2. | *S. aureus* | + | + | 8 |
| 3. | *E. coli* | ++ | + | 7 |
| 4. | *Pseudomonas* | + + | + + | 8 |
| 5. | *Spore forming bacilli* | + + | + + | 6 |
| 6. | *Streptobacilli* | + | + + | 7 |

+ = Effective

++ = More effective


## 4. DISCUSSION

The results of the qualitative phytochemical screening test on *tagetes* leaf extract showed that marigold leaves contain alkaloids, flavonoids, saponins, and tannins. According to Devika (2012) research, it was found that marigold leaves contain cardio glycosides, phenolics and coumarins. Furthermore, marigold leaves contain glycosides and terpenoids (S. S. Thorat et al, 2010 & Ali et al, 2017).

The aim of the researchers, conducting research on anthocyanin, because ofanthocyanin can function to prevent atherosclerosis by inhibiting the atherogenesis process and also inhibiting the process of blood clots (L.N. Samber et al, 2013), while for betacyanin because betacyanin contains antiradical effects and high antioxidant activity (S. M. Putri et al, 2016).

The purpose of researchers doing research on quinones is because quinones can help prevent heart disease and osteoporosis (N. El-Najjar et al, 2011).

The motive of researchers doing research on alkaloid is because alkaloid compounds have long been known, and have also been used as drugs that have a lot of potential, for example,

the name of the drug derived from alkaloids is atropine which has an anticholinergic effect, there is also scopolamine which has an antiemetic effect and many others have various effects such as antidepressants, antimalarial, antibacterial, antipyretic, analgesic, anti-inflammatory as for antidiabetic, antihypertensive and anticancer (B. Debnath et al, 2018).

The objective of researchers doing research on coumarin is because Coumarin itself can be used as an anti-coagulant, antiplasmodial and can also be used as an anticancer (P. K. Jain et al, 2012).

The purpose of researchers doing research on flavonoids is because the main antioxidants in food are flavonoids, which are known to prevent cardiovascular disease by reducing the oxidation of low-density lipoproteins and they also doing research on phenolics is because phenolic compounds have fascinated scientists internationally for their peculiar activities, such as anti-inflammatory, antioxidant power, and anti-carcinogenic properties (R. Meccariello et al, 2021).

The reason of researchers doing research onsteroids is because steroids have a similar effect to others such as antiinflammatory, anticancer but for steroids it has a cardioprotective effect (K. K. J. Senthil et al, 2016).

The researchers focused on terpenoids is because terpenoids also have the same effect and have advantages such as anti-mutagenic, anticholinesterase, anti-tyrosinase and anti-diabetic properties (S. K. Malik et al, 2017).

The purpose of researchers doing research on cardiac glycosides is because cardiac glycosides are well known and have been used as drugs such as digitoxin, digoxin, ouabain, oleandrin which have long been used for the treatment of heart disease (S. Patel et al, 2016).

Then purpose of researchers doing research on saponin is because saponins have long been known and used as natural detergents. However, saponins also have biological activity which can be used for anti-inflammatory and immune stimulating remedies (D. Kregiel et al, 2017).

But based on research conducted by Kaur (2013) on marigold plant extracts which are also in accordance with my research which can be used as antiviral, antibacterial, anti-inflammatory and can be used as cancer treatment.

The antibacterial activity of leaf extract against *Pseudomonas, Bacillus, S.aureus, Klebsiella* and *E.coli*. The flavonoids contains antibacterial activity against all tested strains & show minimum zone of inhibition (Rama and Madhavan et al, 2011).

## 5. CONCLUSION

Based on the discussion and results of the research entitled "Phytochemical Screening and Antioxidant Capacity Test of Marigold Leaf & flower Extract, "*Tagetes erecta L.",* the following conclusions can be drawn -

The present phytochemical screening of the marigold leaves and flower proved to contain flavonoid, terpenoids, alkaloid, quinones, steroids, glucosides, tanin and coumarins bioactive compounds which are of medicinal value and have a definite physiological action on the human body.

Further investigation like antibacterial susceptibility test, the total antioxidant capacity of marigold leaf & flower extract worked against some gram positive and negative microbial strains. Extract of marigold show clear inhibition zone against bacterial strains like *Bacillus subtils, S. aureus, E. coli, pseudomonas, spore forming bacilli* and *Streptobacilli.* In this study, we investigated that flavonoids show maximum zone against bacterial strains as compared to leaves extract.

## ACKNOWLEDGEMENT

The authors are thankful to our college “College of Applied Education and Health Sciences, Meerut (CAEHS College) for their support and providing facility & technical support to carry out the above work.

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