JNCS Journal of Nepal Chemical Society January 2026, Vol. 46, No. 1, 94-102
ISSN: 2091-0304 (print)
DOI: https://doi.org/10.3126/jncs.v46i1.91126
RESEARCH ARTICLE

Phytochemical Screening, GC-MS Profiling, and Antimicrobial Evaluation of Marigold (*Tagetes erecta* L.) Leaf Extract of Kathmandu, Nepal

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(Received: December 30, 2025, revised: January 23, 2026 accepted: January 25, 2026)

Abstract

Tagetes erecta L. holds a significant traditional and medicinal value in Nepali culture which is locally known as “Sayapatri”. In spite of the therapeutic potential, the scientific validation of *T. erecta* of Nepali origin has not been properly recorded. In this work, the leaves of *T. erecta* was used to obtain the ethanolic extract using the Soxhlet apparatus. The leaf extract was used for preliminary phytochemical screening, which revealed the presence of essential bioactive compounds, including alkaloids, flavonoids, tannins, terpenoids, and polyphenols. For the detailed analysis, Gas Chromatography- Mass Spectroscopy (GC-MS) analysis of the extract was conducted which revealed the presence of compounds such as neophytadiene (55.42%), 9,12,15-octadecatrienoic acid (11.16%), pentadecanoic acid (10.91%), and phytol (6.72%) as major bioactive components. Further, the antimicrobial efficacy of the extract was tested against five pathogenic strains by agar-well diffusion method, two gram positive bacteria *B. subtilis* ATCC 6051 & *S. aureus* ATCC 6538P , two gram-negative bacteria *E. coli* ATCC 8739 & *K. pneumonia* ATCC 700603, and one fungal strain *C. albicans* ATCC 2091. The antimicrobial test results were recorded by measuring the Zone of Inhibition (ZOI) where moderate inhibitory effects of 1.1 cm was measured on *E. coli, B. subtilis, S. aureus, and C. albicans,* and no activity was observed against *K. pneumonia.* These results showcase the promising antimicrobial potential of *T. erecta* grown in Nepal.

Keywords: *Tagetes erecta* L. (Sayapatri); Phytochemical Screening; Antimicrobial tests; GC-MS analysis.

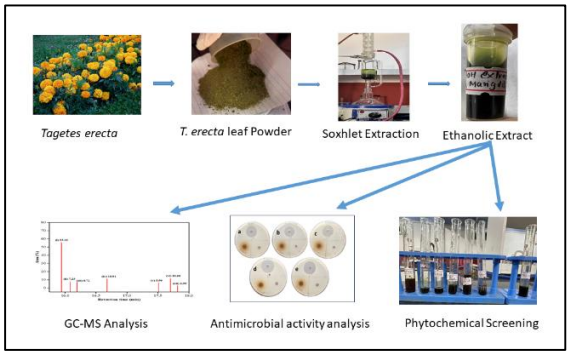
Introduction

The growing resistance of bacteria towards conventional antibiotics has shifted the focus of scientists to explore natural sources of medicine more closely [1]. Plants used in traditional healthcare are again being investigated for their rich supply of active health-boosting substances [2]. African marigold, identified by the scientific name *T. erecta*, is an example of an herbal species with established uses in traditional medicine and modern pharmaceuticals [3]. Even though it is native to Central America, *T. erecta* is grown in South Asia mainly for its ornamental appearance and therapeutic benefits [4].

Traditionally, marigold has been used to treat cuts, skin infections, wounds, and stomach pain [3], [5].

Pharmacologically, the bioactive compounds identified in *T. erecta*, including flavonoids, tannins, and terpenoids. These compounds have been recorded to provide antimicrobial, antioxidant, anti-inflammatory, larvicidal, and anticancer properties [6], [7]. Many studies on *T. erecta* have discovered that it contains important groups of compounds such as flavonoids, alkaloids, tannins, saponins, glycosides, terpenoids, and phenolics [8]. These metabolites are known to have been asscoiated in microbial infections, reducing oxidative stress, and overall having wound healing properties [9]. Likewise, GC-MS analyses of *T. erecta* have showed the presence of compounds like neophytadiene, thiophenes, and stigmasterol, which are linked to anti-inflammatory effects [10]. Nevertheless, although *T. erecta* has been studied in India [11], Mexico [12], Pakistan [13], and Bangladesh [14], its cultivation under Nepal's specific environmental conditions may result in different phytochemical compositions and unique bioactivities [15]. Whereas other researchers in their specific countries have shown that marigold leaf extracts, especially those extracted using ethanol, are abundant in alkaloids, flavonoids, tannins, terpenoids, and polyphenols, this detailed phytochemical profiling has not been reported yet on *T. erecta* grown in Nepal. Thus, the proposed research intends to address this research gap and investigate the phytochemical profile of *T. erecta* growing in the Nepalese environmental conditions, which can be added to the knowledge of the possible medicinal use of this plant.

The extracts of *T. erecta* L. leaves grown in Nepal are investigated using an integrated approach which includes preliminary phytochemical screening, GC-MS analysis to identify the main bioactive components, and antimicrobial testing against selected microbes. The aim of this paper is to analyze whether marigold grown in Nepal exhibits similar or superior bioactivity compared to those from other regions, thereby validating its role in antimicrobial applications.



Scheme 1: Graphical representation of the study design illustrating ethanolic leaf extraction from *T. erecta,* phytochemical profiling, and antimicrobial evaluation.

Materials and Methods

Materials

T. erecta leaves, ethanol, Soxhlet extractor, round-bottom flask, heating mantle, condenser, beaker, muslin cloth as extraction thimble.

Methods

Collection of the Leaves and Plant Identification

T. erecta leaves were collected from Kathmandu area, and the plant specimen was taxonomically identified by National Herbarium and Plant Laboratories, Godwari, Lalitpur, Nepal. They were first washed by tap water to remove dirt and debris, which was followed by rinsing with distilled water for thorough cleaning. The cleaned leaves were shade dried at room temperature until fully dehydrated. Once dried, the fine powder was obtained by grinding the dried leaves in mechanical grinder. The powdered leaves were kept in an air-tight zip-lock bag for storage and future use.

Preparation of Ethanolic *T. erecta* Leaf Extract

40 g of dried leaf powder was accurately measured and enclosed in a muslin cloth thimble, which was then inserted into the Soxhlet extractor with 400 mL of ethanol in

round bottom flask. The extraction was continued for 4 hours below 80 °C till the solvent in the chamber became clear which indicates the completion of the extraction. The resulting solution was filtered through Whatman No. 1 filter paper and the clarified extract was evaporated in water bath to obtain the crude solid extract.

Phytochemical Screening

A portion of the crude solid extract was subjected to preliminary phytochemical screening to detect any presence of bioactive compounds like flavonoids, alkaloids, carbohydrates, tannins, saponins, and phenolic substances, following the procedures described by G. E. Trease [16] and A. J. Harborne [17].

Gas Chromatography – Mass Spectroscopy (GC-MS) Analysis

10 mg of the plant extract was dissolved in n-hexane, and 5 µL of the resulting extract was added to 995 µL of n-hexane for injection. The analysis was conducted using a Shimadzu GC-MS system equipped with an SH-I-Sil MS capillary column. The injection was performed in splitless mode. Data acquisition and analysis were performed using Shimadzu Lab Solutions software (GCMS Release 4.53), and compound identification was achieved by comparing the EI mass spectra of the peaks with the NIST/EPA/NIH mass spectral library (2020), producing the total ion chromatogram (TIC).

Antimicrobial Assay

The antimicrobial screening of the plant's leaf extract was evaluated against five pathogenic microbial strains. The organisms tested were two Gram-negative bacteria (*Escherichia coli* ATCC 8739 & *Klebsiella pneumoniae* ATCC 700603), two Gram- positive bacteria (*Bacillus subtilis* ATCC 6051 & *Staphylococcus aureus* ATCC 6538P), and one fungal strain *Candida albicans* (ATCC 2091).

The antimicrobial activity test of the plant extract was conducted using the agar-well diffusion method [18]. 25 mL of sterilized MH agar (Sisco Research Laboratories Pvt. Ltd., India) medium for *E. coli*, *K. pneumoniae*, *B. subtilis*, *S. aureus,* and *C. albicans* was transferred into each sterilized petri dish. The entire agar surface of the media plates was uniformly cotton-swabbed with 0.1% inoculum suspension, ensuring even distribution of the organism over the agar surface. The wells (9 mm diameter, 3 mm depth) were loaded with 100 µL of sample solution dissolved in DMSO (concentration 100 mg/mL). DMSO, as a negative control, and standard kanamycin solution (5 mg/mL, 10 µL loaded) and standard itraconazole solution (20 mg/mL, 10 µL loaded), as a positive control, were used in the respective wells. The media plates were then incubated at 37 °C for 24 hours. The antimicrobial results were observed after 24 hours. The inhibition zones formed were measured with a ruler in centimeters.

Results and Discussion

Phytochemical Screening

Qualitative analysis of the extract revealed that *Tagetes erecta* leaf is rich in alkaloids, flavonoids, carbohydrates, tannins, and phenolic substances. These bioactive constituents are often cited for the positive effects they have on medicine. In particular, flavonoids and phenolics help protect cells from damage and reduce inflammation [19]**,** but alkaloids more commonly give certain plants antimicrobial, analgesic and antimalarial properties [20]. Even though carbohydrates mainly support the structure or energy needs of the body, they may help modulate the immune system [21] Some phytochemicals may not be properly soluble in the ethanol, this prevents the proper extraction of such compounds [22]**.** Many environmental factors like, the quality of soil, temperature, elevation and the season, play the crucial role in the concentration of secondary metabolites in plants [23].

Table 1: Phytochemical Screening results of ethanolic leaf extract of *T. erecta.*

Phytochemicals	Observed results	Inferences
Alkaloids (Conc. H_2SO_4 test)	Color change observed	+++
Flavonoids	Yellow color turned colorless	+++
Carbohydrates	Brick red ppt	+++
Saponins	Foam did not persist for several minutes	---
Tannins	Green color observed	+++
Polyphenols	Green color observed	+++
Glycoside	No pink color	---
Terpenoids	Reddish brown color	+++
Protein	No white ppt	---
Resins	Absence of resin formation	---
Fixed oils and fats	No oil and stain in filter paper	---
Gums and Mucilage	No white or cloudy ppt	---

Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The GC-MS chromatogram in (**Figure 1**) presented in the study identifies a range of bioactive compounds which indicates the richness in phytochemical constituents *T. erecta* leaves extract of Nepal. The most abundant compound was found to be neophytadiene (55.42%) that is a diterpene hydrocarbon widely known for its antimicrobial and anti-inflammatory properties [24]. Other notable constituents included 9,12,15-octadecatrienoic acid (Z, Z, Z)- (11.67%) commonly known as linolenic acid which is a class of essential fatty acid with anti-inflammatory and antioxidant activities [25], [26] , and pentadecanoic acid (10.91%), a saturated fatty acid reported to show anticancer properties [27]. Phytol (3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol), a diterpenoid alcohol present at 6.72%, is also recognized for its antimicrobial, antifungal, and cytotoxic effects [28], [29]. Additional compounds such as, 2-Undecyloxirane and Z, Z-8,10-hexadecadien-1-ol, known for their bioactivity. The presence of wide range of diverse chemical constituents correlates the strong antibacterial and antifungal activity observed in the study. These findings supports the therapeutic potential of *T. erecta* as a source of natural antimicrobial agents and validates the traditional use of *T. erecta* in Nepali culture. The chromatogram details are presented in **Table 2** along with their chemical class.

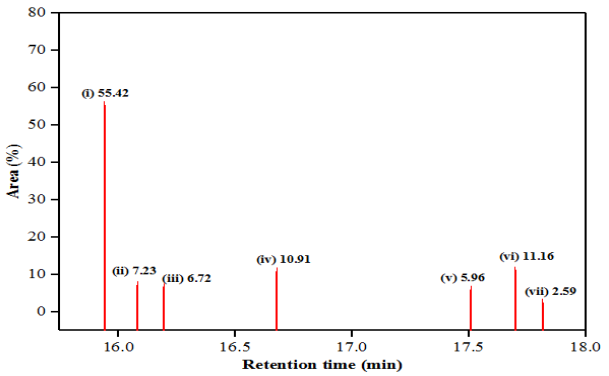
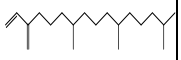
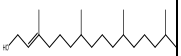
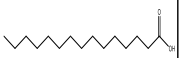
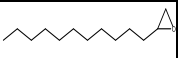
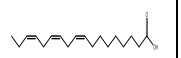
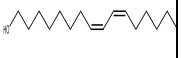


Figure 1: GC-MS chromatogram of *T. erecta* leaves extract of Nepal presenting the percentage area of the major phytochemical components.

Table 2: Area percentage of major compounds of *T. erecta* Leaf extract in GC-MS chromatogram of Nepal and their structures.

S. N	Retention Time	Area %	Chemical Compounds	Chemical Class	Structure
1	15.940	55.42	Neophytadiene	Diterpene hydrocarbon (Terpene)	
2	16.082	7.23	E,E-2,13-Octadecadien-1-ol	Unsaturated long-chain alcohol (Fatty alcohol	
3	16.196	6.72	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Diterpene alcohol (e.g., Phytol	
4	16.679	10.91	Pentadecanoic acid	Saturated fatty acid	
5	17.509	5.96	2-Undecyloxirane	Epoxide (Cyclic ether)	
6	17.698	11.16	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Polyunsaturated fatty acid (PUFA) (e.g., α-Linolenic acid)	
7	17.815	2.59	Z,Z-8,10-Hexadecadien-1-ol	Unsaturated fatty alcohol	

Antimicrobial Activity Analysis

The *T. erecta* leaves extract was analyzed against different five microbial starins,

including both Gram-positive and Gram-negative bacteria, as well as s fungal strain. The extract demonstrated moderate inhibitory effects, with a zone of inhibition of 1.1 cm against *E. coli, B. subtilis, S. aureus, and C. albicans,* as shown in the **Table 3**. The result indicated that the *T. erecta* leaves extract possesses antimicrobial properties, consistent with previous studies reporting the antimicrobial activity against similar pathogens [30], [31], [32]. The extract inhibited *E. coli* and *S. aureus*, likely due to the bioactive compounds such as flavonoids and polyphenols present in the extract **(Table 1)** which may have disrupted the microbial cell membrane and fungal ergosterol biosynthesis [33]. Also, the presence of the compound neophytadiene (55.42%) **(Table 2)**, a terpenoid known to disrupt the microbial membrane, especially against gram-positive bacteria and fungi [34]

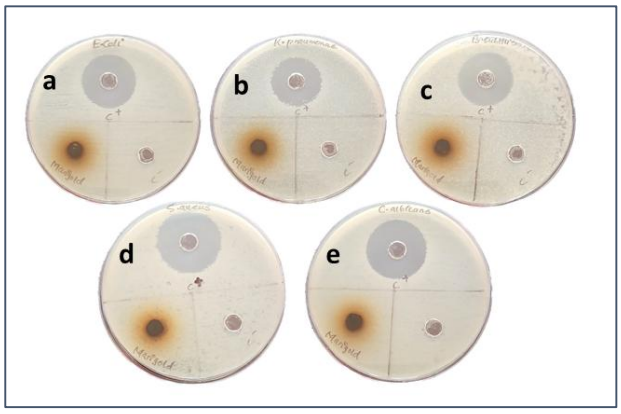


Figure 2: Photographs showing ZOI of *T. erecta* Leaf extract against Gram-negative bacteria **a.** *E. coli* & **b.** *K. pneumonia,* Gram-positive bacteria **c.** *B. subtilis* & **d.** *S. aureus* and Fungus **e.** *C. albicans.*

However, the marigold extract exhibited no inhibitory effect against *K. pneumoniae*, with no visible zone of inhibition. This result may contradict the inhibitory effect of marigold extract, which showed effective inhibition against *K. pneumonia.* But the resistance of *K. pneumoniae* may be due to the thick polysaccharide capsule and efflux pump system that reduced the penetration of the extract [35]. The absence of saponins and glycosides, which are known for their microbial effect against gram-negative bacteria [36], may explain the lack of zone of inhibition zones against *K. pneumoniae*. These findings support the continued exploration of marigold potential as a natural antimicrobial agent in the fight against resistant pathogens.

Table 3: Antimicrobial analysis of *T. erecta* leaves extract against selected microbial strains based on ZOI.

Strain	Reference culture	Type	Positive control (c+) cm	*T. erecta leaf* extract cm
E. coli	ATCC 8739	Gram –ve	2.7	1.1
K. pneumoniae	ATCC 700603		2.5	0
B. subtilis	ATCC 6051	Gram +ve	2.7	1.1
S. aureus	ATCC 6538P		2.8	1.1
C. albicans	ATCC 2091	Fungus	2.8	1.1

The bar graph in **Figure 3** illustrates the antimicrobial efficacy results of *T. erecta* against the selected microbial strains. It provides a clear visual comparison of antimicrobial effects of extract on the basis of ZOI. *T. erecta* species have cosmopolitan distribution across different regions worldwide across Asia, Europe, and Americas. The geographical factors can have great impacts on its phytochemical constituents, its concentrations, and therapeutic applications [23]. **Table 3** shows a comparative overview of the *T. erecta* of Nepali along with the different medicinal properties of *T. erecta* species on the basis of their territorial patterns. The present work focuses on the antimicrobial properties of *T. erecta* native to Nepal and the findings further reinforce its therapeutic validation and the potential for future pharmacological applications.

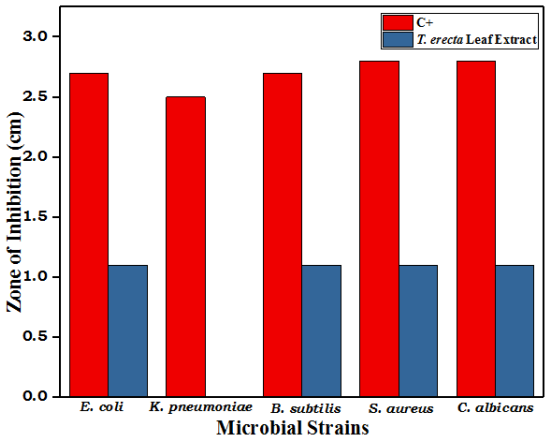


Figure 3: Bar graph illustration of the antimicrobial efficacy of *T. erecta* leaves extract against different microbial strains.

Table 4: Geographical distribution and therapeutic applications of *T. erecta* species.

SN	Species	Geographic Distribution	Therapeutic Applications
1	*T. erecta*	Nepali origin; distributed across China and Southeast Asia	Antibacterial and Antifungal Activities **(This work)**
2	*T. erecta*	Northeastern Brazil	Essential oil exhibited Antibacterial activities [37]
3	*T. minuta*	Nepal and other regions	Antimicrobial activity of essential oil and extracts [38]
4	*T. minuta*	South America and globally naturalized	Anti-inflammatory activity [39]
5	*T. erecta*	Mexico	Petal Extracts; Antioxidant and Cytotoxic Effects against Ovarian Cancer Cell Lines [40]
6	*T. erecta*	India, Iran, and other regions	Antibacterial and Antifungal [41], [42]
7	*T. minuta*	South Africa	Antibacterial and antioxidant activities from essential oil [43]
8	*Tagetes patula*	Asia, Europe, and other regions	Antioxidant, anti-inflammatory, antimicrobial, and anti-parasitic activities [7]
9	*T. lucida*	Mexico and Central America	Antimicrobial activity [44]
10	*T. rupestris* & *T. filifolia*	Argentina	Essential oils with anti-insect activity [45]
11	*T. patula*	France	Anti-inflammatory effects against uropathogenic E. coli infection (Kidney protection)[46]

Conclusions

This study gives the better understanding of the phytochemical make-up and the antimicrobial properties of *T. erecta* leaves cultivated in the environmental conditions of Nepal. The initial phytochemical profiling was found to contain a variety of pharmaceutically valuable secondary metabolites and especially the flavonoids, alkaloids, tannins and polyphenols which have varied bioactivities. Bioactive compounds were further identified on GC-MS analysis with neophytadiene being the most dominant and other phytochemical including phytol and octadecatrienoic acid. These components are probably the causes of moderate antimicrobial effect in *E.coli, B. subtilis, S. aureus, and* C. *albicans*, which validates the traditional use of the marigold in the treating infections. The extract was inactive against *K. pneumoniae,* and this could be due to lack of microbial resistance factors or special phytochemicals such as saponins and glycosides. As compared to the similar works by other countries [47], the paper reports not only the similarity but also the diversity in the bioactivity of *T. erecta* which confirms the importance of the role of both geographical and environmental factors in shaping the phytochemical composition. The findings support the therapeutical value of Nepalese marigold and confirm the need to explore the process of its production as an antimicrobial substance.

Acknowledgements

We acknowledge Urbana School of Science (An Undertaking of Name), Putalisadak, Kathmandu, for funding of this research work.

Author's contribution statement

B. B. Magar: Conceptualization, Sample collection, Investigation, Formal analysis, Visualization, Writing -original draft. **S. Adhikari, O. M. Shrestha, and A. Thadarai:** Sample collection, Investigation, Data curation, Validation, Writing – original draft. **S. Bhandari and I. Shahi:** Data interpretation (antimicrobial results), Writing – review & editing. **S. Shrestha:** Formal analysis (GC–MS analysis), Writing – review & editing. **R. Pandit:** Validation, Writing – review & editing, Finalization of the manuscript, **M. M. Shrestha and N. Rai:** Conceptualization, Supervision, Resources, Funding acquisition, project administration, Writing- review & editing,

Visualization, Finalization of the manuscript

Conflict of interest

The authors declare no conflict of interest to this work.

Data availability statement

All the data and results will be made available on a request.

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