Article

Phytochemical characterization of fresh and air-dried ethanolic leaf extract of African marigold *Tagetes erecta* (L.)

Ralf Benjo G. Morilla, **Mark Anthony J. Torres**, **Cesar G. Demayo**

Department of Biological Sciences, College of Science and Mathematics, MSU-Iligan Institute of Technology, 9200 Iligan City Philippines

E-mail: ralfbenjo.morilla@g.msuiit.edu.ph, cgdemayo@gmail.com

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Abstract

Tagetes erecta (L.), also known as African marigold, has a lengthy history of traditional medicinal use due to its presumed therapeutic properties. The leaves of *T. erecta* (L.) have been used in traditional medicine to treat various health issues, including arthritis, joint pain, fever, skin disorders, and allergies. This study aims to investigate the medicinal potential of *T. erecta* (L.) leaves by evaluating their biological properties and phytochemical composition. Fresh and air-dried ethanolic leaf extracts of *T. erecta* (L.) were prepared and phytochemically screened for bioactive compounds. Using a reduction power assay, the antioxidant activity of both extracts was determined, and the results were compared to the antioxidant activity of vitamin C. Additionally, Gas Chromatography-Mass Spectrometry (GCMS) was utilized to analyze the chemical composition of the extracts and identify potentially bioactive compounds. The biological functions with the greatest number of compounds were identified using seriation and cluster analysis. The *T. erecta* (L.) leaf extracts contained bioactive compounds, including saponins, tannins, flavonoids, alkaloids, and steroids, according to phytochemical analysis. The antioxidant activity of the air-dried ethanolic leaf extracts was greater than that of the fresh extracts. Nonetheless, even at higher concentrations, the antioxidant activity of the ethanolic leaf extracts was inferior to that of vitamin C. The GCMS analysis identified 12 compounds in the extracts, where 10 are known to have specific biological functions. This study provides scientific support for the traditional medicinal applications of *T. erecta* (L.) leaves. The presence of bioactive compounds in the extracts and their antioxidant properties suggests that there may be a scientific basis for the purported efficacy of traditional medicine. *T. erecta* (L.) shows considerable promise as a valuable resource in traditional medicine and warrants additional research into its potential pharmacological applications.

Keywords *Tagetes erecta* (L.); phytochemical screening; folk medicine; anti-inflammatory; GCMS.

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1 Introduction

Plants and herbs are used extensively for their benefits, including having few adverse effects and being inexpensive (Miraj and Kiani, 2016). Compared to conventional therapy options, many are easy to obtain. Only 6% of the 250,000–400,000 plant species have evaluated their biological activity, and only 15% have examined their phytochemical composition. This indicates the necessity for pharmacological evaluation of herbal medicines (Goyal et al., 2007). *Tagetes erecta* (L.), a yellow-colored, local variety of Amarillo reported to have numerous pharmacological and therapeutic benefits, has been widely used as a medication. Locally referred to as Amarillo in the Philippines, *T. erecta* (L.) is a robust, branching herb native to Mexico and other warmer regions of America; it has been neutralized abroad in the tropics and subtropics, including India and Bangladesh (Karwani and Sisodia, 2015). This plant is popular as a remedy for colds and bronchitis (Ghani, 1998), gastrointestinal issues (Kirtikar and Basu, 1993), carbuncles, eye infections, and muscular spasms, and for its anti-nociceptive and anti-inflammatory actions (Shinde et al., 2009). The extract from the flowers and leaves are reported to have antibacterial, antimicrobial, insecticidal, wound-healing, hepatoprotective, antioxidant, and anti-diabetic properties (Karwani and Sisodia, 2015; Verma and Verma, 2012; Padalia and Chanda, 2015; Shetty et al., 2015). Studies on the phytochemistry of several portions of *T. erecta* (L.) have revealed a variety of chemical components, including thiophenes, alkaloids, saponins, flavonoids, carotenoids, and triterpenoids (Faizi and Naz, 2004; Munhoza et al., 2014). Gallic acid, gallicin, quercetagetin, 6-hydroxykaempferol-O-hexoside, patuletin-O-hexoside, and quercetin were among the antioxidants detected in *T. erecta* (L.) extracts; quercetagetin was determined to be the most potent antioxidant (Gong et al., 2012; Wang et al., 2016).

Despite the extensive traditional use of *T. erecta* (L.) to treat various conditions like rheumatism, irregular menstruation, indigestion, colic cough, anemia, and muscular and bone discomfort (Shetty et al., 2009; Shetty et al., 2015), there remains a dearth of scientific research to provide a solid rationale for its traditional therapeutic applications.

In light of the existing knowledge gap, this study aimed to assess the biological characteristics of fresh and air-dried ethanolic leaf extracts of *T. erecta* (L.). Our primary objectives were to conduct phytochemical screening to identify potential medicinally effective compounds, evaluate the ferric reducing antioxidant power (FRAP) assay's ability to detect reducing antioxidant activity in the extracts, and analyze the bioactive substances in *T. erecta* (L.) leaf extracts using gas chromatography-mass spectrometry (GC-MS). By focusing on the local traditional practices for treating illnesses, this research seeks to provide a scientific basis for the efficacy of these practices and contribute to the growing body of knowledge on natural medicine.

2 Materials and Methods

2.1 Informal interview and field observation

A survey was conducted in Kapatagan, Lanao del Norte's mountainous area, to determine the traditional medicinal applications of *T. erecta* (L.) leaves (Fig. 1). This area was chosen due to its historical utilization of herbal remedies. Inhabitants were interviewed using semi-structured queries designed to elicit pertinent information for this study. The questionnaire encompassed a diverse range of aspects, including traditional preparation methods, application techniques, and the therapeutic use of *T. erecta* (L.) leaves. The snowball sampling technique was used in this investigation. Before the survey, the primary informants were briefed. By employing this comprehensive survey approach and sampling technique, the study endeavors to unearth valuable information regarding the traditional uses of *T. erecta* (L.) leaves. The gathered knowledge from the community's wisdom keepers holds immense potential to enrich the scientific understanding of herbal medicine promoting the sustainable use of traditional remedies in modern healthcare practices.

Fig. 1 Map of Kapatagan, Lanao del Norte, Philippines.

2.2 Collection and authentication of plant materials

The collection and authentication of plant materials, specifically the fresh foliage of *Tagetes erecta* (L.), were gathered from the mountainous villages of Kapatagan, Lanao del Norte, Philippines (Fig. 2). To ensure accurate identification, Professor Jaime Guihawan, a renowned curator in the Department of Biological Sciences at MSU-IIT, verified the plant specimens. Subsequently, the freshly gathered leaves were carefully washed with water and left to air-dry naturally for several weeks, preserving their botanical integrity. Upon drying, the leaves underwent further processing by grinding them into a fine powder using a mechanical processor, guaranteeing uniformity and consistency in the samples. The powdered leaves were stored in impermeable containers to maintain their potency and prevent any potential degradation. These containers were then meticulously kept in a cool, dry, and dark environment at room temperature, safeguarding the phytochemical composition of the plant material.

Fig. 2 The *Tagetes erecta* (L.) plant used in this study

2.3 Extract preparation for fresh and air-dried sample

The extraction was conducted in the Graduate Research Laboratory of the MSU-IIT Department. For the air-dried leaf extraction, 250 grams (g) of *T. erecta* (L.) pulverized leaves were steeped for 48 hours in 1.25 liters (l) of ethanol containing 99% ethanol. After three days, the suspension was filtered using Whatman filter paper No. 1. The filtrate was concentrated at approximately 45°C using a rotary evaporator. Fresh ethanolic leaf extraction followed the same procedure. The obtained viscous crude extracts were preserved in containers for phytochemical screening, ferric reducing antioxidant power (FRAP) assay, and Gas Chromatography–Mass Spectrometry (GC-MS) Analysis.

2.4 Ethanolic extract partitioning

Using standard partitioning techniques, the ethanolic extract was partitioned at the Department of Chemistry, MSU-IIT. The ethanolic extract of *T. erecta* (L.) leaves was partitioned with 1 water to 1 hexane to produce an aqueous and hexane-soluble fraction. The hexane-soluble extract was concentrated using a rotary evaporator, yielding 7.70 grams, which was then refrigerated until use. Like the hexane-soluble extract, the resulting crude ethanol extract was partitioned with 1:1 water: chloroform to produce an aqueous portion and chloroform-soluble extract. The chloroform-soluble extract was concentrated using the rotary evaporator and refrigerated until further use. To obtain an aqueous amount and an ethyl acetate-soluble extract, ethyl acetate was partitioned with water in a ratio of 1:1. The liquid was then concentrated in a rotary evaporator and continuously refrigerated until use.

2.5 Phytochemical screening

The phytochemical screening of the fresh and air ethanolic leaf extracts of *T. erecta* (L.) was conducted using the standard phytochemical methods described by Macabeo et al. (2005), which were modified based on the laboratory analysis of the Department of Chemistry, MSU-Iligan Institute of Technology, Lanao del Norte, Philippines. The qualitative evaluation of phytochemicals (i.e., alkaloids, saponins, flavonoids, tannins, cyanogenic glycosides, steroids, and anthraquinones) was recorded using a 3-point scale (+turbid, ++moderate, and +++heavy) based on Handbook of Philippine Medicinal Plants (De Padua et al., 1977).

2.6 Detection of saponins

Saponins were detected in ethanolic extracts of *T. erecta* (L.) using the froth test method. In a test vessel containing an equal volume of water, two (2) milliliters of plant extract were added. The plant extract was agitated for approximately thirty seconds. The formation of a two-centimeter-tall foam determined the presence of saponin. The froth lasted for half an hour.

2.7 Detection of alkaloids

The detection of alkaloids was accomplished by transferring an adequate quantity of plant extract to an evaporating dish and evaporating it in a scalding water bath until the dish was nearly dry. The dish was removed from the bath when it was nearly dried and then chilled. 5-10 ml of 2M HCl and 0.5 g of sodium chloride crystal were added to the dish. It was submerged in a cauldron of boiling water for approximately five (5) minutes. The solution was then chilled and filtered. The residue on the filter paper was rinsed with 2M HCl. The combined filtrate was divided into three (3) equal sections after being rinsed and divided into three (3) equal portions. Wagner's reagent was added to the first portion, Mayer's reagent was added to the second portion, and the third portion served as the control. The formation of a brown precipitate with Wagner's reagent and a white precipitate with Mayer's reagent indicated a positive result. The results were shown as follows: (-) for the absence of precipitate; (+) for a turbid solution only; (++) for moderate precipitate formation; and (+++) for substantial precipitate formation.

2.8 Detection of flavonoids

The crude ethanolic extract was transferred to an evaporating vessel before completely evaporating in a boiling

water immersion. The extract was chilled and delipidized with hexane until it became transparent. The defatted extract was dissolved in 10 ml of 80 percent alcohol before being filtered and divided into two equal portions. 0.5 ml of 12M HCl was introduced in the first portion, while the second portion served as the control. Two test containers were placed in the heated water bath, and a color change was observed. The presence of red in the solution indicated that the results were favorable. The reaction was observed for two (2) hours due to the sluggish development of the red color. The following results were recorded: (-) no color change; (+) presence of a very light red hue, (++) moderate red hue, and (+++) production of a dark red hue.

2.9 Detection of tannins

After transferring the crude ethanolic extract to an evaporating vessel, it was nearly evaporated to dryness in a boiling water reservoir. The extract was chilled and added to 20ml of boiling water, after which 2-3 droplets of a 10% NaCl solution were added. The solution produced was filtered. The leftovers were rinsed with water. The combined filtrate was recovered, cleansed, and divided into three (3) equal portions. 1-3 drops of 1% ferric chloride were added to the first filtrate, 1-3 drops of Gelatin-Salt reagent were added to the second, and the third filtrate served as a control. (1) The formation of a black or blue-black precipitate with ferric chloride and (2) a white precipitate with the Gelatin-sodium chloride test indicated a positive result. The results were recorded as follows: (-) for the absence of precipitate, (+) for a turbid solution only, (++) for moderate precipitate formation, and (+++) for substantial precipitate formation.

2.10 Detection of steroids

The crude extract was almost completely evaporated in a scalding water bath in the evaporating container. The dried crude extract was cooled and defatted with hexane. The extract was mixed with 3 to 5 milliliters of ferric chloride reagent and filtered. They were separating the filtrate into two equal portions. One (1) ml of concentrated sulfuric acid was added through the test tube's wall as the first component. Positive results were indicated by forming a brown (sometimes blue or green) rim at the aqueous extract's boundary. The presence of 2-deoxy carbohydrates indicated the presence of sulfuric acid.

2.11 Detection of anthraquinones

In the evaporating container, the crude extract was nearly evaporated to dryness in a bath of scalding water. The crude extract was cooled with hexane and defatted. Then 10ml of purified water was added. The resulting solution was combined and filtered after being agitated. Five (5) ml of benzene was used to extract the filtrate twice. A few minutes were required to separate the aqueous and benzene layers. Using a transfer pipet, the benzene layer was separated and deposited in a test container containing one (1) milliliter of ammonia reagent, which was then shaken for a few seconds. The hue was spotted in the aqueous stratum. Indicating the presence of anthraquinones, developing a reddish-pink hue in the solution's aqueous layer was the determining factor in the outcome.

2.12 Detection of cyanogenic glycosides

One (1) ml of *T. erecta* (L.) leaf extract was added to a 20-milliliter test tube with four to five droplets of chloroform. Above the solution was a sheet of picrate paper. The test vial was placed in an inverted dropper and submerged in the hot water. The instantaneous formation of red color on the surface of the picrate paper indicated positive results for cyanogenic glycosides.

2.13 Ferric Reducing Antioxidant Power (FRAP) assay

The Ferric Reducing Antioxidant Power (FRAP) assay was employed to assess plant extracts' reducing power and antioxidant activity. The method is based on the ability of a compound to act as an antioxidant by converting the ferric-ferricyanide complex to the ferrous-ferricyanide complex. A modified version of Oyaizu's (1986) method was used in this study. In brief, 2.5 ml of various plant extract solutions were combined with 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at

50°C for 20 minutes. After incubation, 2.5 ml of 10% trichloroacetic acid (TCA) was added to the mixture, followed by centrifugation at 1000 g for 10 minutes. The solution's upper layer (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1%). The absorbance was measured at 510 nm using an ultraviolet spectrophotometer. An increase in the absorbance indicates a higher reducing power, indicating greater antioxidant activity.

Each test was performed in triplicate, and the results were reported as the mean. Ascorbic acid was used as a positive control and underwent a similar procedure. In the FRAP assay, a potential antioxidant reduces ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), forming a blue complex (Fe^{2+}/2, 4, 6, tripyridyl-s-triazine or TPTZ) that enhances absorption at 593 nm. Higher absorption at this wavelength indicates a higher reducing power and, consequently, greater antioxidant activity of the phytochemical.

For each test extract sample at varying concentrations (10-100 µl) in 0.2 M phosphate buffer (pH 6.6) along with equal quantities of 1% (w/v) potassium ferricyanide, the reaction was incubated at 50°C for a specific time. The reaction was stopped by adding equal volumes of 10% (w/v) trichloroacetic acid (TCA) solution, followed by centrifugation at 3000 rpm for 20 minutes. The absorbance was measured at 700 nm after mixing the supernatant with an equal volume of distilled water and 0.1% (w/v) ferric chloride solution. The increasing absorbance with higher concentrations signifies the extract's enhancing reducing power (Jayasri, 2009). By applying the FRAP assay in this study, we sought to elucidate the antioxidant potential of the tested plant extracts, contributing to the scientific understanding of their bioactive properties and potential applications in promoting health and wellness.

2.14 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed with slight modifications to the protocol of Chipiti et al. (2015) to elucidate the chemical composition of the ethanolic extract. The analysis used an Agilent Technologies 7890A GC system coupled to a 5975C Mass Selective Detector. An HP-5MS capillary column (30 m x 0.25 mm internal diameter, 0.25 µm film thickness) was employed to separate the constituents present in the extract. Chloroform was used to dilute the extract before injection into the GC-MS system. The injector temperature was set at 320 degrees Celsius, and helium served as the carrier gas. The initial oven temperature was set to 70°C and programmed to increase at 10°C/min until reaching 280°C, with a pause duration of 4 minutes at each increment. Injections were performed in split mode with a 100:1 split ratio, and 1 µL of the diluted extract was injected. The mass spectrometer operated in electron ionization mode with a 70 eV electron ionization voltage and an electron multiplier voltage of 1859 V. Additional MS operating parameters included an ion source temperature of 230°C, a quadrupole temperature of 150°C, a solvent delay of 3 minutes, and a scan range of 33-550 amu.

Compounds in the ethanolic extract were identified by comparing their mass spectra at specific retention times to reference standards from the National Institute of Standards and Technology (NIST) library. To determine significant matches, a similarity index of at least eighty percent was applied (Kulathilaka et al., 2014). The total operating duration of the GC-MS analysis was 45 minutes, providing a comprehensive and reliable profile of the chemical constituents in the *T. erecta* (L.) leaf extract.

3 Results

Based on the testimonies of the primary informants, the ethnopharmacological uses of *Tagetes erecta* (L.) leaves include anti-inflammatory (skin diseases, fever, headache, and joint pains/arthritis) and antihistamine (for skin allergies and itching) properties, as shown in Table 1.

Table 1 Ethnopharmacological uses of *Tagetes erecta* (L.) leaves in Kapatagan, Lanao del Norte, Philippines.

	Preparation	Modes of application	Traditional medical use
1	Infusion	Internal (oral)	For the treatment of headache, fever, joint pains/arthritis
2	Infusion	External (rubbing)	Skin diseases and allergies
3	Infusion	External (compresses)	Arthritis

The remedies were only prepared by infusion in heated water. Headache, fever, and joint symptoms are treated with an intravenous infusion. Massaging the affected area by external application treats skin diseases and allergies, whereas arthritis is treated with compresses applied to the affected area. This information gathered from the primary informants is consistent with the traditional uses of *Tagetes erecta* (L.) reported by Shinde et al., 2009, such as treating headaches, fever, and joint pains/arthritis.

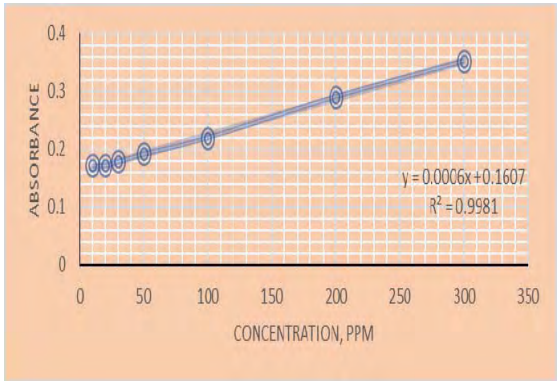
Table 2 displays the qualitative preliminary screening of medicinally essential metabolites in the fresh and air-dried ethanolic leaf extracts of *T. erecta* (L.). The plant's antioxidant, anti-inflammatory, analgesic, anticancer, and antibacterial properties may be due to alkaloids, flavonoids, tannins, and steroids. Tripathi et al. (2017) reported the occurrence of saponins, tannins, flavonoids, alkaloids, steroids, and anthraquinones. Except for the anthraquinones, all of the secondary metabolites reported by Tripathi et al. (2018) match the laboratory analysis performed by the Department of Chemistry at the MSU-Iligan Institute of Technology. In addition, Ashokkumar et al. (2010) and Ramya et al. (2012) provide evidence for the presence of alkaloids, tannins, saponins, and flavonoids.

Table 2 Screening of the fresh and air-dried ethanolic leaf extracts of *T. erecta* (L.) for secondary metabolites

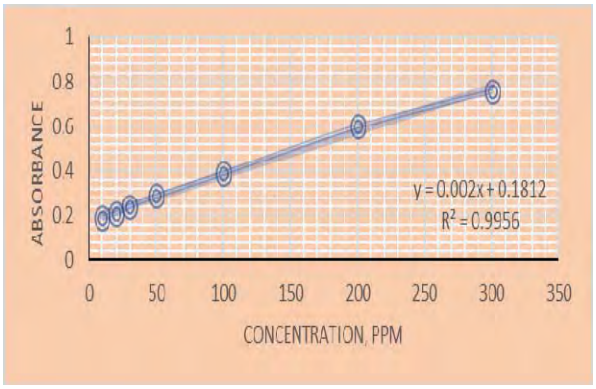
	saponins	tannins	flavonoids	alkaloids	anthraquinones	cyanogenic glycosides	steroids
Air-dried sample	(+)	(+++)	(++)	(+++)	(-)	(-)	(+++)
Fresh sample	(++)	(+++)	(+++)	(++)	(-)	(-)	(+++)

(+) indicates present: +turbid, ++moderate, +++heavy; (-) indicates absent

The medicinal properties of plants are due to the presence of various constituents, including alkaloids, saponins, flavonoids, glycosides, phenols, and sterols. Tannins are beneficial for treating inflamed and ulcerated tissues, cancer, and antiseptics (Patel et al., 2011). Flavonoids protect against allergies, inflammation, free radicals, platelet aggregation, pathogens, ulcers, hepatotoxins, viruses, and tumors. Saponins exhibit anti-inflammatory, anticoagulant, anti-diabetic, antioxidant, aldose reductase inhibitory, and cholesterol-binding properties (Patel et al., 2012). The absence of anthraquinones and cyanogenic glycosides may indicate that this plant has no or minimal toxic effects, as cyanogenic glycosides can cause poisoning that results in severe gastric irritations and damage (Sarker and Nahar, 2007). In contrast, anthraquinones and their derivatives can cause diarrhea, vomiting, and abdominal discomfort (Li et al., 1916-1917).



A.



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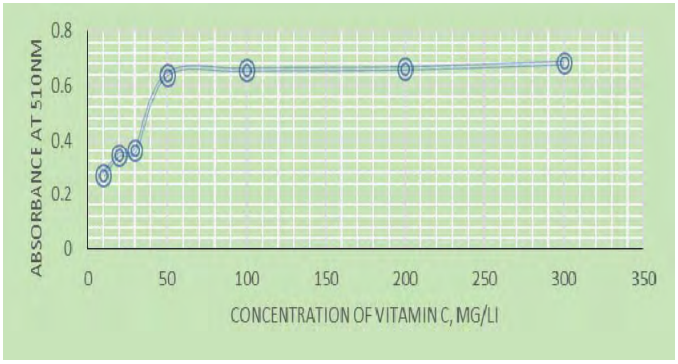

C.

Fig. 3 Absorbance of (A) fresh ethanolic leaf extract, (B) air-dried ethanolic leaf extract, and (C) vitamin C (control) of *T. erecta* (L.) at 510 nm with increasing concentration indicating the reducing power of the extract.

The ferric reducing/antioxidant power (FRAP) assay method can be utilized to determine the reducing power of plant extracts. The assay is based on a compound's reducing activity (antioxidant). The ability of each plant extract to convert the ferric–ferry cyanide complex to the ferrous–ferry cyanide complex was determined using Oyaizu's (1986) method. The reducing power of *T. erecta* (L.) ethanolic leaf extracts is shown as a function of their concentration in Fig. 3. In this assay, reducers (antioxidants) convert the Fe^{3+}/ferricyanide complex to the ferrous form (Ferreira et al., 2007). The Ferric Reducing Antioxidant Power (FRAP) assay results provided valuable insights into the antioxidant activity of *T. erecta* (L.) ethanolic leaf extracts compared to the control Vitamin C. In Fig. 3C, the EC50 value of Vitamin C was determined to be 35.12 ppm, indicating a remarkably potent antioxidant activity. This observation validates the efficacy of Vitamin C as a positive control in the assay, consistent with its well-known antioxidant properties. Figures 3A and 3B revealed intriguing differences between the reducing power of *T. erecta* (L.) fresh ethanolic leaf extracts (565.5 ppm) (Fig. 3A) and air-dried ethanolic leaf extracts (159.4 ppm) (Fig. 3B). The air-dried extracts exhibited significantly higher reducing power values than the fresh extracts. This finding suggests that drying may concentrate or preserve certain antioxidant compounds in the leaves, resulting in enhanced antioxidant activity.

Despite the increasing concentration of *T. erecta* (L.) extracts, Vitamin C consistently demonstrated more potent antioxidant activity than the ethanolic leaf extracts, as observed in Fig. 3. This finding highlights the superior antioxidative capabilities of Vitamin C compared to the plant extracts and emphasizes the significance

of this well-known antioxidant in various applications. Table 3 further supports the notion that air-dried extracts serve as a more potent source of antioxidants than fresh extracts. The increased potency may be attributed to the possible concentration of specific antioxidant compounds during drying. The difference in antioxidant activity between the fresh and air-dried extracts underscores the importance of processing methods in preserving or enhancing the biological activity of herbal extracts. These results agree with previous studies that suggest drying processes can influence the antioxidant potential of medicinal plants. The presence of various phytochemicals, such as phenolic hydroxyl or methoxyl groups, flavones hydroxyl, keto groups, and unbound carboxylic groups, may contribute to the observed antioxidant activity in both fresh and air-dried extracts (Pratt and Hudson, 1990). Epidemiological studies suggest that antioxidant-rich plants play a protective function in health and disease, and their consumption reduces the risk of cancer, cardiovascular disease, hypertension, and stroke (Tripathi et al., 2018).

Table 3 Equivalent concentration of extracts that can reduce the same amount of ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+})

Vitamin C (ppm)	Fresh (ppm)	Air-dried (ppm)
10.000	31.396	15.698
20.000	57.452	39.829
30.000	93.223	59.918
50.000	288.367	164.834
100.000	449.915	296.421
200.000	563.291	453.311
300.000	2838.841	579.325

As shown in Table 3, the air-dried ethanolic leaf extracts of *Tagetes erecta* (L.) exhibited higher antioxidant activity than the fresh extracts. The reason behind this phenomenon could be attributed to the concentration and preservation of active antioxidant compounds during the air-drying process. Several studies have shown that the drying process can affect the chemical composition of plant extracts and their antioxidant potential. When plant materials are air-dried, the moisture content is reduced, leading to a higher concentration of active compounds present in the extracts. This increase in concentration may result in a higher antioxidant capacity compared to fresh extracts, where water content might dilute the antioxidant compounds.

Additionally, the drying process can help stabilize certain compounds, including antioxidants, by reducing enzymatic reactions and microbial growth that may occur in fresh plant materials. This preservation of active compounds during drying may contribute to the increased antioxidant potency of the air-dried ethanolic leaf extracts (Arefin et al., 2015).

It's important to note that while the study mentioned above specifically discusses the antioxidant activity of *Tagetes erecta* (L.) flower extracts, similar trends have also been observed in other plant species. Due to the concentration and preservation effects, the drying process has been reported to enhance the antioxidant potential in various plant extracts. However, it is also essential to consider that the antioxidant capacity of plant extracts can vary depending on factors such as the plant species, environmental conditions, and extraction methods used. Therefore, further research and additional studies on *Tagetes erecta* (L.) and other plant species would be valuable to confirm and better understand the reasons behind the increased antioxidant potency after air-drying.

GC-MS analysis of compounds was performed on a fresh sample of *T. erecta* (L.) ethanolic leaf extract as

depicted in Fig. 4. The GC-MS analysis of a fresh sample of *T. erecta* (L.) leaf extract revealed the presence of four compounds. The GC-MS analysis of the fresh sample of *T. erecta* (L.) ethanolic leaf extract, as illustrated in Fig. 4, provided valuable insights into the chemical composition of the extract.

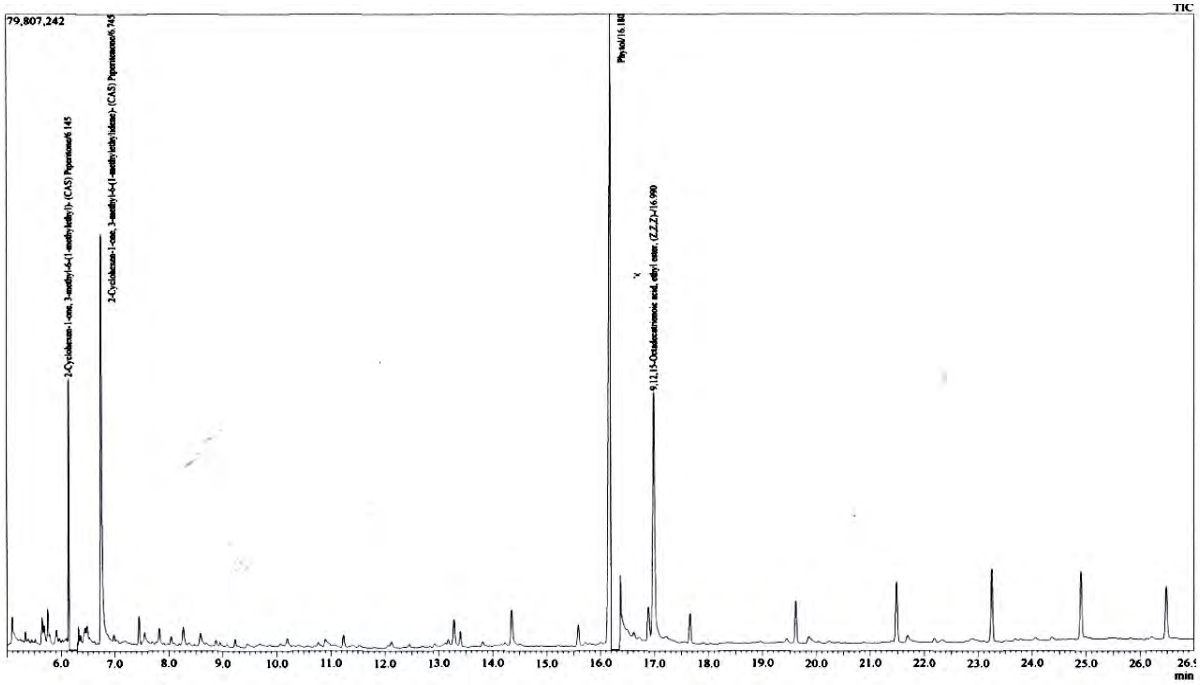

Fig. 4 GC-MS chromatogram of a fresh sample of *T. erecta* (L.) ethanolic leaf extract

The chromatogram displayed four prominent peaks, each corresponding to specific compounds detected in the extract as shown in Table 4. Through rigorous analysis of their retention time (RT), similarity index (SI), molecular formula, base peak (BP), and mass peak (MP), as well as referencing their mass spectra with the Wiley 7 and NIST library, these four phytoconstituents were successfully characterized and identified. The first peak, with a retention time of 6.145 minutes and SI of 95, was conclusively identified as Piperitone. The second peak, corresponding to Piperitenone, was confirmed with a retention time of 6.745 minutes and a SI of 91. Phytol, the third peak, was distinctly identified with a SI of 90 and a retention time of 16.180 minutes. Lastly, the fourth and final peak, identified with the longest retention time of 16.990 minutes and with a SI of 94, was unequivocally determined to be Ethyl linoleate.

Identifying these four compounds adds to the growing body of knowledge regarding the chemical constituents of *T. erecta* (L.) leaves. These findings are particularly significant due to the potential bioactive properties of the identified phytoconstituents. Piperitone, for instance, has been recognized for its diverse pharmacological activities, including antimicrobial and antioxidant properties, which make it an attractive target for further investigation and potential applications in various industries, including pharmaceuticals and natural products. Likewise, Piperitenone, Phytol, and Ethyl linoleate exhibit intriguing biological activities that warrant further exploration. Phytol, a diterpene alcohol, has demonstrated anti-inflammatory and antinociceptive effects in various studies. At the same time, Ethyl linoleate, an ester of linoleic acid, holds promise as a potential skin moisturizer due to its emollient properties. It is important to emphasize that identifying these compounds lays the foundation for future studies on the therapeutic potential of *T. erecta* (L.) leaves and their possible applications in natural medicine and drug development. However, further

investigations are required to elucidate their exact biological activities, potential synergistic effects, and underlying mechanisms of action.

Table 4 List of 4 phytoconstituents identified from ethanolic leaf extract of *T. erecta* (L.) fresh sample through GC-MS.

S/N	Phytochemical compound	BP	MP	RT (min)	SI	Formula	Biochem. properties	Reference
1	Piperitone	82.05	91	6.145	95	$C_{10}H_{16}O$	Antimicrobial Anti-inflammatory Antioxidant	Teymouri and Alizadeh, 2018 Singh et al.,2017 Sökmen et al., 2004
2	Piperitenone	150.15	304	6.745	91	$C_{10}H_{14}O$	Antimicrobial, Antioxidant, Anti-inflammatory, Hypotensive, and Insecticidal properties	Teymouri and Alizadeh, 2018
3	Phytol	71.05	408	16.180	90	$C_{20}H_{40}O$	Antimicrobial, anticancer, cancer preventive, diuretic anti-inflammatory Antioxidant	Sermakkani and Thangapandian, 2012 Santos et al., 2013
4	Ethyl linolenate	79.05	343	16.990	94	$C_{20}H_{34}O_2$	Antiinflammatory, InsectifugeHypocholesterolemic, Cancer preventive, Nematicide, Hepatoprotective, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, and Anticoronary Antioxidant	Sermakkani&Thangapandian, 2012 Miyake and Shibamoto, 1997

GCMS detected the presence of bioactive compounds with antioxidant properties. These include Piperitone (Sokmen et al., 2004), Piperitenone (Teymouri and Alizadeh, 2018), Phytol (Santos et al., 2013), and Ethyl linolenate (Miyake and Shibamoto, 1997). Antioxidants protect the body from reactive oxygen species (ROS), which are produced during oxidative metabolism and inflammation and cause oxidative damage to membrane lipids, proteins, and DNA (Mulgund et al., 2015). These compounds' antioxidant properties can be used to counteract the pathological and detrimental effects of free radicals (Yanishlieva et al., 2006). In addition, antioxidants contribute to the body's defense against cardiovascular disease, cancer, arthritis, asthma, and diabetes (Peter and Shylaja, 2012).

Ethanolic leaf extracts of air-dried *T. erecta* (L.) samples were also subjected to GC-MS analysis of chemical compounds. The GC-MS analysis of an air-dried sample of *T. erecta* (L.) leaf extract revealed the presence of a total of 10 compounds. Figure 5 depicts the GC-MS chromatogram of the 10 peaks of the detected compounds. The chromatogram analysis of the air-dried ethanolic leaf extract of *T. erecta* (L.)

revealed the presence of ten prominent peaks, and the components corresponding to these peaks were identified as follows. The first established peak was identified as Alpha-Terpinolene with a retention time of 5.050 min and a similarity index of 96. The second peak is Piperitone with a similarity index of 95 and a retention time of 6.145 min. The third prominence as being Beta-caryophyllene with a retention time of 7.455 min and a similarity index of 96. Phytol was identified as the fourth peak with a retention time of 12.050 and a similarity index of 90. The fifth peak was identified as an ethyl ester of hexadecanoic acid with a similarity index of 95 and a retention time of 14.355. The sixth peak, with a retention time of 16.160 and a similarity index of 98, was identified as trans-Phytol. Ethyl linoleate was deemed the seventh apogee, with a retention time of 16.890 and a similarity index 94. The eighth peak was identified as 7-Tetradecenal (Z) with a 16.985 RT and 87 SI. The ninth peak, with an RT of 17.380 and a similarity index of 94. The tenth and final peak is Squalene with a similarity index of 95 and a retention time of 25.815. The GC-MS analysis of the air-dried ethanolic leaf extract of *Tagetes erecta* (L.) resulted in the identification and characterization of 10 phytoconstituents based on their retention time (RT), similarity index (SI), molecular formula, base peak (BP), mass peak (MP), and biochemical properties with references. Among these phytoconstituents, Alpha-Terpinolene was identified with the shortest retention time of 5.050 min, while Squalene was identified with the longest retention time of 25.815 min. Phytoconstituents are natural chemical compounds in plants with diverse biological activities and potential therapeutic applications. The GC-MS analysis is a valuable technique for identifying and quantifying these compounds in plant extracts. It allows for precisely determining their molecular characteristics and provides insights into their potential biological activities. The identification of Alpha-Terpinolene and Squalene in the ethanolic leaf extract of *Tagetes erecta* (L.) is significant, as these compounds have been reported to exhibit various biological activities. Alpha-Terpinolene is known for its antioxidant properties and has been studied for its potential antimicrobial and antifungal effects (Konappa et al., 2020). On the other hand, Squalene is a naturally occurring triterpene that has been shown to possess anti-inflammatory and immunomodulatory properties (Thamer and Thamer, 2023).

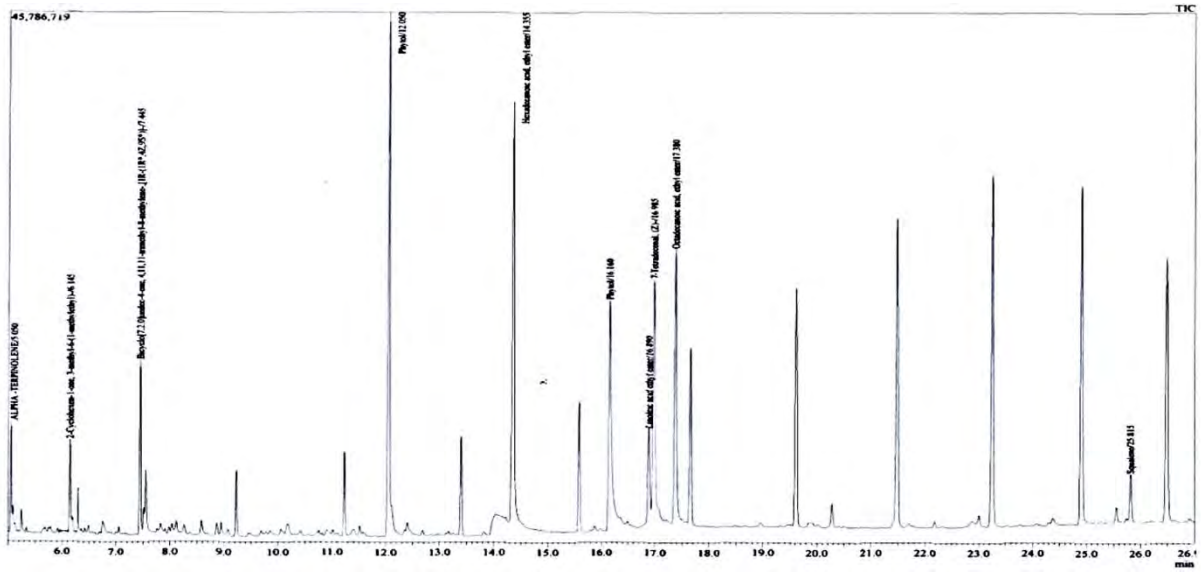

Fig. 5 GC-MS chromatogram of the air-dried sample of *T. erecta* (L.) ethanolic leaf extract.

GCMS also detected the presence of bioactive compounds with antioxidant properties in the air-dried ethanolic leaf preparations of *T. erecta* (L.). Terpinolene (Dorman et al., 2000), Beta-caryophyllene (Legault and Pichette, 2007), Hexadecanoic acid, ethyl ester (Priya et al., 2012), and Squalene (Sunita and Manju, 2017) are among these compounds. In addition, the results indicate the presence of antimicrobial bioactive compounds. These include Piperitone (Teymouri and Alizadeh, 2018), Phytol (Sermakkani and Thangapandian, 2012), Hexadecanoic acid, ethyl ester (Abubakar and Majinda, 2016), trans- Phytol (Sermakkani and Thangapandian, 2012), and 7-Tetradecenal, (Z) (Canli et al., 2017). An antimicrobial substance is any natural, semisynthetic, or synthetic origin that destroys or inhibits the growth of microorganisms while causing minimal or no harm to the host. Not all antimicrobials are antibiotics, but all antibiotics are antimicrobials (Kümmerer, 2009).

Table 5 List of 10 phytoconstituents identified from ethanolic leaf extract of *T. erecta* (L.) air-dried sample through GC-MS.

S/N	Phytochemical compound	BP	MP	RT (min)	SI	Formula	Biochem. properties	Reference
1	Terpinolene	93.05	270	5.050	96	$C_{10}H_{16}$	Anticancer Antioxidant Antifungal Larvacidal	Harada et al., 2012 Dorman et al., 2000 Hammer et al., 2004 Conti et al., 2012
2	Piperitone	82.05	91	6.145	95	$C_{10}H_{16}O$	Antimicrobial Anti-inflammatory	Teymouri and Alizadeh, 2018 Singh et al., 2017
3	Beta-caryophyllene	41.05	269	7.455	96	$C_{15}H_{24}$	Anti-inflammatory,Anticancer Antibiotic, Antioxidant, Anticarcinogenic and local anaesthetic activities	Legault& Pichette, 2007
4	Phytol	68.05	325	12.050	90	$C_{20}H_{40}O$	Antimicrobial, Anticancer, Cancer preventive, Diuretic and Anti-inflammatory	Sermakkani and Thangapandian, 2012
5	Hexadecanoic acid, ethyl ester	88.05	229	14.355	95	$C_{18}H_{36}0_2$	Inhibits phagocytosis, Lubricant and Anti-androgenic Antioxidant, 5- α-reductase, Pesticide and Antifibrinolytic, Hemolytic, nematicide,antialopecic and Antimicrobial Antioxidant, Hemolytic,	Sativum et al., 2012 Priya et al., 2012 Abubakar and Majinda, 2016 Tyagi and Agarwal, 2017

							Hypocholesterolemic, Flavor, Nematicide and Anti-androgenic	
6	trans- Phytol	71.05	298	16.160	98	$C_{20}H_{40}O$	Antimicrobial, Anticancer, Cancer preventive, Diuretic and Anti-inflammatory	Sermakkani and Thangapandian, 2012
7	Ethyl linoleate	67.05	318	16.890	94	$C_{20}H_{36}O_2$	Hepatoprotective, antihistaminic, hypocholesterolemic, anti-eczemic Anti-inflammatory properties	Wu et al., 2010 Park et al., 2014
8	7-Tetradecenal, (Z)	55.05	372	16.985	87	$C_{14}H_{26}O$	Antimicrobial	Canli et al., 2017
9	Stearic acid, ethyl ester	88.05	332	17.380	94	$C_{20}H_{40}O_2$	No activity reported.	
10	Squalene	69.05	286	25.815	95	$C_{30}H_{50}$	Antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant, Pesticide, Chemo preventive, and Lipoxygenaseinhibitor	Sunita and Manju, 2017

Based on the results of the GC-MS analysis of the air-dried ethanolic leaf extract of *Tagetes erecta* (L.), one of the ten compounds, ethyl ester of stearic acid, has no reported biological activities. Stearic Acid ethyl ester is a 20-carbon saturated fatty acid esterified product of Stearic Acid. However, the discussion also indicates that this compound has no reported biological activities. Stearic Acid ethyl ester has been studied for its effects on cells, and it has been shown to induce apoptosis and interfere with the cell cycle in the G2/M and S phases (Hasaba et al., 2003). Saturated fatty acids (SFAs), including Stearic Acid ethyl ester, have been reported to be essential for the biological activities of lipopolysaccharides. Additionally, SFAs can induce NFκB (nuclear factor κB) activation and Cox-2 expression (Hasaba et al., 2003). Despite its involvement in cell cycle interference and apoptosis induction, there are no reports of specific biological activities attributed to ethyl ester of stearic acid. The lack of reported biological activities suggests this compound may not have significant therapeutic or pharmacological effects. However, further research is necessary to explore potential actions that may not have been reported yet.

According to the results of the study, both fresh and dried ethanolic extracts of *T. erecta* (L.) leaves contained 15 bioactive compounds, with antimicrobial (8), anti-inflammatory (7), anticancer (5), and antioxidant (5) compounds containing the highest number (Fig. 6). Based on these findings, the results may provide information regarding the safety and credibility of the *T. erecta* (L.) leaf as a medicinal plant source and support its traditional applications. Furthermore, other phytoconstituents in the extract may contribute to

its overall pharmacological profile and therapeutic potential. However, to fully understand the biological activities and medicinal significance of the identified phytoconstituents, further studies are needed, including in vitro and in vivo experiments and the isolation and characterization of individual compounds.

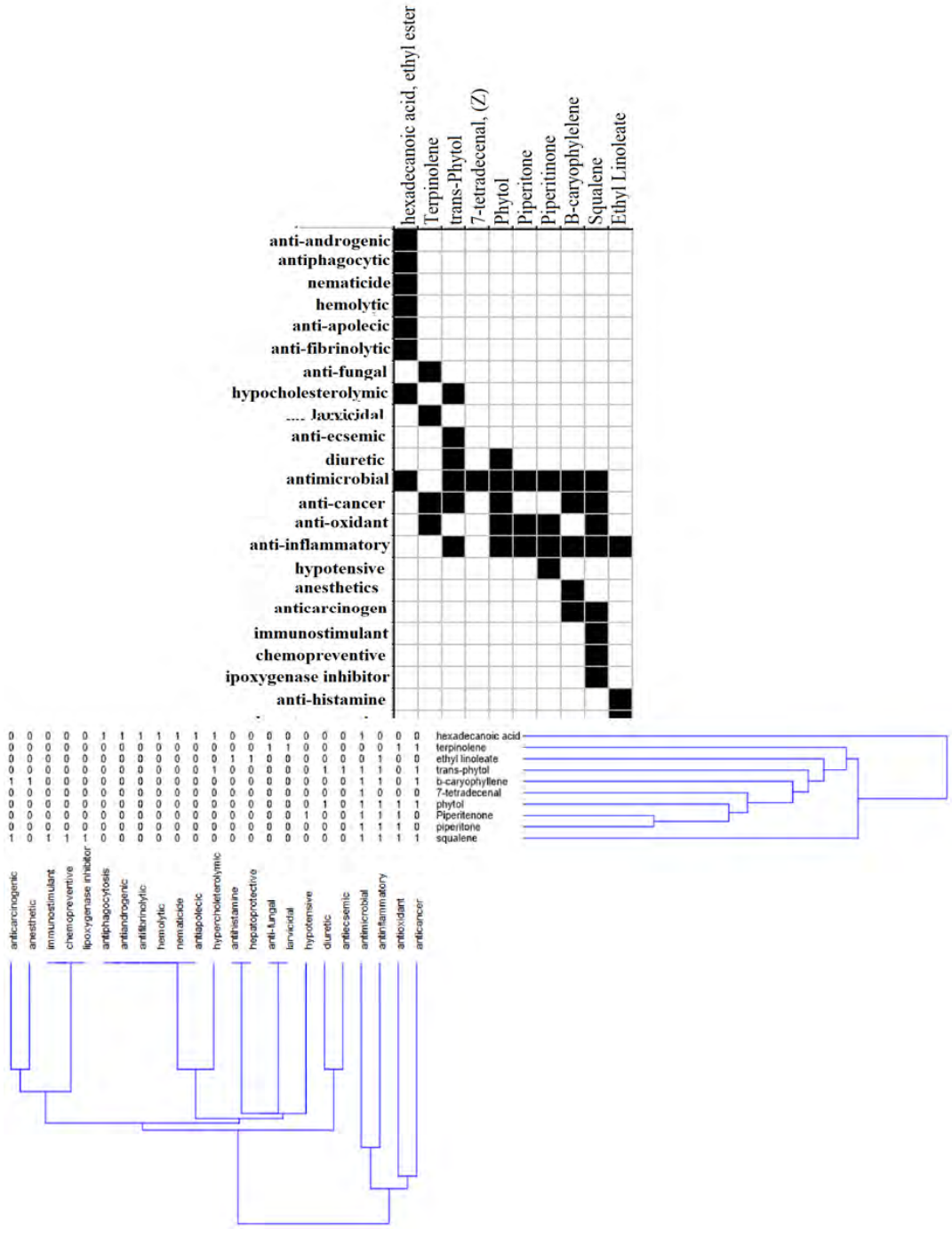

Fig. 6 Seriation and Clustering of compounds and their reported biological functions.

4 Conclusion

The current study provides valuable insights into the ethnomedicinal value of *T. erecta* (L.). The study suggests that the diverse phytoconstituents identified in the extracts may contribute to the plant's observed ethnopharmacological functions. These findings support the traditional medicinal uses of *T. erecta* (L.) leaves and offer a basis for further exploring its potential therapeutic applications in managing various health conditions. Future research may involve isolating and characterizing specific compounds better to understand their individual contributions to the observed effects.

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