Article

Ethnomedicinal use and qualitative analysis of phytochemicals from an ethanolic extract of the fresh and dried leaves of the Mexican sunflower (*Tithonia diversifolia*)

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Abstract

The ethnomedicinal use and evaluation of an ethanolic extract of the fresh and dried leaves of Mexican Sunflower (*Tithonia diversifolia*) were investigated for antimicrobial and antioxidantproperties by phytochemical, ferric-reducing antioxidant power (FRAP) assay and GC-MS analysis. Phytochemical analysis of this plant confirms the presence of various phytochemicals like saponins, steroids, alkaloids, flavonoids, and tannins. The total antioxidant activity measured by ferric reducing antioxidant power (FRAP) assay showed the potential use of this plant as antioxidant source. Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed a total of thirteen known compounds in *T. diversifolia.* Only hexadecanoic acid and ethyl ester, identified by GC-MS, are known to have antimicrobial and antioxidant properties. These findings demonstrate that ethanolic extract from fresh and dry leaves of *T. diversifolia* exhibits medicinal properties and, thus, is a good source of natural health substances.

Keywords *Tithonia diversifolia*; antimicrobial; antioxidant; ferric reducing antioxidant power (FRAP); gas chromatography-mass spectrometry (GC-MS).

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1 Introduction

Tithonia diversfolia, or Mexican sunflower, is a member of the family Asteraceae. This species is a native plant from Mexico and Central America, a woody herb or succulent shrub, stoloniferous, annual, or perennial that can reach a height of 2 to 3 meters (Gualberto et al., 2011). It escaped from cultivation and is now growing

wild in many tropical regions (CABI, 2014). In Kenya, the Mexican sunflower is one of the most famous indigenous fodder tree species in the sub-humid highlands, where it is frequently coppiced and even uprooted (Roothaert et al., 1997). It is a fast-growing plant that tolerates drought and can rapidly form large herbaceous shrubs (CABI, 2014). This plant is known to adapt to most soils; thus, they are found in disturbed areas, abandoned and wastelands, along roadsides and waterways, and on cultivated farmlands (GISD, 2012; Olabode et al., 2007). This plant is easy to grow and does not require fertilizer or special attention (Devide, 2013). Studies have shown that the leaf of *T.diversifolia* is considered to have most of the active constituents (Orwa et al., 2009). Some studies show extracts from this plant contain bioactive compounds that have anti-inflammatory activity (Rungeler et al., 1998), antioxidant (Picincu, 2018), and anti-amoebic and spasmolytic activities (Tona et al., 1998, 2000). In this research, we explore other studies on the traditional uses of this plant species and also assess the presence of bioactive substances that may provide scientific explanations for the traditional use of the plant.

2 Methodology

2.1 Sampling site

Laturan is a barangay in the municipality of Libona, in the province of Bukidnon (Fig. 1). Its population, as determined by the 2015 Census, was 2,927. This represented 6.66% of the total population of Libona. The population of Laturan fell from 3,473 in1990 to 2,927 in 2015, a decrease of 546 people. The latest census figures in 2015 denote a positive growth rate of 0.04%, or an increase of 6 people, from the previous population of 2,921 in 2010. Laturan is situated at approximately 8.2958, 124.7678, on the island of Mindanao. Elevation at these coordinates is estimated at 693.3 meters or 2,274.6 feet above mean sea level.

Fig. 1 (a) Map of the Philippines, (b) study area.

Fig. 2 a. *Tithonia diversifolia* plant b. flower c. leaves d. front view.

2.2 Collection of plant material

Fresh and healthy leaves of *T. diversifolia* was collected from Barangay Laturan, Libona, Bukidnon, Philippines, in April 2019 (Fig. 2). The leaveswere separated and washed thoroughly with sufficient water. For air-dried samples, the plant leaves were dried under shade for two weeks and powdered in an electrical grinder. The collected plants were brought tothe laboratory ofthe Department of Biological Sciences. For fresh samples, the leaves were weighed, ground, and soaked in ethanol.

2.3 Preparation of crude ethanolic extract

The plant extract was prepared based on a modified procedure. Two hundred (200) grams of powdered fresh and dry *T. diversifolia* leaves were soaked in one (1) liter of absolute ethanol for two weeks with stirring. Using Whatman filter paper No. 1 (Whatman, UK), the supernatant was filtered, and the filtrate was concentrated using a rotary evaporator to a temperature of about 45 °C. The crude extract was collected and allowed to dry at room temperature completely. The obtained viscous crude extract was stored in storage vials for the different tests.

2.4 Ethanolic extract partitioning

The ethanolic extract partitioning was performed using the standard partition methods described by Geetha (2014) at the Department of Chemistry, MSU-IIT.

2.5 Phytochemical screening

The phytochemical screening of the ethanolic extract of *T. diversifolia* variety was executed using the standard phytochemical methods described by Aguinaldo et al. (2005) that was modified according to the Laboratory Manual for the UNESCO Sponsored Workshop on the Phytochemical, Microbiological, and Pharmacological Screening of Medicinal Plants at the Department of Chemistry, U.P. Diliman. A 3-point scale [+ turbid, ++ moderate, and +++ heavy] in scoring was based on the Handbook of Philippine Medicinal Plants by de Padua et al. (1981).

2.6 Saponins

The froth test method was used to determine the presence of saponins in the *T. diversifolia* crude extract. Two (2) milliliters of the plant extract were added from an equal volume of water in the test tube. The solution in the test tube was shaken for about thirty (30) seconds. The presence of the saponins in the plant extract was

indicated by the formation of froth about two (2) cm in height, which lasted for thirty (30) minutes.

2.7 Alkaloids

A sufficient amount of the plant extract was transferred into an evaporating dish and was evaporated to almost dry under a boiling bath. As the plant extract in the evaporating dish hadnearly dried, it was removed from the boiling bath and was cooled off. 5-10 mL of 2M HCl and 0.5g of sodium chloride crystal were added to the evaporating dish. It was placed again under a boiling water bath for about five (5) minutes. The resulting solution was cooled and filtered. The residue was washed in the filter paper with 2M HCl. The combined filtrate was washed and divided into three (3) equal parts. The first part was added with 3-5 drops of Wagner's reagent, the second part was added with Mayer's reagent, and the third part of the filtrate served as a control. The positive result was determined by the formation of a brown precipitate with Wagner's reagent and a white precipitate formed with Mayer's reagent. The results were expressed as follows: (-) for the absence of precipitate; (+) if the solution becomes turbid only; (++) if the precipitate form is only in moderate amount; and (+++) if the precipitate form is heavy.

2.8 Flavonoids

The crude extract was transferred in an evaporating dish and was evaporated to almost dry under a boiling water bath. Then, the extract was cooled and defatted with hexane until it became clear. The defatted extract was dissolved with 10 mL of 80% alcohol and was filtered. The filtrate was then divided into two equal parts. A 0.5 mL of 12M HCl was added to the first part, and the second part served as the control. The two test tubes were placed in the hot water bath, and the change of color was then observed. A positive result was determined by the occurrence of the red color of the solution. The reaction was observed for about two (2) hours since the development of the red color was very slow. The results were recorded as follows: (-) no change of color occurs; (+) if the red coloration is very light; (++) if the red coloration is moderate; and (+++) if dark red color is produced.

2.9 Tannins

The crude extract was transferred to the evaporating dish and was evaporated to almost dry under a boiling water bath. The extract was cooled and added with 20mL of boiling water, followed by the addition of 2-3 drops of a 10% NaCl solution. The resulting solution was filtered, and the residue was washed with water. The combined filtrate was washed and divided into three (3) equal parts. The first filtrate was added with 3-5 drops of 1% ferric chloride, the second part was added with 3-5 drops of Gelatin-Salt reagent, and the third part served as a control. Indication of the positive result was observed with the formation of black or blue-black colored precipitate with ferric chloride and white precipitate with Gelatin-sodium chloride test. The results were recorded as follows: (-) for the absence of precipitate; (+) if the solution becomes turbid only; (++) if the precipitate form is only in moderate amount; and (+++) if the precipitate form is heavy.

2.10 Steroids

The crude extract was evaporated to almost dryness under a boiling water bath in the evaporating dish. The dried crude extract was cooled and was defatted with hexane. The extract was added with 3-5mL of ferric chloride reagent and was filtered. The filtrate was divided into two equal parts. The first part was added with one (1) mL of concentrated sulfuric acid through the wall of the test tube. The positive results were determined with the formation of a brown (sometimes blue or green) ring at the boundary region of the aqueous extract. The sulfuric acid was indicated with the presence of 2-deoxy sugars.

2.11 Anthraquinones

Sufficient amounts of the plant extract were evaporated to almost dry under a boiling water bath in the evaporating dish. The dried crude extract was cooled and defatted with hexane. It was then added with 10mL of distilled water, and the resulting solution was stirred and filtered. The filtrate was extracted twice with 5mL

of benzene. The extract was stood for a few minutes to separate the aqueous and benzene layers completely. The benzene layer was separated using a transfer pipet, placed in a separate test tube containing one (1) mL of ammonia reagent, and shaken for a few seconds. The color was observed in the aqueous layer. The presence of anthraquinones was determined by the development of a reddish-pink color in the aqueous layer of the solution.

2.12 Cyanogenic glycosides

One (1) mL of the plant extract was placed in a 20 mL test tube and was added with 4-5 drops of chloroform. The test tube was placed in the hot water bath. A picrate paper was suspended above the solution. The test tube was covered with a dropper in an inverted position. The immediate red coloration of the picrate paper indicated positive results for cyanogenic glycosides.

2.13 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was performed following the protocol of Chipiti et al.(2015) with modifications to identify the compounds present in the ethanolic extract. The extract was diluted with chloroform and subjected to an Agilent Technologies 7890AGC system coupled with (an Agilent) 5975C Mass Selective detector. An HP-5MS capillary column (30 m x 0.25 mm internal diameter, 0.25 μm film thickness) was then applied. The carrier gas will be helium with the injector temperature at 320°C. The initial oven temperature will be at 70°C, which was programmed to increase to 280°C at the rate of 10°C/min with a hold time of 4 min at each increment. Injections of 1 μL were made in split mode with a split ratio of 100:1. The mass spectrometer was operated in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. Other MS operating parameters were as follows: ion source temperature 230°C, quadrupole temperature 150°C, solvent delay 3 min and scan range 33-550 amu. The compounds were identified by direct comparison of the mass spectrum of the analyte at a particular retention time to that of a reference standard found in the National Institute of Standards and Technology (NIST) library.

2.14 Ferric reducing antioxidant power assay

The ferric-reducing antioxidant power assay of the essential oil was carried out according to Reddy and Grace's method (2016). Two (2) mL of each sample and standard solution were spiked with 2.5 mL of 1% Ferricyanide solution. Then, the mixture was kept at 50 degrees Celsius in a water bath for 20 minutes. After cooling, 2.5 mL of 10% trichloroacetic acid was added and centrifuged at 300 rpm for 10 minutes. 2.5 mL of supernatant was mixed with 2.5 mL of distilled water and 1 mL of 0.1% ferric chloride and kept for 10 minutes. The control was prepared similarly, excluding the samples. The absorbance of reducing solutions was measured at 510 nm.

3 Results and Discussion

Based on the literature, traditional medicinal uses of *T.diversifolia* in different countries are shown in Table 1. Different plant parts, ranging from the leaf and stem, were used to treat many different kinds of ailments. Variations in the preparations of the plant parts have similarities and also differences among the users (Table 1).

To identify the biochemical substances in the plant leaves, characterization of *T. diversifolia*plant extracts from fresh and air-dried samples were also tested for the presence of saponins, steroids, tannins, flavonoids, cyanogenic glycosides, anthraquinones. Other bioactive compounds were analyzed using GC-MS Analysis.

Table 1 Some ethnomedicinal uses of *Tithonia diversifolia.*

Country	Plant Part(s) used	Traditional uses and ethno-botanical reports	Preparation	References
Central America	Leaf	Wounds and hematomas	Leaf extracts applied externally	(Stuart et al., n.d.)
Nigeria	Aerial	Malaria	Hot water extract	(Calzada et al., 1978)
Taiwan	Leaf	Hepatitis	Oral decoction	(Johns et al., 1995)
	Stem	Hepatitis	Oral decoction	
Tenejepa	Leaf	Diarrhea, cough and abdominal pain	Boiled in water, tea applied, and drunk	(Stepp, 2018)
Cameroon	Leaf	Measles	Infusion, ground into small pieces, mixed with water, and then drunk	(Kamden et al., 1986)
Costa Rica	Leaf	Wound	Dried leaves applied externally	(Kuo et al., 1997)
Thailand	Leaf	Gastrointestinal disorders	Oral decoction	(Johns et al., 1995)
	Stem	Gastrointestinal disorders	Oral decoction	
Kenya	Leaf	Antiplasmodial activity, gastrointestinal disorders	Pound and add cold water, oral decoction	(Mukungaet al, 2016)
		Malaria and typhoid	Pounded together, burned, ash soaked in water and drunk	(Odhiambo et al., 2011)
	Stem	Gastro-intestinal disorders	Oral decoction	(Johns et al., 1995)
Africa	Leaf	Constipation, indigestion, sore throat	Infusion	(Stuart et al., n.d.)

3.1 Phytochemical result

The fresh and dry leaf extract of *T.diversifolia* (Mexican Sunflower) was analyzed for the presence of alkaloids, anthraquinones, cyanogenic glycosides, flavonoids, steroids, saponins, and tannins. All results of the phytochemical analysis are shown in Table 2. Results displayed in fresh leaves heavy precipitate (+++) to saponins, steroids, flavonoids with turbid (+) of tannins, and alkaloids with a low detection (-) of anthraquinones and cyanogenic glycosides do not imply that the two compounds are absent. In dry leaves showed heavy precipitate (+++) to saponins, steroids, flavonoids with moderate amount (++) of alkaloids with turbid (+) of tannins with low detection (-) of anthraquinones and cyanogenic glycosides does not imply that the two compounds are absent.

3.2 Saponins

The phytochemical screening of the presence of the saponins in the fresh and dry leaf extract of *T. diversifolia*

showed that the sample contains a heavy precipitate. The presence of saponins exhibits antimicrobial properties in the leaf extracts of *T. diversifolia* (Arabski et al., 2012). These chemicals help to reduce cholesterol levels, kill disease-causing bacteria, scavenge oxidative stress, inhibit tumor growth, and act as antioxidants (Picincu, 2018). It has also been shown to have hypolipidemic and anticancer activity (Sarker and Nahar, 2007).

Table 2 Phytochemical constituents of ethanolic extract of *T. diversifolia* (Mexican Sunflower).

Compound	Fresh		Dried	
	Test Results	Remarks	Test Results	Remarks
Saponins	+++	Intense color/ heavy precipitate	+++	Intense color/ heavy precipitate
Alkaloids	+	Turbid	++	Moderate amount
Flavonoids	+++	Intense color/ heavy precipitate	+++	Intense color/ heavy precipitate
Steroids	+++	Intense color/ heavy precipitate	+++	Intense color/ heavy precipitate
Tannins	+	Turbid	+	Turbid
Anthraquinones	-	Below detection	-	Below detection
Cyanogenic glycosides	-	Below detection	-	Below detection

(+++) Heavy, (++) Moderate, (+) Turbid only, (-) Absent

3.3 Alkaloids

The presence of the alkaloids in the extract showed a moderate amount in dry leaf and turbid in fresh leaf extracts in the sample. This chemical functions in the defense of plants against herbivores and pathogens and is widely exploited as pharmaceuticals, stimulants, narcotics, and poisons due to their potent biological activities (Madziga*et al*., 2010; Doughari, 2012). Alkaloids' biological activities include antimalarial, anticancer, antimicrobial, and antihyperglycemic activities (Kittakoop et al., 2014; Cushnie et al., 2014; Shi et al., 2014).

3.4 Flavonoids

The fresh and dry leaf ethanolic extracts are abundant in flavonoids, showing the development of a red color solution in whicha dark red color is produced. Numerous reports support the use of antioxidant or free radical scavengers as well as quenchers of singlet oxygen formation (Kar, 2007; Ali and Neda, 2011). Flavanoids showed other biological functions as protection against platelet aggregation, microorganisms, hepatotoxins, viruses, tumors, ulcers, inflammation, and allergies (Barakat et al., 1993)

3.5 Steroids

The phytochemical screening of the fresh and dry leaf extracts of *T. diversifolia* (Mexican Sunflower) showed that the sample contains steroids with heavy presence (+++) of the formation of a brown ring. Steroids have been reported to have broad antimicrobial activity (Gutierrez et al., 2015).

3.6 Tannins

The phytochemical screening of fresh and dry leaf extracts contains tannins with turbid only (+) in the sample determined by the formation of black or blue-black colored precipitate with ferric chloride and white

precipitate with Gelatin-sodium chloride test. It is helpful in wood healing (Kar, 2007) as an astringent and antimicrobial (Singhal, 2001). Some studies reported that tannins are one of the ingredients found in plant-based medicines (Haslam, 1996). It is also used in the dyestuff industry as caustics for cationic dyes (tannin dyes) and production of inks, textile dyes, antioxidants in beverages, and antiviral and antitumor activity (Haslam, 1996; Khanbabaee and Van Ree, 2001; Kakiuchi et al., 1986). Tannins can also inhibit HIV replication (Kashiwada et al., 1992)

3.7 Anthraquinones

The phytochemical screening of the fresh and dry leaf extract of *T.diversifolia* showed that the sample does not contain anthraquinones; there is an absence (-) of the development of a reddish-pink color in the solution. The lack of Anthraquinones may indicate that the fresh and dry leaf extracts of *T.diversifolia* do not have antimicrobial, antiparasitic, insecticidal, fungicidal, and antiviral properties (Yadav et al., 2019); however, other compounds present may also exhibit antimicrobial and antioxidant activity.

3.8 Cyanogenic glycosides

The absence of cyanogenic glycosides may indicate less or no toxicity of fresh and dry leaf extracts of *T. diversifolia*. The solution does not show immediate formation of red color at the surface of the picrate paper. This plant is not capable of generating hydrocyanic acid, which is a violent poison when ingested. This chemical compound is responsible for its antitussive properties.

3.9 Free Reducing Antioxidant Power Assay

Antioxidant activity is a complex procedure that usually happens through several mechanisms and is influenced by many factors, which cannot be fully described with one single method (El Jemli*et al.,* 2016). Antioxidants include substances such as flavonoids, tannins, and vitamin C, which are known to have beneficial health effects, such as lowering the risk of cancer, heart disease, and neurodegenerative disorders, and are abundantly found in plants. One way to measure antioxidant capacity is by ferric-reducing antioxidant power (FRAP). The results of antioxidant activity were expressed as $EC_{0.50}$ value, which is defined as the effective concentration of antioxidants at the absorbance equal to 0.500. The reducing capacity of ethanolic extract of the fresh and dry leaf of *T.diversifolia*is performed using Fe^{3+} to Fe^{2+} reduction assay. The ferrous sulfate was prepared by phenanthroline reagent and buffer. A UV-spectrophotometer measured it, and the solution became an orange color from a colorless one that can be read at 510 nm. The extract plus the ferric ion (Fe^{3+}) undergoesa reduction reaction, forming a ferrous ion (Fe^{2+}). In order to detect if there is a presence of ferrous ionsin the resulting solution, a phenanthroline reagent is added, forming a ferrous phenanthroline complex, which indicates an orange color. The result of antioxidant property evaluation of the fresh and dry leaves of the plant sample showed 59.14 ppm from dry and 217.87 ppm from fresh based on FRAP compared to ascorbic acid (control);it is clear that dry leaves havethe strongest antioxidant activity since the equivalent power extract is closer to Vitamin C. The equivalent concentration of extract (in ppm) that can reduce the same amount of ferric ion Fe^{3+} to the ferrous ion Fe^{2+} is listed in Table 3.

The absorbance of fresh and dry leaf extract of *T. diversifolia* at 510 nm with increasing concentration indicates the reducing power of the extract (Fig. 3 and 4).

The results of the present investigation revealed that the ethanolic extract of fresh and dry leaves of *T. diversifolia* has antioxidant properties.

Table 3 Equivalent concentration of extract that can reduce the same amount of Ferric ion (Fe^{3+}) to the Ferrous ion (Fe^{2+}).

	Reducing power of Extracts, ppm	
Vitamin C (ppm)	**(dry)**	**(fresh)**
10	7.87	-317.24
20	20.62	109.78
30	37.08	213.92
50	70.34	272.02
100	59.68	205.82
200	64.19	211.55
300	64.12	233.57
500	59.14	217.87

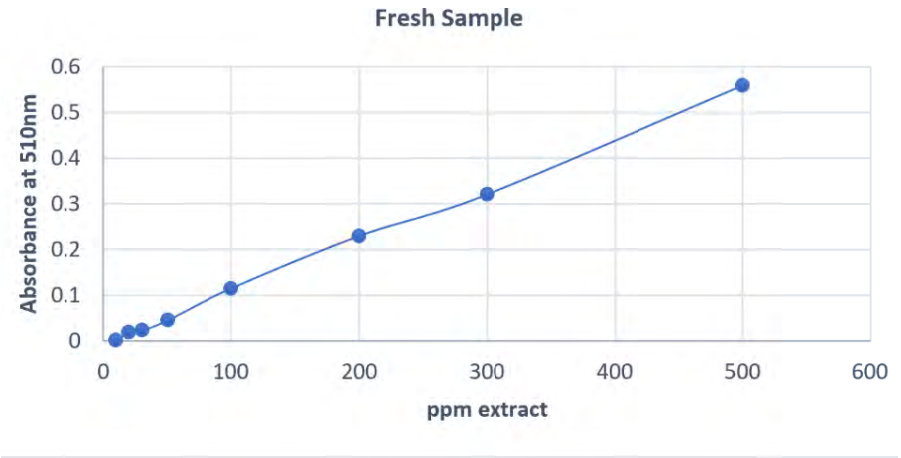

Fig. 3 Absorbance at 510nm and concentration of the Fresh Leaves Extract.

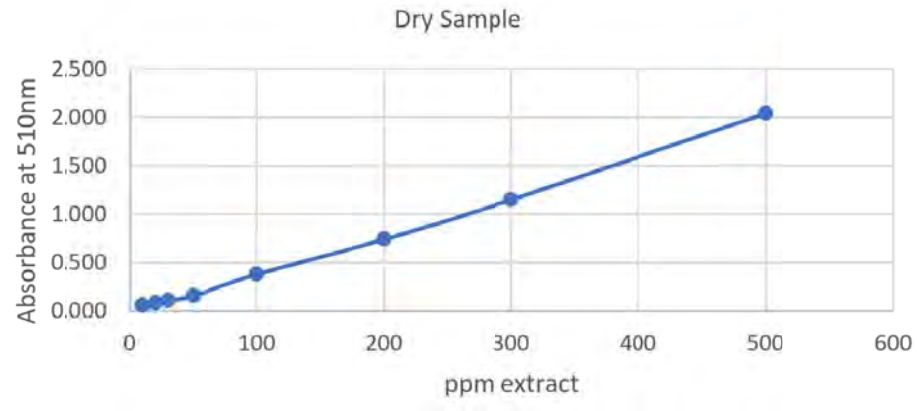

Fig. 4 Absorbance at 510 nm and concentration of the Dry Leaves Extract.

3.10 Identification of phytoconstituents compounds of ethanolic extracts of *T. diversifolia* fresh leaves by GC-MS

Gas chromatography and mass spectroscopy analysis of compounds were carried out in fresh leaf ethanolic extract of *T. diversifolia*. The six (6) phytoconstituents on the ethanolic extracts of *T. diversifolia* are listed in Table 4. The GC-MS chromatogram of the six peaks of the compounds detected is shown in Figure 5. Chromatogram GC-MS analysis of the ethanolic extract of *T.diversifolia*showed the presence of six major peaks, and the components corresponding to the peaks were determined as follows. The components includehexadecanoic acid, ethyl ester, phytol, linoleic acid ethyl ester, 9,12,15-octadecatrienoic acid, ethyl ester, (Z,Z,Z), 1,4-Methanoazulene-9-methanol,decahydro-4,8,8-trimethyl-[1S-(1.alpha., 3a.,4.alpha.,8a., beta., 9R)] and 4,8,13-Cyclotetradecatrene-1,3-diol,1,5,9-trimethyl-12-(1-methylethyl). The chemical structure of bioactive compounds isolated from the fresh ethanolic leaf extracts of *T.diversifolia* is seen in Fig. 6.

Table 4 Six phytoconstituents identified from ethanolic leaf extract of *Tithonia diversifolia* fresh samplethrough GC-MS.

S/N	Phytochemical Compound	MW	Formula	Biochemical properties	Reference
1	Hexadecanoic acid, ethyl ester	284	$C_{18}H_{36}O_2$	Anti-inflammatory Antimicrobial activity Antioxidant, Hypocholesterolemic acid, Pesticide and Antiandrogenic activity	Aparna et al., 2012; Manilal et al., 2009; Duke, 2007
2	Phytol	296	$C_{20}H_{40}O$	Anti-inflammatory Antiproliferative activity	Silva *et al.*, 2014; Ghout*et al.*,2018
3	Linoleic acid ethyl ester	308	$C_{20}H_{36}O_2$	Anti-inflammatory, Antimicrobial	Marimuthu *et al.*, 2014
4	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)	306	$C_{20}H_{34}O_2$	Antimicrobial activity, Antiulcer Antioxidant	Koga *et al.*, 1996
5	1,4-Methanoazulene-9-methanol,decahydro-4,8,8-trimethyl-[1S-(1.alpha.,3a.,4.alpha.,8a.,beta.,9R)]	222	$C_{15}H_{26}O$	Anti-inflammatory activity Anticholinesterase	Ahmad et al., 2016
6	4,8,13-Cyclotetradecatrene-1,3-diol,1,5,9-trimethyl-12-(1-methylethyl)	306	$C_{20}H_{34}O_2$	Antimicrobial activity	Cruickshank et al., 1977

MW = molecular weight.

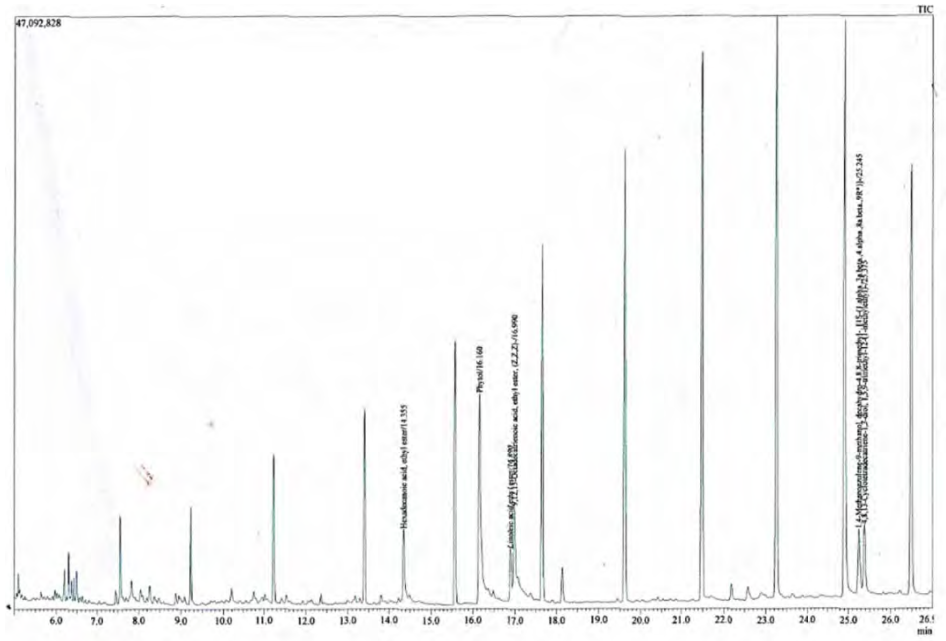

Fig. 5 GC-MS Chromatogram showing the Biochemical Components from the fresh leaf ethanolic extracts of *Tithonia diversifolia*.

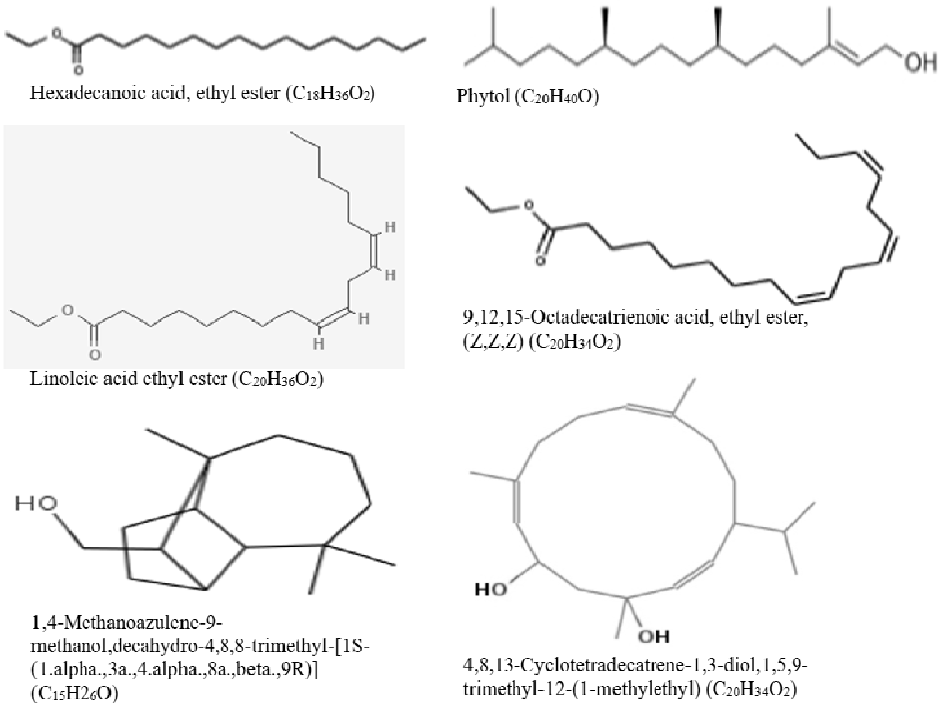

Fig. 6 Chemical structure of bioactive compounds isolated from the fresh ethanolic leaf extracts of *T. diversifolia.*

3.11 Identification of phytoconstituents compounds of ethanolic extracts of *T. diversifolia* dry leaves by GC-MS

Gas chromatography and mass spectroscopy analysis of compounds were carried out in dry leaf ethanolic extracts of *T. diversifolia*. The eight (8) phytoconstituents on the ethanolic extracts of *T. diversifolia* are listed in Table 5. The GC-MS chromatogram of the eight peaks of the compounds detected is shown in Figure7. Chromatogram GC-MS analysis of the ethanolic extract of *T.diversifolia* showed the presence of eight major

peaks, and the components corresponding to the peaks were determined as follows. The components include caryophyllene, 1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1a.alpha.,4a.alpha., 7.a.beta.,7b.alpha.)], caryophyllene oxide, 4-camphenylbutan-2-one, phytol, 1-(3-isobutyryl-bicyclo[1.1.1] pent-1-yl)-2-methylpropan-1-one, squalene and 2,6,6,10-tetramethyl-undeca-8,10-diene-3,7-dione. The chemical structure of bioactive compounds isolated from dry ethanolic leaf extracts of *T.diversifolia* is seen in Fig. 8.

Table 5 Eight phytoconstituents identified from ethanolic leaf extract of *Tithonia diversifolia* dry samplethrough GC-MS.

S/N	Phytochemical compound	MW	Formula	Biochemical properties	Reference
1	Caryophyllene	204	C15H24	Antimicrobial, Antifungal activity	Agboola et al., 2016
2	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-,[1ar-(1a.alpha.,4 a.alpha.,7.a.beta.,7b.alpha.)]	220	C15H24O	Antioxidant	Cavar et al., 2008
3	Caryophyllene oxide	220	C15H24O	Antimicrobial, Antifungal activity	Agboola et al., 2016
4	4-Camphenylbutan-2-one	206	C14H22O	No activity reported	-
5	Phytol	296	C20H40O	Anti-inflammatory activity	Silva et al., 2014
6	1-(3-Isobutyryl-bicyclo[1.1.1]pent-1-yl)-2-methylpropan-1-one	208	C13H20O2	Analgesic, Anti-inflammatory, and Anticancer activities	Caliskan et al., 2012
7	Squalene	410	C30H50	Antioxidant, anticancer,chemopreventive,ga stro- preventive and hepatoprotective effects, pesticide, antitumor, sunscreen properties	Sunitha et al., 2001; Ukivaet al., 2002; Katerere et al., 2003
8	2,6,6,10-Tetramethyl-undeca -8,10-diene-3,7-dione	236	C15H24O2	No activity was reported.	-

MW = molecular weight.

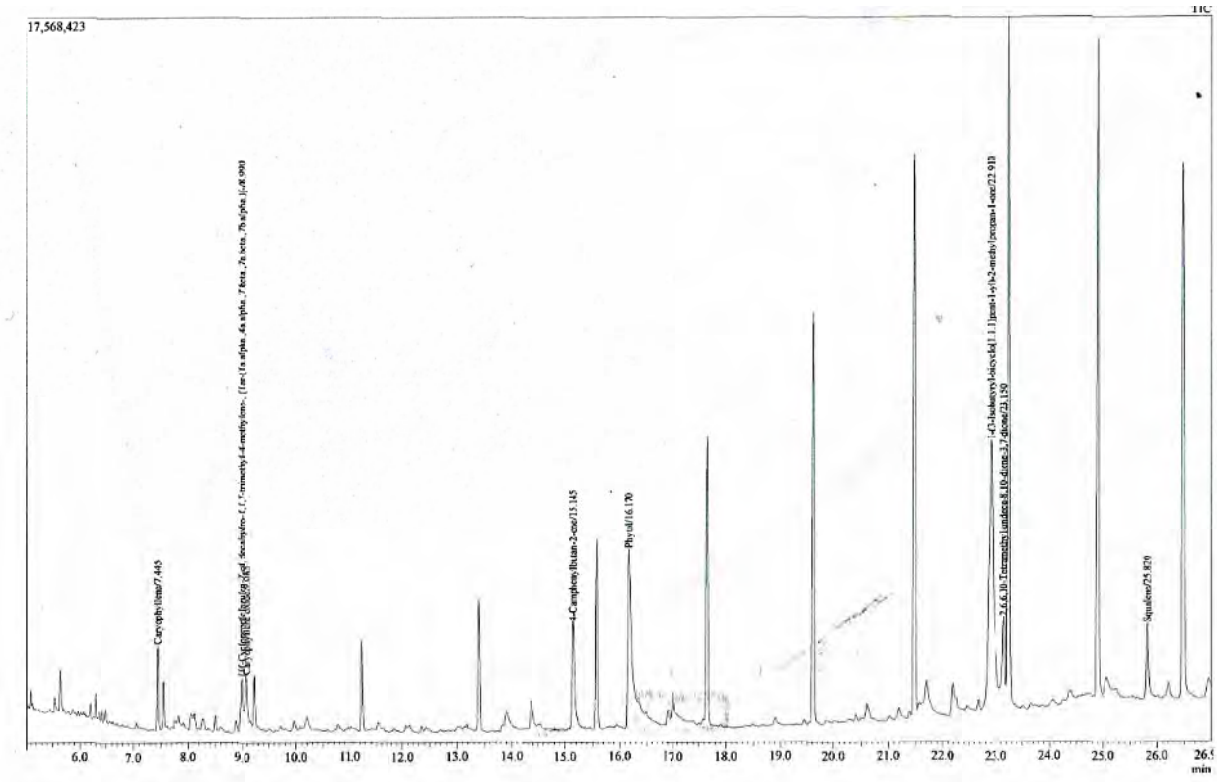

Fig. 7 GC-MS Chromatogram showing the Biochemical Components from the fresh leaf ethanolic extracts of *Tithonia diversifolia.*

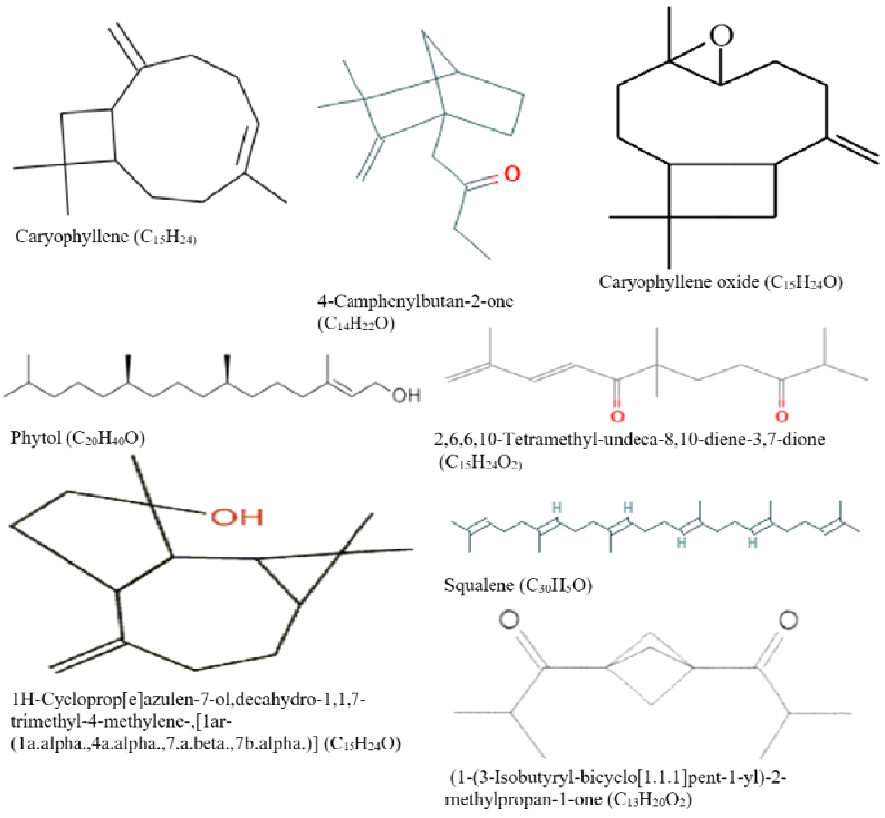

Fig. 8 Chemical structure of Bioactive compounds isolated from dry ethanolic leaf extracts of *Tithonia diversifolia.*

The GC-MS analysis of fresh and dry ethanolic extracts of *T. diversifolia*showed the presence of 13 biochemical compounds, 11 of which were reported to possess various bioactivities. Of the 11 compounds, six compounds were reported to have anti-inflammatory activities (hexadecanoic acid, ethyl ester; Phytol; Linoleic acid ethyl ester; 1,4-Methanoazulene-9-methanol,decahydro-4,8,8-trimethyl- [1S-(1.alpha.,3a., 4.alpha.,8a.,beta.,9R)]; 1-(3-Isobutyryl-bicyclo[1.1.1]pent-1-yl)-2-methylpropan-1-one. Antimicrobial activity was reported in six compounds (hexadecanoic acid, ethyl ester; Linoleic acid ethyl ester; 9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z); 4,8,13-Cyclotetradecatrene-1,3-diol,1,5,9-trimethyl-12-(1-methylethyl); Caryophyllene; Caryophyllene oxide. Antioxidant properties were reported in four compounds (Hexadecanoic acid, ethyl ester; 1,4-Methanoazulene-9-methanol,decahydro- 4,8,8-trimethyl-[1S-(1.alpha.,3a.,4.alpha.,8a.,beta.,9R)];1H-Cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4-methylene-[1ar-(1a.alpha.,4a.alpha.,7.a.beta.,7b.alpha)]; and Squalene. The presence of the substances may explain the scientific basis of the many medicinal claims of the anti-inflammatory, antimicrobial, and antioxidant properties of the plant.

4 Summary and Conclusion

Phytochemical analysis of fresh and dried leaf ethanolic extracts of Mexican Sunflower (*T. diversifolia*) revealed the presence of bioactive compounds such as saponins, tannins, steroids, flavonoids, and alkaloids. GC-MS analysis identified important substances in the extracts, such as hexadecanoic acid, ethyl ester, phytol, squalene, and caryophyllene. These substances, including the extracts' potent antioxidant properties, indicate that the plant has the potential to be a valuable source of biologically active molecules. The results may also provide the scientific basis for the various ethnomedical applications of the plant.

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