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COMPARATIVE ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS, FREE RADICAL SCAVENGING ACTIVITY AND GC-MS ANALYSIS OF LEAF AND FLOWER EXTRACT OF *TITHONIA DIVERSIFOLIA* (HEMSL.) A. GRAY

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Keywords:

Phytochemical, GC-MS, *Tithonia diversifolia*, leaf extract, Flower extract, DPPH

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ABSTRACT: The secondary plant metabolites possess therapeutic value, thus play a significant role in human health and general wellbeing. The present study aimed to estimate the secondary metabolite concentration and to evaluate the antioxidant potential of *Tithonia diversifolia* leaf and flower extract in methanol. The phytochemical compounds in the extracts were characterized through Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The leaf extract exhibited sixteen bioactive compounds, and flower extract revealed thirteen compounds with valuable activity. The major phytocompounds in leaf extract were pentacosane (22.01%), gamma-sitosterol (15.80%) and pentatriacontane (11.95%) whereas in flower extract showed methyl linolelaidate (19.55%), methyl palmitate (18.73%), 1-dotriacontanol (15.35%), 5- eicosene, (E)- (11.90%). The compounds were found to be dissimilar in both leaf and flower. Quantitative phytochemical analysis of leaf extract was found to contain a high concentration of phenols, flavonoids and saponins, whereas total alkaloids were maximum in flower extract. In *in-vitro*, the antioxidant activity was evaluated using 1, 1-diphenyl-2- picrylhydrazyl (DPPH) assay. Both leaf and flower extract showed remarkable antioxidant scavenging activity with an IC_{50} value of 120.264 µg/ml and 121.7µg/ml, respectively. The findings of this study provide an insight into the phytochemistry and antioxidant property of methanol extract of leaf and flower of *T. diversifolia*. Such properties may be of great importance in alleviating the chronic effect of oxidative stress, and it can be recommended as a plant of pharmaceutical importance.

INTRODUCTION: *Tithonia diversifolia* (Hemsl.) a. gray is commonly called the *Mexican sunflower*, tree marigold, or the Japanese sunflower. It is a subtropical plant belonging to the Asteraceae family. It is native to Mexico and Central America[1] and was subsequently introduced in Africa, Australia, and Asia [2].

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It is an invasive, woody shrub, reaching to heights of about 2-3 m, and the mature stem bears bright yellow to orange colored flowers and is aromatic. In Mexico and Nigeria, the plant was used by many ethnic groups in their folk medicine, especially stem and leaf extracts of *T. diversifolia* are taken orally for Malaria [3].

It is also used in the treatment of diabetes, diarrhea, liver disease, and stomach ache in Indonesia. In India, dried leaf powder is used in skin infection and for healing wounds [4]. The main active compound isolated from aerial parts of *T. diversifolia* showed sesquiterpene lactones *i.e.* tagininins (Tagitinins A, C, E and F), titho-

folinolide [5, 6] diversifolin [7] and diversifolide found in roots [8]. The predominant constituents in the volatile oil of leaf and flower found to contain α-pinene, β-pinene, β-caryophyllene, germacrene D, (Z)-β-ocimene and limonene [9].

Gas Chromatography-Mass Spectrometry (GC-MS), an analytical technique, is gaining more importance in the screening of phytochemicals. It is the first step to understand the chemical profile of the plant sample. GC-MS has become a highly recommended advanced technology to identify and quantify the secondary metabolites, even at low concentrations [10]. As there is no report about the GC-MS analysis of *T. diversifolia* plant from India, an attempt has been made to quantify the secondary metabolites, antioxidant assay, and identification of chemical constituents of methanol extract of *T. diversifolia* leaf and flower using GC-MS.

MATERIALS AND METHODS:

Collection of Plant Material and Extraction: Leaves and flowers of *T. diversifolia* were collected from HMT quarters, Bangalore, Karnataka, India. The plant was authenticated by Dr. V. Rama Rao, Taxonomist, Regional Ayurveda Research Institute for metabolic disorders (Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, and Government of India), Bengaluru and Karnataka with the authentication number RRCBI-mus 203.

The collected leaves and flowers were shade dried, powdered and extracted separately with absolute methanol using Soxhlet apparatus for 16 h at a temperature not exceeding the boiling point of the solvent. The extracts were filtered and concentrated under reduced pressure at 40 °C using a rotary flash evaporator and stored at 4 °C until further use.

Quantitative Phytochemical Analysis: Quantification of phytochemical analysis was carried out with a focus on comparing secondary metabolites present in leaf and flower extract of *T. diversifolia*

Determination of Total Phenols by Folin-Ciocalteu Reagent Method: Total phenolic content (TPC) was determined by the folin-ciocalteu reagent method [11]. To 1 ml of each extract, 5 ml of (1:10) folin-ciocalteu reagent and [4] ml of Na_2CO_3 (7.5%) were added. The above solution was incubated for 30 min at 20 °C and absorbance were read at 765 nm. Gallic acid was used as a reference standard (20-100 µg/ml). The TCPs were determined using a linear regression equation obtained from the standard plot of gallic acid. The TPC was calculated as mean SD (n=3) and expressed as mg/g gallic acid equivalent (GAE) of extract.

Estimation of Total Flavonoid Content (TFC) by Aluminium Chloride Method: Quercetin was used as a standard to construct the calibration curve. 0.5 ml of different aliquots of a standard solution of quercetin (20, 40, 60, 80 and 100 µg/ml) was mixed with 2 ml of distilled water, 0.15 ml of 5% sodium nitrite and allowed to stand for 6 min at room temperature later, 0.15 ml of 10% aluminum chloride solution was added. After 6 min of incubation, 2 ml of 4% w/v sodium hydroxide was added, and volume was made up to 5 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm using a UV-visible spectrophotometer against blank. The amount of flavonoid was calculated from the linear regression equation obtained from the quercetin calibration curve. The flavonoid content was calculated as mean SD (n=3) and expressed as mg/g of quercetin equivalent (QE) of extract[12].

Estimation of Alkaloid: Atropine was used as the standard alkaloid to construct the calibration curve. Briefly, 10 mg of atropine was dissolved in methanol and then diluted to 200, 400, 600, 800, and 1000 µg/ml. The diluted standard solution of atropine or plant extracts (1 ml) of different concentrations was separately mixed with 2 ml of 2 N hydrochloric acid and 5 ml of chloroform. Vortex the above solution vigorously and take out the chloroform layer using a micropipette. To the separated chloroform layer, add 5 ml of bromocresol green (BCG) solution (7 mg of BCG was dissolved in 3 ml of 2 N sodium hydroxide then the volume was made up to 100 ml with distilled water) and 5 ml of sodium phosphate buffer (Ph = 4.7). The mixture was vortexes for 5 min, and the yellow color complex was formed at the bottom of the test tube. Pipette out the yellow complex, and the absorbance of the reaction mixture was measured at 470 nm in the UV-Vis spectrophotometer against blank. The total alkaloid concentration was calculated from the linear

regression equation obtained from the atropine calibration curve [13].

The alkaloid content was calculated as mean SD (n=3) and expressed as mg/g of atropine equivalent (AE) of extract.

Quantitative Determination of Saponins: Different aliquots of standard saponin quillaja (1 mg/ml) were taken in different test tubes, and the volume was made up to 1 mL with absolute methanol in all the test tubes later, 500 µL of 8% vanillin, 500 µL of 72% sulphuric acid was added and incubated at 60 °C for 10 min. After incubation, the absorbance was read at 544 nm using a UV-Vis spectrophotometer. The samples were also processed similarly by taking 1 mL of each sample. The standard graph was plotted, and the amount of saponin in each sample was calculated using the linear regression equation. The total saponin content (TSC) was calculated as mean SD (n=3) and expressed as mg/g of quillaja equivalent (QJE) of extract [14].

Assay of free Radical Scavenging Activity by DPPH Method: The free radical scavenging activity of different concentrations of plant samples of *T. diversifolia* and standard ascorbic acid was estimated by Vasundhara *et al.,* 2017 method [15]. One milliliter of various concentrations (1, 10, 25, 50, 75, 100 µg/ml) of the sample or standard ascorbic acid was taken in a separate test tube. Three milliliters of 1 mmol/L DPPH solution prepared in absolute methanol was added to each test tube. The solvents were mixed and kept in the dark at 37 °C for 15 min to complete the reaction. The blank was prepared without sample or ascorbic acid. The absorbance was read at 517 nm using a UV-Vis spectrophotometer. The percentage of free radical inhibition activity of sample and positive control ascorbic acid was calculated by using the following formula.

Free radical inhibition activity (%) = Ac - As × 100 / Ac

Where Ac - Absorbance of control and As - Absorbance of the sample at 517 nm.

The concentration of the sample required to scavenge 50% of the DPPH free radical (IC_{50}) was determined from the curve of percent inhibitions plotted against the respective concentration.

GC-MS Analysis: The methanolic leaf and flower fraction of *T. diversifolia* were investigated using Gas Chromatography and Mass Spectrometry (GC-MS) [9]. The Shimadzu GC-MS, of model number QP2010S, was used with a silica column rxi-5 sil MS, of length: 30 m; internal diameter: 0.25 mm; thickness: 0.25 µm. Helium gas (99.99%) was used as carrier gas with split-less injection mode having column flow: 1.0 ml/min, pressure; 65.2 kPa, linear velocity: 36.8 cm/sec; purge flow: 3.0 ml/min and split ratio: 50.0 the injector was maintained at a temperature of 260.0 °C and column oven temperature, at 80.0 °C. The GC instrument was operated at an ion source temperature of 200 °C, with an interface temperature of 280.0 °C and a solvent cut time of 6.50 min. The mass spectrometer was operated from 7.00 min to 50.00 min with an event time of 0.05 sec, and fragments from m/z 50.00 to 500.00 were programmed. The area percentage of each chemical compound was determined by comparing its average peak area to the total area. The software used to run mass spectra, and the chromatogram was GC-MS solution. The spectrum of unknown components was compared with the known components, stored in the database of the National Institute of Standards and Technology (NIST) [11] and WILEY [8]. The identification of the phytochemical compounds was based on the peak area, retention time, and molecular formula.

Data Analysis: Analysis of the experimental data was performed in triplicate and expressed as mean ± sem. Statistical one-way ANOVA was calculated using the software tool graph pad prism 5.01. Differences were considered statistically significant $P<0.05$.

RESULTS AND DISCUSSION: Since the dawn of civilization, plants are used as the main source of medicine in curing a wide range of ailments in humans and animals [16]. Today, many pharmaceutical industries depended directly or indirectly on the floral kingdom to produce an effective drug. Thus, extraction and analysis of plant material play an important role in the development of qualitative herbal formulation [17].

In the aforementioned study, quantification of the phytochemical compounds in the leaf and flower extract of *Tithonia diversifolia* in methanol was

carried out **Table 1** illustrates that leaf extract contained a higher concentration of phenols, flavonoids, alkaloids, and saponins compared to flower extract. A significant difference in the total phenols, total flavonoids, total alkaloids, and total saponins was observed by ANOVA one-way test.

TABLE 1: LINEAR REGRESSION EQUATION OF STANDARD OF TOTAL PHENOLICS, TOTAL FLAVONOIDS, TOTAL ALKALOIDS AND TOTAL SAPONINS CONTENT IN METHANOL EXTRACT OF LEAF AND FLOWER OF *T. DIVERSIFOLIA*. VALUES ARE MEAN ± SEM; P < 0.05.

Qualitative analysis	Linear regression equation of standard	Leaf extract	Flower extract
Total phenolics (mg/g)	Y = 0.0138 × + 0.0244, R^2 = 0.999(Gallic acid)	48.304 ± 2.765	34.101 ± 1.999
Total flavonoids (mg/g)	Y = 0.0029 × + 0.0033, R^2 = 0.999 (Quercetin)	24.78 ± 0.172	21.9 ± 1.0456
Total alkaloids (mg/g)	Y = 0.0003 × + 0.0008, R^2 = 0.999 (Atropine)	43.33 ± 0.577	88.333 ± 0.192
Total saponin (mg/g)	Y = 0.0002 × + 0.0031, R^2 = 0.999 (Quillaja)	694.5 ± 1.528	304.5 ± 2.082

Where y is the absorbance and x is the concentration of the standard in µg/ml

The current findings support the study of Olutobi and Olasupo [18], which confirms the presence of phytochemicals such as alkaloids, flavonoids, phenols, saponins, tannins, and terpenoids in methanol extract of *T. diversifolia* leaves. However, ethanol extract of dried inflorescence collected from Brazil showed the presence of phenol, flavonoids, and tannins [19] similar results were obtained by Essiett and Akpan [20] only differing in the presence of saponins. The variation in the phytochemical composition of *T. diversifolia* may depend on the environmental changes, climatic factors, and geographical distribution [21]. Phenolic compounds can scavenge free radicals and thus gained importance in pharmaceutical, nutraceutical, and herbal industries. Free radicals are the reactive oxygen species created in the body during normal metabolism or introduced from the environment [22]. An imbalance between free radicals and antioxidants in the body leads to oxidative stress involved in the development of chronic diseases such as Parkinson's disease, Huntington's disease, dementia, heart failure, autism, cancer, atherosclerosis, aging-related diseases, *etc.* [23, 24]. **Table 2** and **Fig. 1** summarize the free radical scavenging activity of leaf and flower extract of *T. diversifolia* compared to a standard (Ascorbic acid).

TABLE 2: PERCENTAGE INHIBITION OF METHANOL EXTRACT OF LEAF AND FLOWER OF *T. DIVERSIFOLIA* AND STANDARD ASCORBIC ACID AT VARIOUS CONCENTRATIONS (µG/ML) IN THE DPPH SCAVENGING MODEL VALUES ARE MEAN % INHIBITION ± SEM ; P < 0.0

	% Inhibition		
Concentration (µg/ml)	Ascorbic acid	Leaf extract	Flower extract
10	15.41 ± 0.203	36.96 ± 0.77	13.62 ± 0.400
25	29.49 ± 0.282	38.03 ± 0.776	20.27 ± 0.306
50	49.22 ± 0.147	40.20 ± 0.325	26.40 ± 0.573
75	62.26 ± 0.144	43.99 ± 0.284	38.30 ± 0.553
100	83.26 ± 0.144	48.20 ± 0.037	41.18 ± 0.895
IC_{50} value	54.839	120.264	121.7

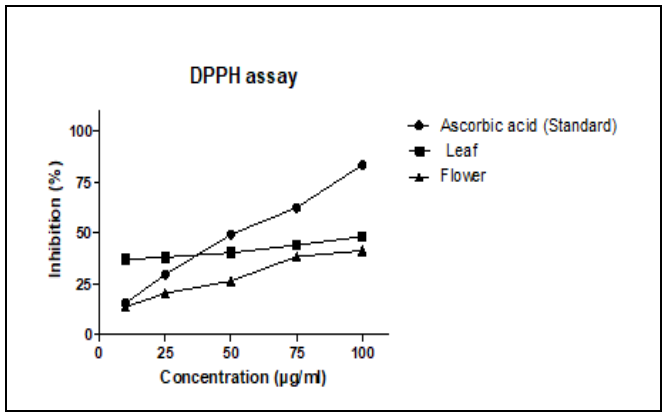
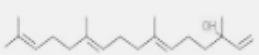
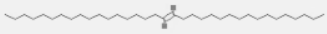
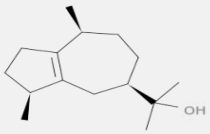
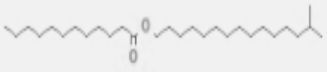
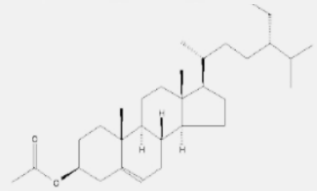
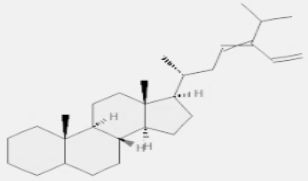
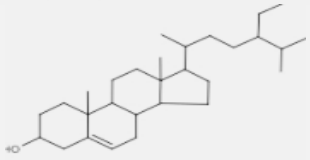


FIG. 1: DPPH SCAVENGING ACTIVITY OF STANDARD ASCORBIC ACID, METHANOL EXTRACT OF LEAF AND FLOWER OF *T. DIVERSIFOLIA*

The half-maximal inhibitory concentration (IC_{50}) of DPPH was calculated under the experimental condition. The leaf extract was found to have a slightly lesser IC_{50} value (120.264 µg/ml) than flower extract (121.7 µg/ml) and ascorbic acid was 54.839 µg/ml. Both the extracts showed less antioxidant property when compared to standard ascorbic acid. Lesser the IC_{50} value stronger the scavenging activity. Scavenging activity of the standard ranged from 15.41% at 10 µg/ml concentrations to 83.26% µg/ml at 100 µg/ml concentrations. Leaf extract was able to scavenge 48.20% at 100 µg/ml concentration, whereas

flower extract depicted 41.18% at 100 µg/ml concentration. Studies have shown that *T. diversifolia* leaves were used in folkloric medicine of Africa to treat neurodegenerative diseases, and scientific data proves that it has antioxidant and cholinesterase inhibitory activity [25]. Thus, the present study signifies that both leaf and flower extract of *T. diversifolia* has the notable effect of free radical scavenging activity. GC-MS analysis of leaf extract of *T. diversifolia* showed sixteen bioactive compounds **Fig. 2** and **Table 3**.

TABLE 3: THE PHYTOCOMPOUNDS OBSERVED IN THE METHANOL LEAF EXTRACT OF *T. DIVERSIFOLIA*

S. no.	Phytochemical compounds and molecular formula	RT (min)	Molecular weight (g/mol)	Area (%)	Nature of compound	Structure
1	Tridecyl acrylate ($C_{16}H_{30}O_2$)	23.581	254.41	4.85	Ester	
2	Neophytadiene ($C_{20}H_{38}$)	26.631	278.5	4.18	Sesqui terpenoid	
3	Phytol, acetate ($C_{22}H_{42}O_2$)	27.513	338.58	1.32	Acyclic diterpene alcohol	
4	17- Pentariacontene ($C_{35}H_{70}$)	38.320	490.9	1.60	Alkene	
5	Pentatriacontane ($C_{35}H_{72}$)	38.891	492.96	11.95	Alkane	
6	Tetracontane ($C_{40}H_{82}$)	39.797	563.08	5.22	Alkane	
7	4- Methyldocosane ($C_{23}H_{48}$)	39.892	324.6	7.89	Alkane	
8	Nerolidol A (Cis or Trans) ($C_{15}H_{26}O$)	40.011	222.37	4.51	Sesqui terpene alcohol	
9	Dodecyl palmitate ($C_{28}H_{56}O_2$)	41.052	424.7	1.60	Ester	
10	Pentatriacontane ($C_{35}H_{72}$)	43.592	492.96	1.77	Alkane	
11	Behenyl chloride ($C_{22}H_{45}Cl$)	43.715	345	3.32	Alkane	
12	Pentacosane ($C_{25}H_{52}$)	44.487	352.69	22.01	Alkane	
13	Gamma- sitosterol ($C_{29}H_{50}O$)	45.728	414.7	15.80	Phyto steroid	
14	2- methyltetracosane ($C_{25}H_{52}$)	46.956	352.7	4.60	Alkane	
15	Stigmasta-5,22-dien-3-ol ($C_{29}H_{48}O$)	47.558	412.7	6.01	Phyto sterol	
16	Beta-sitosterol ($C_{29}H_{50}O$)	49.374	414.71	3.36	Phyto sterol	

The major phytocompounds were pentacosane (22.01%), gamma-sitosterol (15.80%) and penta-triacontane (11.95%) and minor phytocompounds were tridecyl acrylate (4.85%), neophytadiene (4.18%), phytol, acetate (1.32%), 17-penta-riacontene (1.6%), tetracontane (5.22%)-methyldocosane (7.89%), nerolidol a (cis or trans) (4.51%), dodecyl palmitate (1.6%), penta-triacontane (1.77%), behenyl chloride (3.32%), 2-methyltetracosane (4.6%), stigmasta-5,22-dien-3-ol (6.01%) and β-sitosterol (3.36%). Phytocompounds identified from the flower extract revealed thirteen phytochemicals **Fig. 3** and **Table 4**. The major compounds were methyl linolelaidate (19.55%), methyl palmitate (18.73%), 1-dotriacontanol (15.35%), 5-eicosene, (E)-(11.90%) and the minor compounds were squalene (8.83%), dl-alpha-tocopherol (8.58%), 9,12,15- octadecatrienoic acid, methyl ester, (Z,Z,Z)-(4.31%), octacosane (3.09%), methyl lignocerate (2.69%), methyl isostearate (2.15%), cholest- 22- ene- 21- ol, 3,5- dehydro- 6-methoxy-, pivalate (2.11%), phytol, acetate (1.36%), octadecane, 1- (ethenyloxy)- (1.34%). The literature survey reveals that GC-MS analysis of methanol leaf extract (maceration technique) of *T. diversifolia* from Nigeria showed [29] bioactive compounds [26] whereas amana tie [27]observed only two secondary metabolites from ethyl acetate fraction of *T. diversifolia* leaf. However, to the best of our knowledge, there is no report of GC-MS based metabolite profile of methanol extract of *T. diversifolia* leaf and flower from India.

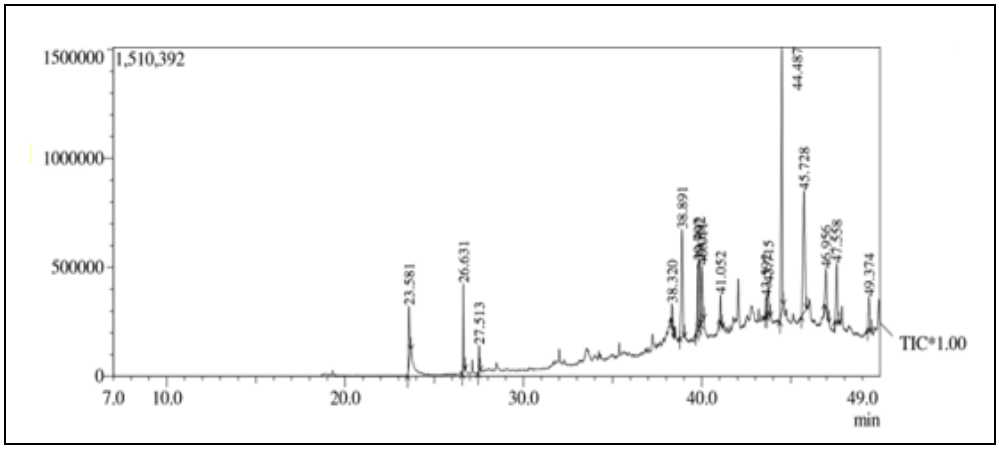


FIG. 2: MASS CHROMATOGRAM OF GC-MS ANALYSIS OF METHANOL LEAF EXTRACT OF *TITHONIA DIVERSIFOLIA*

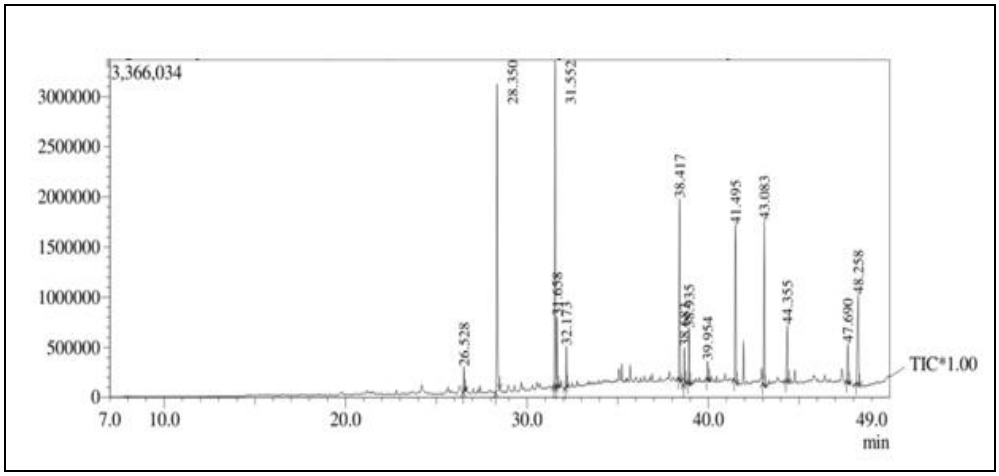
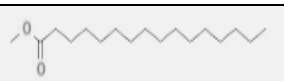
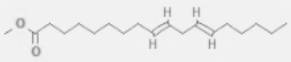
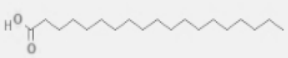
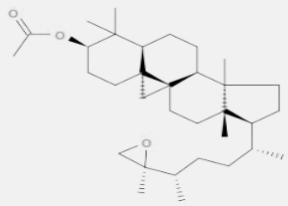
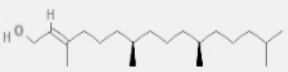
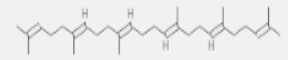
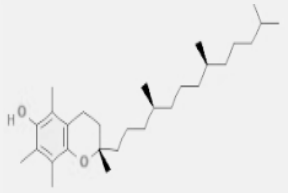


FIG. 3: MASS CHROMATOGRAM OF GC-MS ANALYSIS OF METHANOL FLOWER EXTRACT OF *TITHONIA DIVERSIFOLIA*

TABLE 4: THE PHYTOCOMPOUNDS OBSERVED IN THE METHANOL FLOWER EXTRACT OF *T. DIVERSIFOLIA*

S. no.	Phytochemical compound and molecular formula	RT (min)	Molecular weight (g/mol)	Area (%)	Nature of compound	Structure
1	Phytol, acetate ($C_{22}H_{42}O_2$)	26.528	338.576	1.36	Acyclic diterpene alcohol	

2	Methyl palmitate ($C_{17}H_{34}O_2$)	28.350	270.457	18.73	Fatty acid methyl ester	
3	Methyl linolelaidate ($C_{19}H_{34}O_2$)	31.552	294.479	19.55	Ester	
4	9,12,15- Octadecatrienoic acid, methyl ester, (Z,Z,Z)- ($C_{19}H_{32}O_2$)	31.658	292.4562	4.31	Linolenic acid	
5	Methyl isostearate ($C_{19}H_{38}O_2$)	32.173	298.511	2.15	Ester	
6	5- Eicosene, (E) - ($C_{20}H_{40}$)	38.417	280.54	11.90	Fatty acid	
7	Cholest- 22- ene- 21- ol, 3,5- dehydro- 6- methoxy-, pivalate ($C_{33}H_{54}O_3$)	38.683	498.792	2.11	Steroid	
8	Methyl lignocerate ($C_{25}H_{50}O_2$)	38.935	382.673	2.69	Ester	
9	Octadecane, 1- (ethenyloxy)- ($C_{20}H_{40}O$)	39.954	296.539	1.34	Vinyl Ether	
10	1- Dotriacontanol ($C_{32}H_{66}O$)	41.495 & 44.355	466.879	15.35	Fatty alcohol	
11	Squalene ($C_{30}H_{50}$)	43.083	410.73	8.83	Triterpene	
12	Octacosane ($C_{28}H_{58}$)	47.690	394.772	3.09	Alkane	
13	Dl-alpha-tocopherol ($C_{29}H_{50}O_2$)	48.258	430.717	8.58	Steroid	

The highest phytocompounds were detected in methanol leaf extract [16] followed by flower extract [13]. In this study, the greater antioxidant activity of methanol leaf extract could be correlated to the occurrence of higher quantitative of secondary metabolites **Table 1**. We know that most of the bioactive compounds display several pharmacological activities. Neophytadiene is a good analgesic, antipyretic, anti-inflammatory, anti-microbial and antioxidant compound [28]. Phytol acetate is known to exhibit cancer-preventive property, antimicrobial, anti-inflammatory, and diuretic. Stigmasta-5, 22-dien-3-ol also referred to as stigmasterol shows anti-hepatotoxic, antiviral, antioxidant, hyper-cholesteremic, and cancer preventive [29]. 17-penta-triacontendisplay anti-bacterial, antiviral, anti-oxidant and anti-inflammatory [30-32]. Tetracontane has anti-inflammatory and analgesic activity, whereas pentacosane shows antibacterial property [33]. Nerolidol is sesquiterpene alcohol used a food flavoring agent, anti-microbial, anti-biofilm, anti-oxidant, anti-parasitic, skin-penetration enhancer, skin-repellent, anti-nociceptive, anti-inflammatory, and anti-cancer [34]. It has been reported that gamma-sitosterol have hypolipidemic, antioxidant, antibacterial, anti-diabetic, anti-angiogenic, anti-cancer, antimicrobial, anti-inflammatory, anti-diarrhoeal and antiviral properties [35, 28]. 2-Methyltetracosane is a good free radical scavenger [36]. β-sitosterol, a plant phytosterol having various biological activities such as anti-inflammatory activity, apoptosis inducer, chemoprotective, hypo-cholesterolemic, angiogenic, antimutagenic, anticancer, antioxidant, neuroprotector, antidiabetic [37]. Methyl palmitate is used in the preparation of detergents, emulsifiers, wetting agents, stabilizers, resins, lubricants, plasticizers, and animal feeds [38].

It exhibits a strong acaricidal activity, anti-inflammatory property, protective effect against bleomycin-induced lung inflammation, and inhibits macrophages in rats. Also, it possesses a potent anti-fibrotic effect against carbon tetrachloride-induced liver fibrosis [39-41]. 9, 12, 15-octadecatrienoic acid, methyl ester, (Z, Z, Z) is the linolenic acid compound that acts as an antiinflammatory, hypocholesterolemic cancer-preventive, heaptoprotective, nematicide insectifuge, anti-histaminic antieczemic, antiacne, 5-alpha reductase inhibitor antiandrogenic, anti-arthritic, anti-coronary, insectifuge.

It also has antimicrobial, anticancer, hepatoprotective, anti-arthritic, anti-asthma and diuretic property [42, 43]. 5-eicosene is a fatty acid, exhibits antimicrobial and cytotoxic properties [44]. Octa-decane, 1-(ethenyloxy) is ether and reported as antisepsis [45]. Squalene is a natural 30-carbon isoprenoid compound, seen both in plants and animals.

It is an intermediate metabolite in the synthesis of cholesterol having pharmacological properties such as anti-bacterial, anti-oxidant, anti-tumor, cancer preventive, immunostimulant, chemopreventive, a lipoxygenase inhibitor, pesticide, diuretic [46, 47] reported that octacosane from plant *Couroupita guianensis* L. flower extract showed the highest mortality against spodopteralitura.

Alpha-tocopherol is a fat-soluble vitamin, biologically active form of vitamin E, and essential for the stabilization of biological membranes. Dl-alpha-tocopherol is a potent antioxidant having peroxyl radical scavenging activity and important in protecting cells from oxidative stress [48].

However, some of the other compounds such as tridecyl acrylate, 4-methyldocosane, dodecyl palmitate, pentatriacontane, behenyl chloride, pentacosane, methyl palmitate, methyl linolelaidate, methyl isostearate, cholest-22-ene-21- ol, 3,5-dehydro-6-methoxy-pivalate, methyl lingocerate and 1-dotriacontanol are yet to be described in detail. Nonetheless, extended research is essential in the field of isolation, characterization, and assessment of bioactivity of each compound from *T. diversifolia* to authenticate their pharmacological importance.

CONCLUSION: This is the first scientific data to report the phytochemical profile of leaf and flower extract of *T. diversifolia* from India. The findings of this research give an insight into the bioactive compounds of *Tithonia diversifolia* and its antioxidant properties. The results support the use of *T. diversifolia* in folk medicine to treat different ailments. Thus, it can be concluded that this plant has phytopharmaceutical importance and may serve as the new potential source of herbal drug.

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