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Various Solvent Effects on Phytochemical Screening and Gas Chromatography–mass Spectroscopy Analysis of *Tephrosia Villosa* Leaf Extract Mass

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Abstract

Introduction: Plants produce various chemicals to protect themselves; but recent studies proved that many phytochemicals can also protect humans against infectious diseases. The present study was conducted to identify and characterize the phytoconstituents and gas chromatography–mass spectroscopy (GCMS) analysis of petroleum ether, chloroform, ethyl acetate, ethanol, methanol, and aqueous extract of leaves of *Tephrosia villosa* (L.) Pers. of family Fabaceae. **Materials and Method:** The leaf extracts were subjected to qualitative screening for primary and secondary metabolites as per standard methods; further, GC–MS analysis was carried out for the identification of secondary metabolites. **Result:** Preliminary phytochemical screening of leaf extract of *T. villosa* showed positive result for alkaloids, flavonoids and triterpenoids, saponins, glycosides, gum and mucilages, and fixed oils in methanolic extract which is followed by aqueous extract. In quantitative analysis, the highest amount of phenolic content was obtained in aqueous extract, tannin in petroleum ether, and flavonoids and saponins in ethyl acetate. GC–MS analysis of leaf extract revealed the presence of 53 bioactive phytochemical compounds. **Conclusion:** It can be concluded that the species contain effective phytochemical compounds, need further research on toxicological aspects to develop a safe drug.

Keywords: Aqueous extract, Bioactive compounds, Fabaceae, Gas chromatography–mass spectroscopy, *Tephrosia villosa*

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Introduction

The utilization and trade of medicinal plants tempted their demand at the global level from the pre-historic era. Mainly for primary healthcare, approximately 80–90% of the world's population depends on traditional medicine; most of them occupy the use of plant extracts.[1] Conventional medicines of herbal origin are the naturally occurring substances with a minimum or no processing and have been used to treat various illness. In global health debates, these herbal medicines were becoming significant attention. Traditional medicine has established precautionary, primitive, rehabilitative, and healing role.[2,3] Every plant consists of phytochemical compounds; therefore, they are a potential source of drugs, but a biological screening is necessary to know more about the activities of these compounds. The primary metabolites participate in vital metabolic pathways, and the secondary metabolites are accomplished in plant non vital functions. Secondary metabolites are involved in chemical resistance against pathogens and predators, they also act as allelopathic and photoprotectant agents and assist in pollination and dispersal and in prevention of diseases in the form of medicine in humans. The important bioactive compounds consist of alkaloids, phenols, flavonoids, terpenes, steroids, and glycosides.

Family Fabaceae is a third-largest family of flowering plants having 650 genera and 18,000 species.[4] Genus *Tephrosia* has 400 species throughout the world.[5] Nearly 27 species of *Tephrosia* are reported in India.[6] Plants of this genus are herb to under shrub and grow like a weed. Genus *Tephrosia* is highly known for its richness in bioactive compounds, particularly in flavonoids.[7] The genus *Tephrosia* has been used for the treatment of stomach ache, dropsy, syphilis, rheumatic pain, inflammation, and respiratory disorders and even as an diuretic laxative, and abortifacient.[8] Many species in this genus are poisonous, mainly to fish, because of their high concentration of rotenone. By indigenous cultures, *Tephrosia* species black seeds have been used historically and have been studied in connection with the use of rotenone as an insecticide and pesticide.[9]

For the treatment of dropsy and diabetes, *Tephrosia villosa* is widely used in traditional Indian medicine.[10] The taxon is also used as green manure in coffee and *Hevea* plantations and as a shade crop in tea plantations.[11] Roots, leaves, fruits, and twigs of *T. villosa* showed significant activity against *Culex quinquefasciatus* larvae.[12] Due to the presence of 20 (29)-lupen-3-one, a compound in *T. villosa* leaves showed reduction in glucose level and pancreatic cell regeneration in alloxan-induced diabetes.[13,14] Dehydroxyrotenoid and lupenone were isolated from whole plant along with that four new rotenoid were also isolated.

It was revealed that there is no previous report on phytochemical characterization of this plant from the critical literature survey except methanol. The identification of phytoconstituents through gas chromatography–mass spectroscopy (GC–MS) analysis from crude leaf extract is also absent from this plant. Chemical characterization is required to explore the probable of the plant to

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be used for medicinal purposes, due to lacking knowledge about its phytoconstituents.

The present study was conducted for the identification of bioactive compounds in the leaves of *T. villosa* by qualitative and quantitative phytochemical screening and GC–MS analysis, which might give useful information about this plant for further studies.

Materials and Methods

Plant Collection and Authentication

The plant parts of *T. v*illosa were collected during the month of April 2019 from Nambiyur, Tamil Nadu. The authenticity of the selected plant materials was duly identified and confirmed (vide no.: BSI/SRC/5/23/2016Tech/207) by comparison with reference specimen preserved at Botanical Survey of India, Southern Circle, Coimbatore.

Extract Preparation

One hundred grams of *T. villosa* dried leaves were subjected to Soxhlet extraction using the adjusted methodology of Elwekeel *et al*., 2013.[15] Six polar solvents such as petroleum ether, chloroform, ethyl acetate, ethanol, methanol, and water were used for this extraction. After extraction, the solvent was evaporated by vacuum solvent evaporator. Then, the extracts were stored at 4°C for investigation of phytochemical analysis and pharmacological studies.

Preliminary Phytochemical Studies

Phytochemical qualitative analysis

The petroleum ether, ethyl acetate, chloroform, ethanol, methanol, and water extract were subjected to preliminary phytochemical qualitative screening by various chemical tests such as alkaloids, flavonoids, tannins, steroids, triterpenoids, saponins, glycosides, gum and mucilage, fixed oil, and anthraquinones to analyze the presence of secondary metabolites using standard procedures.

Phytochemical quantitative analysis

Total phenolics and tannins

By Siddhuraju and Becker (2003)[16] method, using Folin–Ciocalteu reagent, the total phenolic content of plant extract was determined. Twenty micrograms of the extract (dissolved in the respective solvent) were taken in a test tube and made up to the volume of 1.0 mL with distilled water in this method. Then in each tube, 0.5 mL of freshly prepared Folin–Ciocalteu phenol reagent (1:1 with water) and 2.5 mL of 20% sodium carbonate solution were added sequentially. For the development of color, the mixtures were shaken and left in the dark at laboratory temperature for 40 min. Using a Shimadzu – UV-160 spectrophotometer (Japan), the absorbance was recorded at 725 nm against the reagent blank. A calibration curve of gallic acid was constructed, and linearity was obtained in the range of 10–50 µg/mL. The total phenol content of the extract was calculated and expressed as gallic acid equivalent (GAE) mg/g extract using the standard curve. As described by Siddhuraju and Manian (2007)[17] using the same extract, tannin content was estimated after treatment with polyvinylpolypyrrolidone (PVPP). One hundred milligrams of PVPP were weighed in a 100 ×12 mm test tube and to this, 1.0 mL distilled water and 1.0 mL of tannin containing phenolic extract were added. The contents were kept at 4°C for 15 min after vortexing. Then, the supernatant was collected by centrifuging (5000 rpm for 10 min at laboratory temperature) the extract. Other than tannins (the tannins would have been precipitated along with the PVPP), this supernatant has only simple phenolics. The phenolic content of the supernatant was calculated, as monitored above and uttered as the content of free phenolics on a dry matter basis. The tannin content of the extract was intended from the above results, as follows:

Tannin (mg GAE/g extract) = Total phenolics (mg GAE/g extract)–Free phenolics (mg GAE/g extract)

Total flavonoid content

Using the method adopted by Zhishen *et al*. (1999),[18] the total flavonoid content was determined spectrophotometrically. 0.5 mL of appropriately diluted extract solution was mixed with 2.0 mL of distilled water and subsequently with 0.15 mL of 5% sodium nitrite solution and maintained for 6 min. Then, 0.15 mL of 10% aluminum chloride solution was added and allowed to stand for 6 min, and finally, 2.0 mL of 4% sodium hydroxide solution was added. Final volume of the content was made up to 5.0 mL with distilled water and was mixed exhaustively. The determination of absorbance was against blank at 510 nm, after 15 min of incubation at laboratory temperature. The total flavonoid content was resolute using a standard curvature with rutin. The mean of the three values was expressed as milligrams of rutting equal (mg RE)/g extract on a dry weight basis.

Total saponins content

Estimation of total saponins content was observed by the method represented based on vanillin-sulfuric acid colorimetric reaction with some modifications. About 250 µL of vanillin reagent (800 mg of vanillin in 10 mL of 99.5% ethanol) was added to the mixture of about 50 µL of plant extract with 250 µL of distilled water. Then, it was mixed well by adding 2.5 mL of 72% sulfuric acid and the solution was kept in a water bath at 60°C for 10 min. Then, the solution was cooled in ice cold water, absorbance was read at 544 nm. Derived from a standard curve, the values were expressed as diosgenin equivalents (mg DE/g extract).[19]

GC–MS Analysis of Extract

The extracts were also subjected to gas chromatography–mass spectroscopy (GC–MS) analysis to find out the bioactive compound of the leaf. The sample was prepared by reconstituting crude extract in *T. villosa* leaf petroleum ether (TVLPE), *T. villosa* leaf chloroform (TVLC), *T. villosa* leaf ethyl acetate (TVLEA), *T. villosa* leaf ethanol (TVLE), *T. villosa* leaf methanol (TVLM) extract, and *T. villosa* leaf aqueous extract (TVLAQ) at the concentration of 1 mg/ml. The GC–MS analysis of leaf extract was done on Shimadzu QP2010 plus system with a thermal desorption system. By a fused silica capillary column having dimensions of 30 m × 0.25 mm × 0.25 µm, GC system was equipped. Helium gas (99.99%) was used as carrier gas at a constant flow rate of 1.21 ml/min in the split ratio 10:0. An

injection volume of 1 µl of the sample was injected into the column and the pressure was reserved at 69.0 k Pa. Ionization energy was set on 70 Ev. The column oven temperature was at first set on 50°C to withhold time of 3 min; and then, oven temperature was increased to 280°C at the rate of 10° withhold time of 24 min. Ion source temperature and interface temperature were 220°C and 270°C, respectively, for GC program. The total running time was 60 min for GC–MS. The sample was analyzed in MS full scan mode with start m/z 50 and end m/z 650 with a scan speed of 1250 after injecting in splitless mode. Reading on unknown mass spectrum GC–MS was completed through comparing the division patterns of the mass spectra with the known and standard compound provided in the database of NIST 16 (National Institute of Standard and Technology) and Wiley 8 library and the compound was identified by their GC retention time. Comparing the average peak area with the total peak area, relative percentage of the amount of each compound was obtained and the name, molecular formula and molecular weight of each detected compound were determined.

Results

Preliminary Phytochemical Screening of *T. villosa*

Preliminary phytochemical screening reveals the presence of primary and secondary metabolites. Among all six extract, more secondary metabolites such as alkaloids, flavonoids, tannins, triterpenoids, saponins, and gum and mucilages was obtained in methanol which was followed by water consisting alkaloids, flavonoids, tannins, triterpenoids, saponins, gum and mucilages, and fixed oils. Results of preliminary phytochemicals screening are shown in Table 1.

Quantitative Analysis of *T. villosa*

T. villosa extract was evaluated for the quantification of various secondary metabolites. Total phenolic was observed (22.29 ± 0.1 mg/g) in aqueous extract, tannins were obtained more (3.32 ± 0.2 mg/g) in petroleum ether extract, whereas the total flavonoids (26.1 ± 0.2 mg/g) and saponins (23.72 ± 0.1 mg/g) were obtained more in ethyl acetate extract. Table 2 shows the amount of secondary metabolites presence in various extracts.

GC–MS Analysis

GC–MS analysis of TVLPE, TVLC, TVLEA, TVLE, TVLM, and TVLAQ extracts was carried out by NIST library. TVLPE extract has 257 phytocompounds with retention time from 14.083 to 29.109 [Figure 1]. Totally, 15 bioactive compounds were identified in TVLPE extract which is tabulated with compound name, molecular formula, molecular weight, CAS number, and its bioactive uses [Table 3]. In TVLC extract, 439 compounds are present with the retention time from 14.288 to 21.741 which are given in Figure 2. Among them, 10 compounds have bioactive

Table 1: Preliminary phytochemical analysis of *Tephrosia villosa*

S. No.	*Compounds*	*Petroleum ether*	*Ethyl acetate*	*Chloroform*	*Ethanol*	*Methanol*	*Water*
1.	Alkaloids	–	–	+	+	+	+
2.	Flavonoids	–	–	–	+	+	+
3.	Tannins	–	–	+	–	+	+
4.	Steroids	+	+	+	–	+	–
5.	Triterpenoids	+	+		+	+	+
7.	Saponins	–	–	–	–	–	+
8.	Glycosides	–	+	+		+	
9.	Gum and mucilages	–	–	–	–	+	+
10.	Fixed oils	+	+		+	+	+
11.	Anthraquinones	–	–	–	–	–	–

Present: +, Absent: –

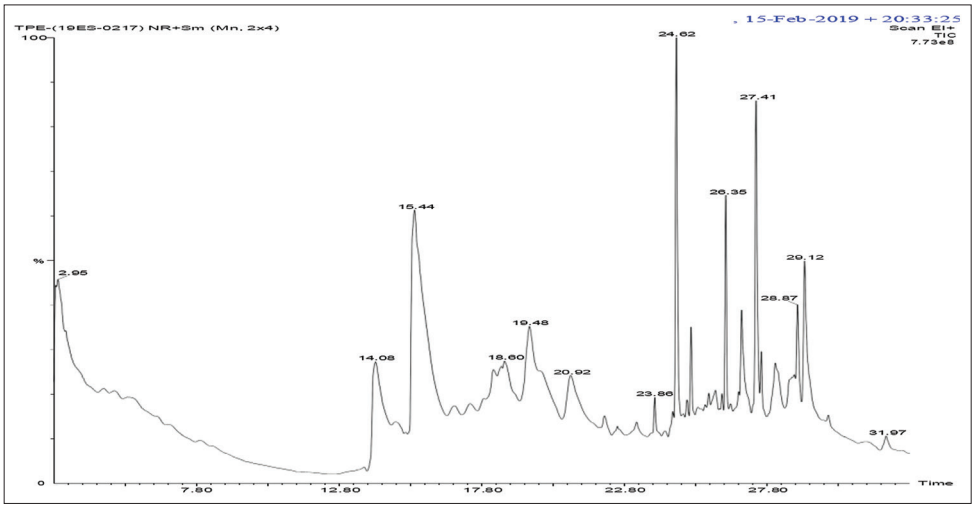

Figure 1: Gas chromatogram phytochemical constitutes of TVLPE extract

uses are represented in Table 4. The result of GC–MS analysis of TVLEA extract has 257 compounds with retention time from 7.580 to 23.572 [Figure 3], which consists of 14 compounds having bioactive uses [Table 5]. TVLE extract has 17 compounds [Figure 4] with retention time 18.620 in which one compound consists of the bioactive uses with molecular formula and molecular weight, which is represented in Table 6. Four bioactive compounds of 89 compounds were identified by GC–MS of TVLM extract, with retention time from 15.824 to 27.128 [Figure 5 and Table 7]. GC–MS analysis of leaf aqueous extract of *T. villosa* has 90 phytochemical constituent with 8 bioactive compounds. Table 8 reported 18 bioactive compounds with molecular formula, molecular weight, and bioactive medicinal uses and retention time from 8.0 to 27.0 [Figure 6].

Table 2: Quantitative determination of secondary metabolites in *Tephrosia villosa*

Sample	*Extracts*	*Total phenolics*	*Tannins*	*Total flavonoids*	*Saponins*
Leaf	PE	8.04±0.1	**3.32±0.2**	11.1±0.1	9.11±0.1
	CH	10.32±0.2	1.06±0.2	3.33±0.2	4.01±0.1
	EA	11.25±0.1	2.29±0.2	**26.1±0.2**	13.72±0.1
	ET	14.48±0.1	1.07±0.2	16.5±0.2	8.13±0.1
	ME	17.21±0.1	1.02±0.1	6.32±0.2	**5.04±0.2**
	AQ	**22.29±0.1**	2.13±0.3	7.09±0.6	8.36±0.2

The bold values are the maximum quantity of the secondary metabolite in each extract

Table 3: Bioactivities of phytocompounds identified in the TVLPE extract by GC–MS

S. No.	*Compound name*	*Molecular formula*	*Molecular weight*	*CAS No.*	*Bioactive use*
1	Myrtenyl acetate	C12H18O2	194.27	3674-03-1	Flavor and fragrance agent[20]
2	Andrographolide	C20H30O5	350.4	5508-58-7	Andrographolide is an alternative treatment to overcome resistant in ER-positive breast cancer through cholesterol biosynthesis pathway[21]
3	Caryophyllene oxide	C15H24O	220.35	13,877-94-6	Antitumor, anesthetic, antibacterial, anti-inflammatory, analgesic, anti-inflammatory, antioxidant[22]
4	Tricyclo[7.1.0.0[1,3]] Decane-2-Carbaldehyde	C11H16O	164.24	39,750-93-1	Antibacterial and anticancer activity[23]
5	(-)-Spathulenol	C15H24O	220.35	6750-60-3	Immunomodulatory effect[24]
6	Farnesene epoxide, E-	C15H24O	220.35	83637-40-5	Antiproliferative activity[25]
7	Pseduosarsasapogenin 5,20-Dien	C27H42O3	414.6	512-04-9	Treatment of amyotrophic lateral sclerosis[26]
8	Cholest-5-en-3-ol (3.beta.)-, acetate	C29H48O2	428.7	604-35-3	Antioxidant activity and antimicrobial activity[27]
9	Squalene	C30H5O	410.7	111-02-4	Antibacterial, antioxidant, antitumor, cancer preventive, immunostimulant, chemopreventive, lipoxygenase inhibitor, pesticide[28]
10	Hexatriacontane	C36H74	507	630-06-8	Radical scavenger[29]
11	Heptacosane	C27H56	380.7	593-49-7	Antibacterial[30]
12	Vitamin E	C29H50O2	430.72	10191-41-0	Fatsoluble antioxidant[31]
13	Pentadecanal	C15H30O	226.4	2765-11-9	Nutrient, stabilizers, surfactants and emulsifier, antibacterial, antioxidant[32]
14	Tetradecanal	C14H28O	380.7	593-49-7	Antibacterial activity[33]
15	Lupeol	C30H50O	426.7	545-47-1	Anti-tumor, cancer preventive, inhibit intestinal cholesterol absorption. Anti-inflammatory[34]

Table 4: Bioactivities of phytocompounds identified in the TVLC extract by GC–MS

S. No.	*Compound name*	*Molecular formula*	*Molecular weight*	*CAS No.*	*Bioactive use*
1	Pseduosarsasapogenin-5,20-Dien	C27H42O3	414	512-04-9	Treatment of amyotrophic lateral sclerosis[26]
2	Caryophyllene oxide	C15H24O	220.35	1139-30-6	Antitumor, anesthetic, antibacterial, anti-inflammatory, analgesic, anti-inflammatory, antioxidant[22]
3	Gamma.-linolenic acid, methyl ester	C19H32O2	292.5	16326-32-2	Antitumor, antioxidant, anti-inflammatory, antidiabetic, antiobesity, treat eczema[35]
4	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	C19H32O2	292	301-00-8	Anti-inflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anti-coronary, insectifuge[20]
5	Methyl 8,11,14-Heptadecatrienoate	C18H30O2	278.4	155273-05-5	Antioxidant[36]
6	Ethyl 9,12,15-Octadecatrienoate	C20H34O2	306.5	1191-41-9	Cell viability[37]
7	Methyl 8,11,14-Eicosatrienoate	C21H36O2	320	21061-10-9	Anti-inflammatory[38]
8	7Oxabicyclo[4.1.0]Heptane, 1-Methyl-4-(2-Methyloxiranyl)-	C10H16O2	168.2	96-08-2	Fragrance compound[39]
9	N-Tetracosanol-1	C24H50O	354.7	506-51-4	Antioxidant[40]
10	Behenic alcohol	C22H46O	326	661-19-8	Hair conditioners, moisturizers, and lubricating oils[41]

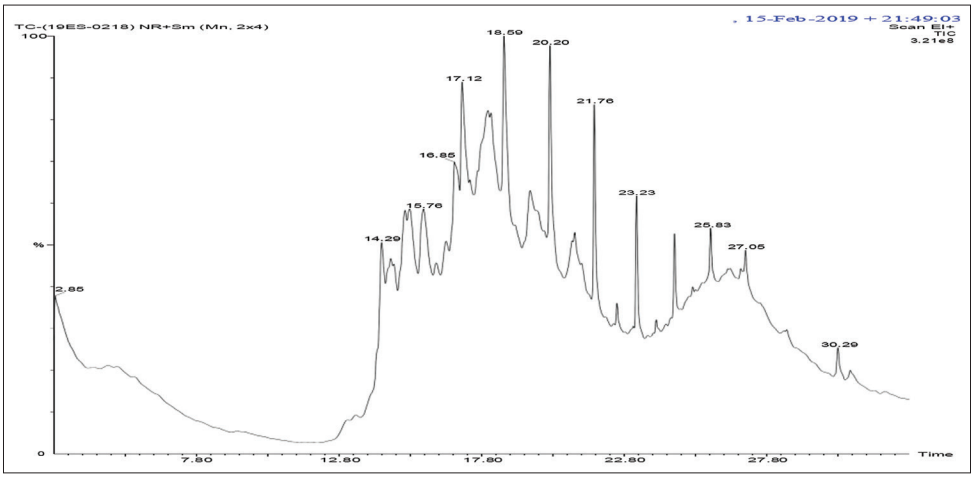

Figure 2: Gas chromatogram phytochemical constitutes of TVLC extract

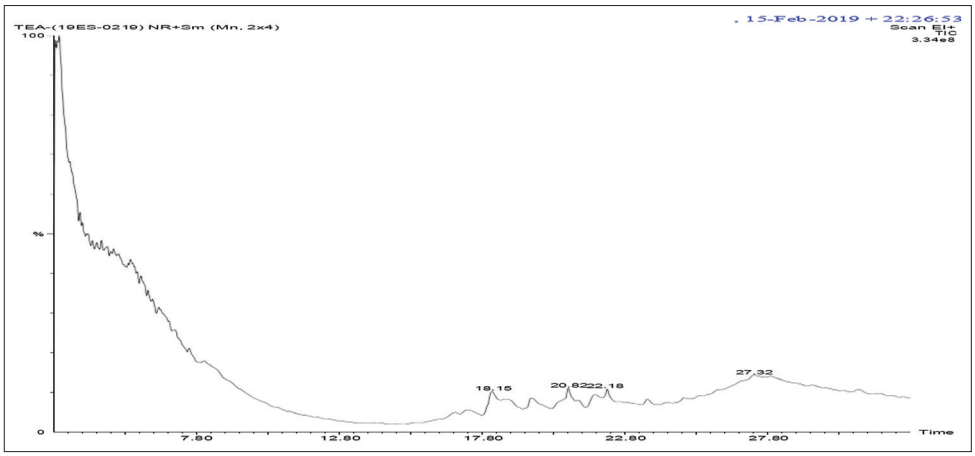

Figure 3: Gas chromatogram phytochemical constitutes of TVLEA extract

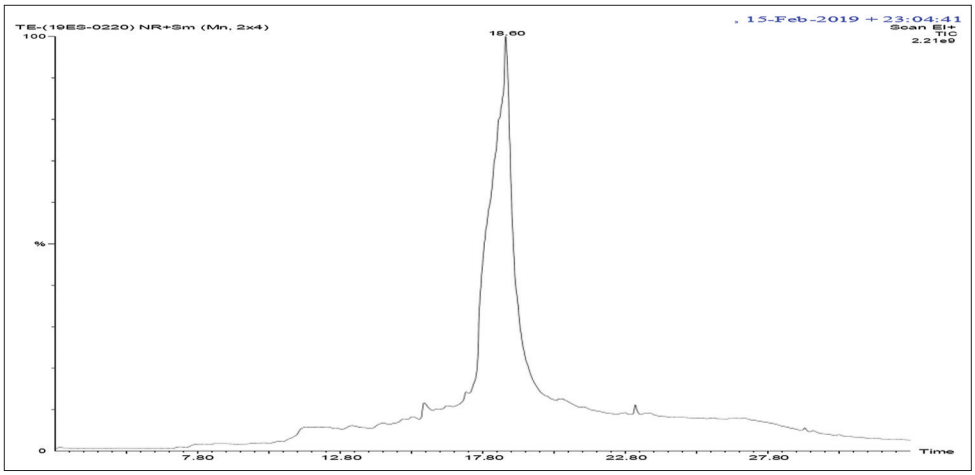

Figure 4: Gas chromatogram phytochemical constitutes of TVLE extract

Discussion

The medicinal and pharmacological actions of medicinal herbs are often depended on the presence of bioactive compounds, the secondary metabolites.[65] Qualitative phytochemical analysis of this plant confirms the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, triterpenes, gum and mucilage, and fixed oils. The results suggest that the phytochemical properties for curing various ailments and possess potential anti-inflammatory, antimicrobial, and antioxidant and leads to the isolation of new and novel compounds. Most alkaloids

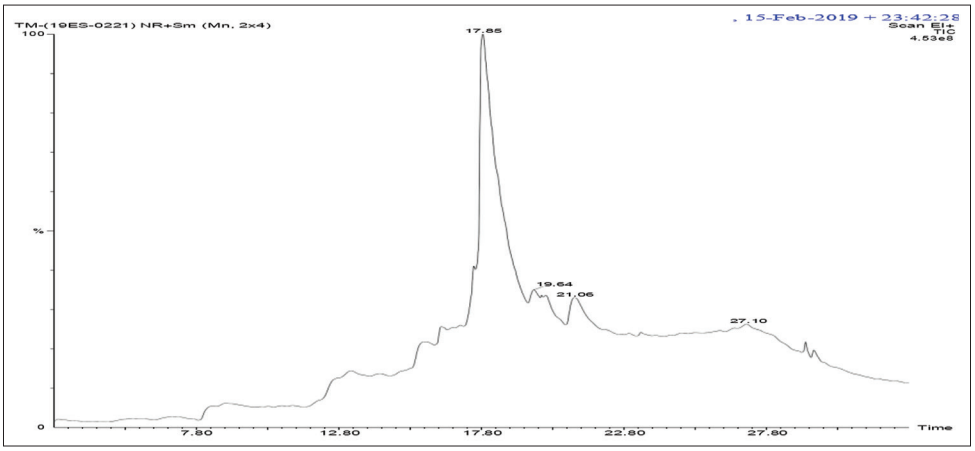

Figure 5: Gas chromatogram phytochemical constitutes of TVLM extract

Table 5: Bioactivities of phytocompounds identified in the TVLEA extract by GC–MS

S. No.	*Compound name*	*Molecular formula*	*Mol. Wt.*	*CAS No.*	*Bioactive use*
1	Pentanoic acid, 3-Methyl-4-Oxo-	C6H10O3	130	6628-79-1	Antibacterial activity[42]
2	(3-Methyl-Oxiran-2-Yl)-Methanol	C4H8O2	88	872-38-8	Catechol – O- Methyl transferase inhibitor, methyl guanidine inhibitor, methyl donor[43]
3	Phytol	C20H40O	296	150-86-7	Antimicrobial, anti-inflammatory, anticancer diuretic[44]
4	N-Nonadecanol-1	C19H40O	284	1454-84-8	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge antihistaminic, antiarthritic, anti-coronary, antieczemic antiacne, 5-alpha reductase inhibitor antiandrogenic[45]
5	1-Eicosanol	C20H42O	298	629-96-9	Antimicrobial, antioxidant[46]
6	1-Octadecyne	C18H34	250	629-89-0	Antimicrobial activity[47] anti-inflammatory agent, antibacterial agent, fragrance[48]
7	Pentadecanal-	C15H30O	226	2765-11-9	Nutrient, stabilizers, surfactants, and emulsifier[49]
8	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	C20H40O	296	7541-49-3	Cancer-preventive antimicrobial, anti-inflammatory, anti-diuretic, antioxidant[50]
9	1-Hexadecyne	C16H30	222	629-74-3	Antibacterial[51]
10	Z-(13,14-Epoxy)Tetradec-11-En-1-Ol Acetate	C16H28O3	268	61886-62-2	Antioxidant, hemolytic[51]
11	Pentadecanal-	C15H30O	226	2765-11-9	Nutrient, stabilizers, surfactants, and emulsifier[49]
12	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	C20H40O	296	7541-49-3	Cancer-preventive antimicrobial, anti-inflammatory, antidiuretic, antioxidant[50]
13	Phytol	C20H40O	296	150-86-7	Antimicrobial, anti-inflammatory, anticancer diuretic[52]
14	Oleic acid	C18H34O2	282	112-80-1	Antimicrobial, antifungal, anticonvulsive, antiadhesive, antiallergic, antianalgesic, antiatherosclerosis, anesthetic, antihelminthic, antianxiety, antibacterial, antiberiberi, antibiotic, anticancer, anticonvulsant, antidiabetic, antidiarrheic, antifertility, antigastric, anti-inflammatory, antiobesity, antioxidant, antiulcer, antitubercellosic, anticold antihepatotoxic, and antiviral activity[53]

Table 6: Bioactivities of phytocompounds identified in the TVLE extract by GC–MS

S. No.	*Compound name*	*Molecular formula*	*Mol. Wt.*	*CAS No.*	*Bioactive use*
1	3-o-methyl-d-glucose	C7H14O6	194	13,224-94-7	Preservative[54]

are very toxic and have a strong bitter taste, and that's the reasons they are used by plant to protect themselves against attacks by microbial pathogens and herbivory and invertebrate pests.[66] Phenolic herb secondary metabolites are widely distributed in herbs and are responsible for color development, pollination, and protection against UV radiation and pathogens.[65,67] Alkaloids are generally toxic to man and many of them have shown physiological activities; hence, they are widely used in medicine.[68] Flavonoids have various proven medicinal properties such as antioxidant, anticancer, anti-inflammatory, antibacterial, and antiviral properties.[69,70] Terpenoids can also be known as isoprenoids which consist of the largest group of herbal secondary metabolites.[67] Terpenoids are involved in defense wound scaling and thermo tolerance of plants as well as in the pollination of seed crops[65] and also used as an antibacterial, antifungal, antimalarial, and antioxidant activity.[71] Plant-based terpenoids are used in food, chemical industries, and pharmaceuticals and also used in the development of biofuel product.[72]

Based on studies, biologically active compounds are found from some of the constituents revealed by GC–MS analysis. They were proven to possess pharmacologic activities which may provide

Table 7: Bioactivities of phytocompounds identified in the TVLM extract by GCMS

S. No.	*Compound name*	*Molecular formula*	*Mol. Wt.*	*CAS No.*	*Bioactive use*
1	Methyl(methyl 4-o-methyl-.alpha.-dmannopyranoside)uronate	C9H16O7	236	2880-95-7	Processing aids and additives[55]
2	Undecanoic acid	C11H22O2	186	112-37-8	Manufacturing of a number of esters, some of which are used in perfumes, more in flavor composition[56]
3	N-Decanoic acid	C10H20O2	172	334-48-5	Acidifier, acidulant, arachidonic acid inhibitor, increases aromatic amino acid decarboxylase activity, increases production of uric acid, anaphylactic, antitumor decrease norepinephrine production, GABAergic, increase NK cell activity, myoneural stimulant[57]
4	Octadecanoic acid	C18H36O2	284	85541-42-0	Antifungal, anti-tumor, antibacterial[58]

Table 8: Bioactivities of phytocompounds identified in the TVLAQ extract by GC–MS

S. No.	*Compound name*	*Molecular formula*	*Mol. Wt.*	*CAS No.*	*Bioactive uses*
1	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	007786-61-0	Anti-inflammatory effect[59]
2	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{17}H_{30}OSi$	278	000096-76-4	Antibacterial and antioxidative[60]
3	Pentadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	7132-64-1	Antioxidant, antifungal, antimicrobial activities[61]
4	Hexadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	284.5	112-39-0	Antioxidant, hypocholesterolemic, nematicide, pesticide, flavor, lubricant, antiandrogenic, 5 alpha-reductase inhibitor[62]
5	9-Octadecenoic acid (Z)-, methyl..	$C_{19}H_{36}O_2$	296.5	112-62-9	Antibacterial[63]
6	trans-13-Octadecenoic acid, meth	$C_{18}H_{34}O_2$	282	13126-39-1	Anti-inflammatory[59]
7	cis-13-Octadecenoic acid, methyl...	$C_{19}H_{36}O_2$	296.5	13126-39-1	Dopaminergic stimulatory activity[64]
8	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.46	541-05-9	Antimicrobial potential, antimicrobial, antioxidant[64]

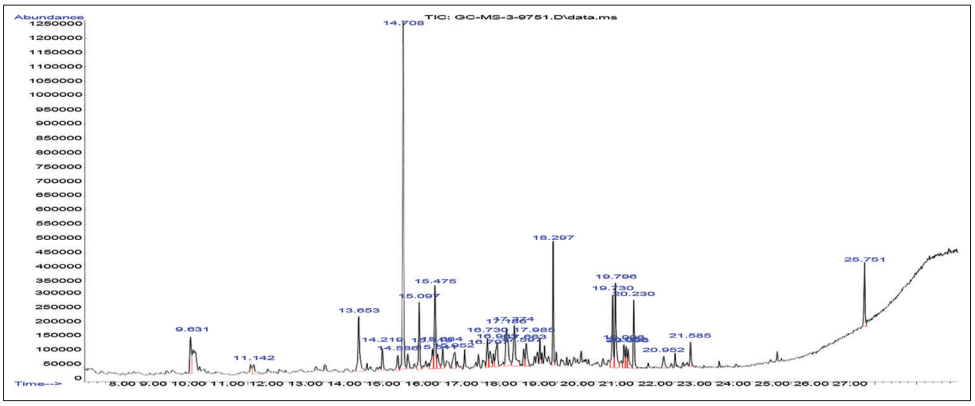

Figure 6: Gas chromatogram phytochemical constitutes of TVLAQ extract

the healing potential of the plant. Phytol was proven to exhibit antinociceptive and antioxidant effects.[14,73] Phytol, precursor of synthetic Vitamin E and Vitamin K, was found to be cytotoxic against breast cancer cell lines (MCF7).[74,75] Other studies revealed that squalene, lupeol possesses various pharmacological properties. Squalene has antioxidant, chemopreventive, antitumor, and hypocholesterolemic activities.[76,77] Lupeol exhibited marked anti-inflammatory, and anticancer properties.[78] Several investigations revealed that lupeol blocks tumorigenesis by affecting molecular growth pathways which are involved in cell proliferation and cell death.[79,80] *In vitro* tests revealed potent anti-mutagenic property of lupeol.[81-83] The compound, caryophyllene oxide, exhibits a wide range of antimicrobial properties.[77] Hexadecanoic acid is known to exhibit strong antimicrobial and anti-inflammatory activity.[84,85]

The present study is the first report on the GC–MS analysis in TVLPE, TVLC, TVLEA, TVLE, TVLM, and TVLAQ extracts. Totally, 53 compounds have been reported to possess interesting biological activities. The GC–MS analysis of various polar solvent extract of *T. villosa* leaf showed the presence of 257, 439, 257, 17, 89, and 90 compounds in the extract of TVLPE, TVLC, TVLEA, TVLE, TVLM, and TVLAQ and each extract has 15, 11, 14, 1, 4, and 8 known bioactive compound, respectively. These bioactive compounds are anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge antihistaminic, antiarthritic, anti-coronary, antieczemic antiacne, 5-alpha reductase inhibitor, antiandrogenic,[45] antioxidant, hypocholesterolemic, nematicide, pesticide, flavor, lubricant, 5-alpha-reductase inhibitor,[62] anti-tumor, antioxidant, anti-inflammatory, antidiabetic, antiobesity, treat eczema[35] resistant in ER-positive breast cancer through cholesterol biosynthesis pathway,[21] treatment of amyotrophic lateral sclerosis, anticancer, and diuretic. Phytocompound and their bioactive applicants of earlier report are presented in Tables 3-8. These biological activities of compounds present in *T. villosa* leaf extract support the medicinal application of the plant. Identification of these compounds in the plant serves as the basis in determining the possible health benefits of the plant leading to further biologic and pharmacologic studies.

Conclusion

The present study was to analyze phytochemical and GC–MS of TVLPE, TVLC, TVLEA, TVLE, TVLM, and TVLAQ extracts. All the extracts have various phytocompounds which have many pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anticancer activities, etc. Further investigations are needed for antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and anticancer activities. The study exhibited the presence of various useful compounds in all the extracts. The availability of various bioactive principles clearly proceeds the purpose of *T. villosa* for various diseases. However, isolation and purification of compounds may proceed to find new active drug.

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