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Gas chromatography mass spectrometry/Fourier transform infrared (GC-MS/FTIR) spectral analyses of *Tithonia diversifolia* (Hemsl.) A. Gray leaves

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Medicinal plants and its products remain the best therapeutic agents for the management of diseases and infections that affect the health of man. Owing to the recorded ethnomedicinal potentials of *Tithonia diversifolia* (Hemsl.) A. Gray, this study was aimed at investigating the gas chromatography combined with mass spectrometry (GC-MS) and Fourier transform infrared (FT-IR) spectral analyses of methanolic extract of leaves of *T. diversifolia* (Hemsl.) A. Gray using standard analytical methods. Results of the GC-MS spectral analysis of methanolic leaf extract of *T. diversifolia* (Hemsl.) A. Gray showed the existence of the following twenty nine (29) bioactive compounds: Ethylene oxide (2.04%), 1-Butanamine, 3-methyl- (0.71%), N-(3-Methylbutyl) acetamide (3.11%), Cyclopentane, 2-n-octyl- (3.33%), Cyclobutanol (0.73%), dl-Phenylephrine (1.53%), Phenylephrine (1.69%), Hexadecanoic acid, methyl ester (2.31%), 1,3-Cyclohexanediol (2.08%), Amphetamine (1.69%), n-Hexadecanoic acid (17.53%), 8-[N-Aziridylethylamino]-2,6-dimethyloctene-2 (0.85%), Benzenemethanol, .alpha.-[(methylamino)methyl]- (0.58%), folic acid (1.32%), acetic acid, hydroxy[(1-oxo-2-propenyl)amino]- (1.11%), cis-Vaccenic acid (19.20%), Octadecanoic acid (7.67%), acetic acid, [(aminocarbonyl)amino]oxo- (0.85%), cis-11-Eicosenoic acid (4.50%), 4-Fluorohistamine (2.20%), 2,3-Dimethoxyamphetamine (0.74%), Benzeneethanamine, 2-fluoro-.beta. ,5-dihydroxy-N-methyl- (2.62%), 2-Propenamide, N-(1-cyclohexylethyl)- (1.73%), Erucic acid (5.82%), Acetamide, 2,2,2-trichloro- (0.85%), 2-Methoxy-N-methylethylamine (2.45%), acetic acid, chloro-, pentyl ester (4.97%), p-Hydroxynorephedrine (1.61%) and Metaraminol (1.28%); with Cis-vaccenic acid, n-Hexadecanoic acid and Octadecanoic acid as the most predominant bioactive compounds residents in the plant whereas that of Fourier transform infrared (FT-IR) showed the existence of alcohols, phenols, aldehydes, ketones, alkanes and primary amines. Based on these findings, it is opined that the methanolic extract of leaves of *T. diversifolia* (Hemsl.) A. Gray could be relied upon in traditional medicine for the management of certain infections and diseases that plague man especially those that dwell in rural areas.

Key words: Fourier transform infrared (FTIR), gas chromatography mass spectrometry (GC-MS), bioactive compounds, methanol extract, functional group, *Tithonia diversifolia* (Hemsl.) A. Gray.

INTRODUCTION

Medicinal plants and its products have been known over the years as effective ingredients for the management of diseases. The essentiality of these plants is due to bioaccumulation of chemical substances (Sofowora,

1999). These substances generally referred to as phytochemicals, have been known to target biochemical pathways (Okereke et al., 2017). Other advantages of herbal products over synthetic drugs include affordability, bioaccumulation, higher safety margin, efficacy and quality (Ezekwesili et al., 2014). The concentrations of these phytochemicals in medicinal plants are affected by certain climatic factors and the time of harvest (Cetin et al., 2010). Currently, there is high dependence on the intake of traditional medicine. According to the report of the World Health Organization in 2008, about 80% of the world's population relies solely on herbal preparations for their primary health care (Derwich et al., 2009). These and others reasons suggested over the years have made herbal products better therapeutic agents over synthetic drugs in the treatment and management of diseases.

Over the years, a lot of methods have been adopted to screen phyto-constituents present in medicinal plants. Some of these methods (Trease and Evans, 2002; Harbone, 2008) do not give information on the molecular structures, weights, formula, functional groups and biological activities of the plants. This limitation has led to the introduction of high throughput techniques of gas chromatography combined with mass spectrometry (GS-MS) and Fourier transform infrared (FT-IR). GC-MS and FT-IR are analytical and high throughput techniques which determine the different bioactive compounds and functional groups respectively present in biological samples. They are very sensitive and reliable when compared to preliminary phytochemical screening methods.

A pile of information has been published elucidating the bioactivity of medicinal plants used by indigenous people of Nigeria to manage diseases and infections. *T. diversifolia* (Hemls.) A. Gray happens to be one of such plants selected for possible phytochemical screening in this study. The plant, commonly known as Mexican Sunflower or the Tree Marigold, belongs to the Family Asteraceae and Order Asterales. It is locally known as Jogbo or Agale among the Yorubas and Izondiri in some eastern parts of Nigeria. In traditional medicine, the extracts of the various parts of the plant have been reported to exhibit anticancer, anti-bacterial, anti-proliferation and antidiabetic effects (Victor et al, 2004; Miura et al., 2005; Oyewole et al., 2008). Several folkloric claims on *T. diversifolia* have been established over the years, however, it is paramount to highlight the bioactive compounds responsible for these observations. Therefore, the thrust of this study was to isolate, identify and characterize possible bioactive compounds residents in the methanolic leaf extract of *T. diversifolia* (Hemsl.) A. Gray using high throughput techniques of GC-MS and FT-IR.

MATERIALS AND METHODS

Plant collection, identification and processing

T. diversifolia (Hemsl.) A. Gray leaves were harvested within the vicinity of Abia State University, Uturu, Isiukwuata Local Government Area of Abia State Nigeria. Uturu is located at the northern region of Abia State. The leaves of the plant were taken to the Department of Plant Science and Biotechnology (PSB) of the University for proper identification by an experienced botanist. Upon identification, the leaves were sorted to eliminate dirt, dust and other extraneous materials. They were washed in distilled water and then allowed to air dried. Upon drying, the leaves were pulverized into fine powder and kept in a polythene bag at room temperature prior to use.

Extraction of plant sample

Exactly two grams (2.0 g) of the pulverized leaf sample of *T. diversifolia* (Hemsl.) A. Gray was dispensed in fifty milliliter (50.0 ml) of methanol with gentle stirring for 72 h. The sample was kept in the dark for 72 h with intermittent shaking. After extraction, the solution was filtered through Whatmann No. 1 filter paper. The extract was concentrated with a rotary evaporator till dry powder was obtained, transferred to glass vials and kept at 4°C before use. The final residue produced was then subjected to GC-MS analysis (Kalimuthu and Prabakaran, 2013) and FT-IR analysis (Krishnaveni and Saranya, 2016).

Gas chromatography-mass spectrometry analysis (GC-MS)

The methanol extract of *T. diversifolia* (Hemsl.) A. Gray leaves were analyzed through GC-MS for the identification of different compounds. The GC-MS analysis was carried out by using Clarus 500 (Perkin - Elmer) Gas chromatograph equipped and coupled to a mass detector Turbo mass gold (Perkin - Elmer) spectrometer with an Elite - 5MS (5% Diphenyl / 95% Dimethyl poly siloxane, 30 m × 0.25 mm × 0.25 µm df) of capillary column. The oven was set to an initial temperature 110ºC for 2 min, further increased up to 200^{0}C at the rate of 10°C/min. Finally temperature was raised up to 280ºC, at the rate of 5ºC/min for 9 min. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1 ml/min. An aliquot of 2 µl of sample was injected into the column with the injector temperature at 250ºC (Split ratio of 10:1). The electron ionization system with ionizing energy of 70 eV was used. Mass spectral scan range was set at 45 to 450 m/z.

Fourier transform infrared (FT-IR) analysis (FT-IR)

The sample was ground in a mortar to reduce the average particle size to 1 to 2 microns. Exactly 0.1 mg of finely pulverized sample was mixed with ground KBr. This mixture was then placed onto the face of a KBr plate with the second window on top. With a gentle circular and back-and-forth rubbing motion of the two windows, the

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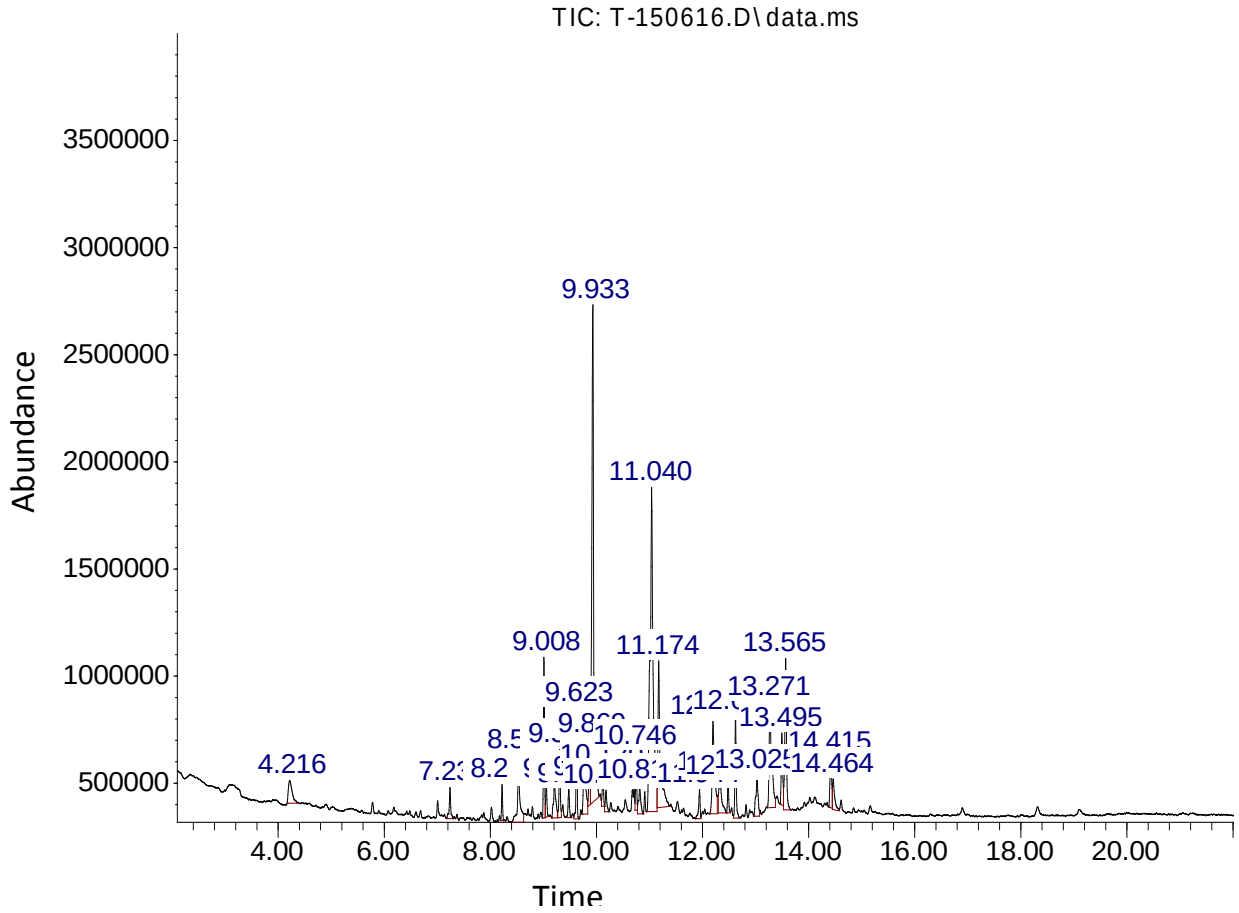

Figure 1. Mass chromatogram of methanolic extract of leaves of *Tithonia diversifolia* (Hemsl.) A. Gray.

mixture was evenly distributed between the plates until it became slightly translucent. The sandwiched plates were placed in the spectrometer to obtain a spectrum. The Fourier transform infrared spectrum was recorded using Bruker Tensor 27 spectrometer in the wavelength range 400 to 4000 cm^{-1} by KBr pellet technique with a resolution and scanning speed of 4 cm^{-1} and 2 mm/s, respectively.

Identification of components

Interpretation of mass spectrum obtained from GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 82,000 patterns. The spectrum of the unknown component was compared with the spectra of the known components stored in the NIST library. The name, molecular weight, molecular formula and structure of the components of the test materials were ascertained.

Identification of functional groups

The FTIR spectrum was used to identify the functional groups of the active components present in plant sample based on the peaks values in the region of IR radiation. When the plant extract was passed into FTIR, the functional groups of the components were separated based on its peaks ratio.

RESULTS

A total of twenty nine (29) compounds were identified from the methanolic leaf extract of *T. diversifolia* (Hemsl.) A. Gray. The identification of the phytochemical compounds was confirmed based on the peak area; retention time and molecular formula were presented in Figure 1 and Table 1. The GC-MS analysis of methanol leaf extract of *T. diversifolia* (Hemsl.) A. Gray showed the presence of the following phytochemicals: Ethylene oxide (2.04%), 1-Butanamine, 3-methyl- (0.71%), N-(3-Methylbutyl)acetamide (3.11%), Cyclopentane, 2-n-octyl- (3.33%), Cyclobutanol (0.73%), dl-Phenylephrine (1.53%), Phenylephrine (1.69%), Hexadecanoic acid, methyl ester (2.31%), 1,3-Cyclohexanediol (2.08%), Amphetamine (1.69%), n-Hexadecanoic acid (17.53%), 8-[N-Aziridylethylamino]-2,6-dimethyloctene-2 (0.85%), Benzenemethanol, .alpha.-[(methylamino)methyl]- (0.58%), Folic Acid (1.32%), Acetic acid, hydroxy[(1-oxo-2-propenyl)amino]- (1.11%), cis-Vaccenic acid (19.20%), Octadecanoic acid (7.67%), acetic acid, [(aminocarbonyl) amino]oxo- (0.85%), cis-11-Eicosenoic acid (4.50%), 4-Fluorohistamine (2.20%), 2,3-Dimethoxyamphetamine

Table 1. List of compounds identified at various retention times from methanolic extract of leaves of *Tithonia diversifolia* (Hemsl.) A. Gray by GC-MS.

S/N	RT	Compound name	MW	Formula	Area (%)
1	4.216	Ethylene oxide	40	C2H4O	2.04
2	8.222	1-Butanamine, 3-methyl-	87	C5H13N	0.71
3	8.532	N-(3-Methylbutyl)acetamide	129	C7H15NO	3.11
4	9.008	Cyclopentane, 2-n-octyl-	180	C13H24	3.33
5	9.056	Cyclobutanol	72	C4H8O	0.73
6	9.206	dl-Phenylephrine	167	C9H13NO2	1.53
7	9.307	Phenylephrine	167	C9H13NO2	1.69
8	9.623	Hexadecanoic acid, methyl ester	270	C17H34O2	2.31
9	9.778	1,3-Cyclohexanediol	116	C6H12O2	2.08
10	9.869	Amphetamine	135	C9H13N	1.69
11	9.933	n-Hexadecanoic acid	256	C16H32O2	17.53
12	10.120	8-[N-Aziridylethylamino]-2,6-dimethyloctene-2	224	C14H28N2	0.85
13	10.677	Benzenemethanol, .alpha.-[(methylamino)methyl]-	151	C9H13NO	0.58
14	10.746	Folic acid	441	C19H19N7O6	1.32
15	10.816	Acetic acid, hydroxy[(1-oxo-2-propenyl)amino]-	145	C5H7NO4	1.11
16	11.040	cis-Vaccenic acid	282	C18H34O2	19.20
17	11.174	Octadecanoic acid	284	C18H36O2	7.67
18	11.944	Acetic acid, [(aminocarbonyl)amino]oxo-	132	C3H4N2O4	0.85
19	19.336	cis-11-Eicosenoic acid	310	C20H38O2	4.50
20	19.593	4-Fluorohistamine	129	C5H8FN3	2.20
21	12.479	2,3-Dimethoxyamphetamine	195	C11H17NO2	0.74
22	12.618	Benzeneethanamine, 2-fluoro-.beta,5-dihydroxy-N-methyl-	185	C9H12FNO2	2.62
23	13.025	2-Propenamide, N-(1-cyclohexylethyl)-	181	C11H19NO	1.73
24	13.271	Erucic acid	338	C22H42O2	5.82
25	13.405	Acetamide, 2,2,2-trichloro-	161	C2H2Cl3NO	0.85
26	13.495	2-Methoxy-N-methylethylamine	89	C4H11NO	2.45
27	13.565	Acetic acid, chloro-, pentyl ester	164	C7H13ClO2	4.97
28	14.415	p-Hydroxynorephedrine	167	C9H13NO2	1.61
29	14.464	Metaraminol	167	C9H13NO2	1.28

(0.74%), Benzeneethanamine, 2-fluoro-.beta, 5-dihydroxy-N-methyl- (2.62%), 2-Propenamide, N-(1-cyclohexylethyl)- (1.73%), Erucic acid (5.82%), Acetamide, 2,2,2-trichloro- (0.85%), 2-Methoxy-N-methylethylamine (2.45%), acetic acid, chloro-, pentyl ester (4.97%), p-Hydroxynorephedrine (1.61%) and Metaraminol (1.28%).

The FT-IR spectroscopic analysis of the methanolic leaf extract of *T. diversifolia* (Hemsl.) A. Gray revealed the presence of alcohols, phenols, aldehydes, ketones, alkanes and primary amines (Figure 2 and Table 2). The absorption at 3283.33 cm^{-1} is due to the O-H bonding of alcohols and phenols present in the extract. The bands between 2918.51 and 2850.16cm^{-1} are due to $C-H_3$, $C-H_2$ and C-H stretching of alkanes; the band at 1598.62 cm^{-1} showed N-H bend of primary amines, the band at 1404.11 cm^{-1} showed $C-H_3$, $C-H_2$ and C-H deformation of alkanes, the band at 1321.32 cm^{-1} showed $A-CH_3$ stretching of aldehydes and ketones, the band at 1243.14 cm^{-1} showed O-C stretching of carboxylic acids and derivatives and the band at 1018.92 cm^{-1} showed C-O stretching of alcohols and phenols.

DISCUSSION

In the present work, exactly twenty nine (29) compounds were isolated from the methanolic leaf extract of *T. diversifolia* (Hemsl.) A. Gray. The identified compounds according to Duke are ethnobotanical and phytochemistry database (1998) possesses many biological properties. Among the identified bioactive compounds, n-Hexadecanoic acid has been reported to possess lubricant, anti-androgenic flavor, hypocholesterolemic, flavor, hemolytic, antioxidant, pesticide, 5-alpha reductase inhibitor activities (Dhanalakshmi and Manavalan, 2014). Hexadecanoic acid, methyl ester, a derivative of palmitic acid has been found to have antioxidant, hypo-cholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha reductase inhibitors.

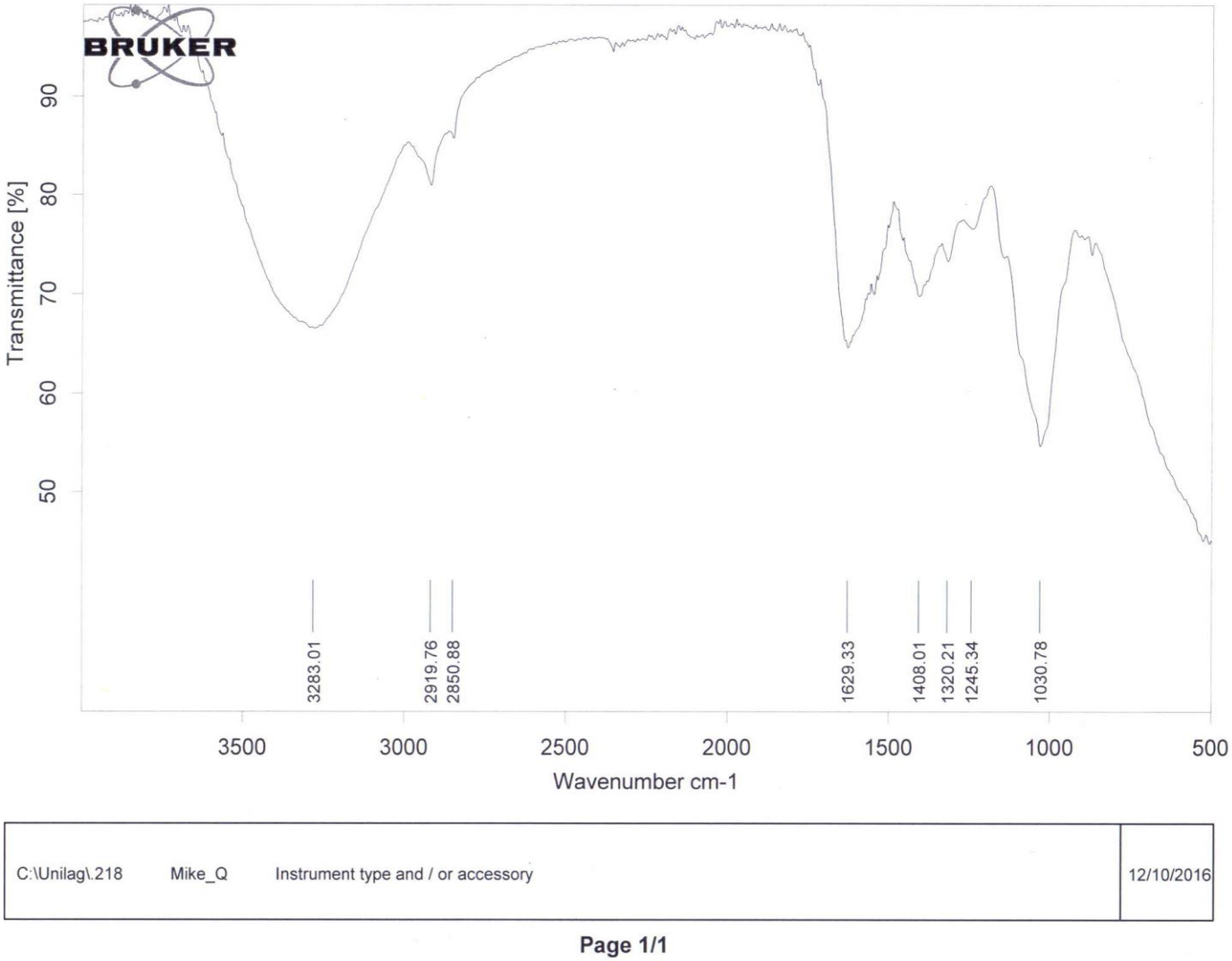

Figure 2. FTIR spectrum of methanolic extract of leaves of *T. diversifolia* (Hemsl.) A. Gray.

Table 2. FTIR analysis of methanolic extract of leaves of *T. diversifolia* (Hemsl.) A. Gray.

S/N	Wave number (cm^{-1})	Functional group
1	1018.92	C-O (Alcohols and phenols)
2	1243.43	O-C (carboxylic acids/derivatives)
3	1321.32	A-CH_3 bending (Aldehydes and ketones)
4	1404.11	CH_2 and CH_3 deformation (alkanes)
5	1598.62	N-H (1^0 Amines)
6	2850.16	CH_3, CH_2 and CH bands (Alkanes)
7	1918.51	CH_3, CH_2 and CH (2 or 3 bands) Alkanes
8	3283.33	O-H (H-bonded) (Alcohols and phenols)

Chandrasekaran et al. (2011) reported that hexadecanoic acid methyl ester has antibacterial and antifungal properties.

Ethylene oxide is used in healthcare industry for sterilization because of its non-damaging effects for delicate instruments and devices that require sterilization

and for wide range of material compatibility. Ethylene oxide is used as a medical sterilant. Harris et al. (1998) observed that ethylene oxide is used as an accelerator of maturation of tobacco leaves and fungicides. Bhattacharya et al. (2014) isolated cis-vaccenic acid from the ethanolic extract of *M. oleifera* leaf. Available data has shown that cis-vaccenic acid has hypolipidaemic and antihypertensive activities.

Octadecanoic acid has antibacterial, soap lubricant and cosmetic potentials. These biochemicals could be responsible for the *in-vivo* activities of the extract of *T. diversifolia* against the proliferation of microorganisms as evidenced in the study of Ogundare (2007), Ayewole et al. (2008) and Anthoney et al. (2015). Phenylephrine is marketed as an alternative for the decongestant pseudoephedrine and clinical trials have shown that phenylephrine, taken orally at the recommended dose, is effective in relieving allergy (Horak et al., 2008). Amphetamine possesses the following recorded pharmaceutical targets weight reduction cognitive enhancers, improvement of brain development and nerve growth (Heal et al., 2013).

The FT-IR analysis is an effective analytical technique for the identification of the functional groups resident in biological sample. The existence of the following functional groups: Alcohols, phenols, aldehydes, ketones, alkanes and primary amines could be responsible for the already established ethnomedicinal properties of *T. diversifolia* (Hemsl.) A. Gray.

Conclusion

The methanolic leaf extract of *T. diversifolia* (Hemsl.) A. Gray has been suggested to be a reservoir of bioactive constituents, which could be used in the treatment of various diseases in future. However, isolation, identification and characterization of individual compounds and their biological activities need to be further unveiled. Based on the current findings, it was therefore concluded that the leaves of *T. diversifolia* (Hemsl.) A. Gray contains bioactive compounds relevant to traditional and clinical medicinal practitioners and as such should be further screened.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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