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Analysis of Bioactive Chemical Compounds of Leaves Extracts from *Tamarindus indica* Using FT-IR and GC-MS Spectroscopy

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Authors' contributions

This work was carried out in collaboration among all authors. Author MAHM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MAHM, GMNO, AZAT and FYSA managed the analyses of the study. Author VP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Tamarindus indica is one of the medicinal plants used in the treatment of various diseases traditionally.
Aims: This study was conducted to identify the phytochemical constituents of *T. indica* leaf extracts.
Methods: Using Fourier-transform infrared spectroscopy (FT-IR) and gas chromatography-mass spectrometry (GC-MS) to identification of bioactive compounds in extracts of *T. indica*.
Results: The FT-IR spectrum confirmed the presence of alcohol group, alkene group, amine group, carbonates, ethers, carboxylic acid and disulfides in both extracts.

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A total of 22 and 38 bioactive phytochemical compounds were identified in the ethanolic and aqueous extracts of *T. indica,* respectively. The major bioactive compounds of the ethanolic extract of *T. indica* leaves were cis-Vaccenic acid, trans-13-Octadecenoic acid, Oleic Acid, Octadecanoic acid, Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester, Eicosanoic acid and Eicosane, 1-Iodo-2-methylundecane, 10-Methylnonadecane. While the major bioactive compounds of the aqueous extract were 3-O-Methyl-d-glucose, Myo-Inositol, 4C-methyl-, Myo-Inositol, 2-C-methyl-, Propane, 2,2-dimethoxy-, 1,3-Dioxolane, Ethanol, 2-(1-methylethoxy)-, and 2-Pentanone, 4-hydroxy-4-methyl-, 2-Hexanol, 2-methyl-, Ethanamine, N-methyl.

Keywords: Tamarindus indica; bioactive chemical compounds; FT-IR; GC-MS.

1. INTRODUCTION

The relationship between humans and plants started from the beginning of the emergence of humans on earth. Plants as a source of medicinal drugs have continued where they play an important role in the maintenance of human health since ancient times [1]. The world population depended on traditional remedies, especially plants, in treating many diseases, where there are about three-quarters of people are treated with traditional medicine [2]. India is the birthplace of the renewed system of indigenous medicine such as Ayurveda, Unani and Siddha [3]. Studies showed that traditional healers in India use 2500 plant species of which100 species are used which serves as regular sources of medicine [4].

The Fabaceae family, commonly is known as the legume, pea, or bean family comprises about 751 genera and 19000 species, which are widely distributed in the world, and the third-largest land plant family in number of species, behind only the Orchidaceae and Asteraceae [5]. They are a rich source of active secondary metabolites [6]. Within this family, the species *Tamarindus indica* and generally recognized as Tamarind and a large and evergreen tree may reach a height of 24 m and diameter 1-2 m [7]. The tamarind flowers are red, pale yellow, and pink. Flowers are present on small buds, where its wide 2.5 cm. The leaves are alternately arranged, reach a length of less than 5 cm. The leaves are bright green, ovular and pinnately veined in some tropical areas. The leaves are frost sensitive [7]. The fruits are thick pods that are between 4 -13 cm and usually arched. Each pod contains 1-10 seeds [8].

T. indica is found in Asia continent, Indian subcontinent and Africa continent also, it is found in high places up to 500 m that is from Burma to Afghanistan, also in woodlands and the arid and semiarid zones up to 47°C [9]. Tamarind has an assortment of the pharmacological action, and it utilized as a part of phytomedicine around the globe for its treating diarrhea [10], antiparasitic such as *Eisonia fatida, Pheretima posthuma, Ascaridia galli, Taenia solium,* and *Entamoeba histolytica* [11-13], *Echinococcus granulosus* [14], antibacterial, antifungal [15], antidiabetic and anticancer activity [16].

Higher plants are sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on the green plants to represent a reservoir of effective chemotherapeutics, which are non-phytotoxic, more systemic and easily biodegradable [11,12]. Plants are rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are important source with a variety of structural arrangements and properties [13]. However, the FT-IR has been used because of the presence of many compounds of secondary metabolites within the extracts. FT-IR is used for the screening of the extracts' constituents, where it is a simple technique and sensitive in evaluating the presence of functional groups which are present in extracts [17]. Where it proved that FT-IR spectroscopy is a reliable and sensitive method for detecting biomolecular composition which is present in plant extracts [18].

GC-MS analysis is a breakthrough in the analysis of phytoconstituents and structure elucidation of these compounds as they have a sensitivity of detecting compounds as low as 1ng [19]. Because of the development of chromatographic techniques like GC-MS, analysis has become easier in analyzing small amounts of chemicals [20].

Due to is lack of sufficient literature on the phytochemical profile and its pharmacological activity for this plant, the present study was

carried out to evaluate GC-MS and FT-IR analysis of extracting this plant in this study.

2. MATERIALS AND METHODS

2.1 Plant Material

T. indica leaves were collected from the campus of Dr. Rafiq Zakaria College for Women-Aurangabad. The leaves were washed under tap water. Then they were dried in the laboratory at room temperature (25-30°C) for two weeks. The leaves were grinded with the mechanical grinder until they became soft powder. The powder was kept in an airtight container to protect the powder from moisture and light.

2.2 Preparation of Plant Extracts

The ethanolic and aqueous extracts of *T. indica* leaves were prepared out using the Soxhlet apparatus for ethanolic extract and magnetic stirrer for aqueous extract as described by earlier researches [21-23].

2.3 FT-IR Analysis of *T. indica* Extracts

FTIR (Bruker, USA) was used for identifying functional groups and the types of chemical bonds that present in extracts. Dried powders of extracts of each plant material were used for FT-IR analysis. Where 10 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare the translucent disc (3 mm diameter). The powdered sample of each plant was loaded in FT-IR, with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.4 GC-MS Analysis of *T. indica* Extracts

GC-MS analysis of the ethanolic and aqueous extracts of *T. indica* leaves performed using Thermo Scientific Triple Quadrupole GC-MS (Trace 1300 GC, Tsq 8000 triple quadrupole MS) equipped with TG 5MS (30 m X 0.25 mm, 0.25 µm) column. Helium was used as the carrier gas at a flow rate of 1 ml/min. using an injection volume of 1.0 µL. Injector temperature was kept at 250^{o}C and ion source temperature was 230°C. The oven temperature was maintained at 50°C isothermal at 280°C, Mass Spectra transfer line temperature. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

3. RESULTS

3.1 Functional Group Analysis by FT-IR of Ethanolic Extract of *T. indica* Leaves

The absorption spectra of ethanolic extract for *T. indica* are shown in the region of 4000-400 cm^{-1} are 12 peaks are derived. The peaks represented the ranges from 3851.78 to 1454.36 peaks were shown in Table 1 and Fig. 1. The peaks at 3851.78, 3740.97 and 3672.72 cm^{-1} represent alcohol compounds. The peaks at 3614.59 and 2359.59 cm^{-1} represents amines. The peak at 2173.79 cm^{-1} represents Alkynes. The peak at 1916.82 cm^{-1} represents carbonyl compounds. The peak at 1743.34 cm^{-1} represents Carboxylic acids compounds. The peaks at 1696.11, 1650.16 and 1520.55 cm^{-1} represent alkenes. The peak at 1454.36 cm^{-1} represents aryl compounds. Interpretation of FT-IR spectra of the isolated compound of ethanolic extract for *T. indica* is presented in Table 1.

3.2 Functional Groups Analysis by FT-IR of Aqueous Extract of *T. indica* Leaves

The absorption spectra of the aqueous extracts of *T. indica* leaves were shown in Fig. 2, which appears the highest number of peaks (14). The peaks at 3815.62, 3741.26, 33672.89 and 3614.03 cm^{-1} represent alcohol. The peak at 2358.97 cm^{-1} represents amines. The peak at 2174.19 cm^{-1} represents alkynes. The peak at 1916.55 cm^{-1} represents carbonyl compounds. The peak at 1743.36 cm^{-1} represents carboxylic acids compounds. The peaks at 1696.09, 1649.97, 1520.63 and 1415.86 cm^{-1} represent alkenes. The peak at 1464.12 cm^{-1} represents aryl compounds. The peak at 1062.08 cm^{-1} represents alkyl-substituted ether compounds. Interpretation of FT-IR spectra of the isolated compound of ethanolic extract for *T. indica* is presented in Table 2.

3.3 GC-MS Analysis of *T. indica* Leaves Ethanolic Extract

Chromatogram GC-MS analysis of *T. indica* ethanolic leaves extract showed the presence of twenty-two peaks Fig. 3. The chemical compound, molecular formula and molecular weight were as shown in Table 3. The major phytochemical constituents were cis-Vaccenic acid, trans-13-Octadecenoic acid,

Oleic Acid, Octadecanoic acid, Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester, Eicosanoic acid, and Eicosane, 1-Iodo-2-methylundecane, 10-Methylnonadecane. Interpretation of GC-MS spectra of the identified compounds of ethanolic extract for *T. indica*. The detailed results are summarized in Table 3.

3.4 GC-MS Analysis of *T. indica* L. Leaves Aqueous Extract

GC-MS analysis of compounds was carried out in aqueous leaves extract of *T. indica* shown in Table 4. The GC-MS chromatogram of the 38 peaks of the compounds detected as shown in Fig 4. The major phytochemical constituents were 3-O-Methyl-d-glucose, Myo-Inositol, 4C-methyl-, Myo-Inositol, 2-C-methyl-, Propane, 2,2-dimethoxy-, 1,3-Dioxolane, Ethanol, 2-(1-methylethoxy)-, and 2-Pentanone, 4-hydroxy-4-methyl-, 2-Hexanol, 2-methyl-, Ethanamine, N-methyl-. Interpretation of GC-MS spectra of the identified compounds of aqueous extract for *T. indica*. The detailed results are summarized in Table 4.

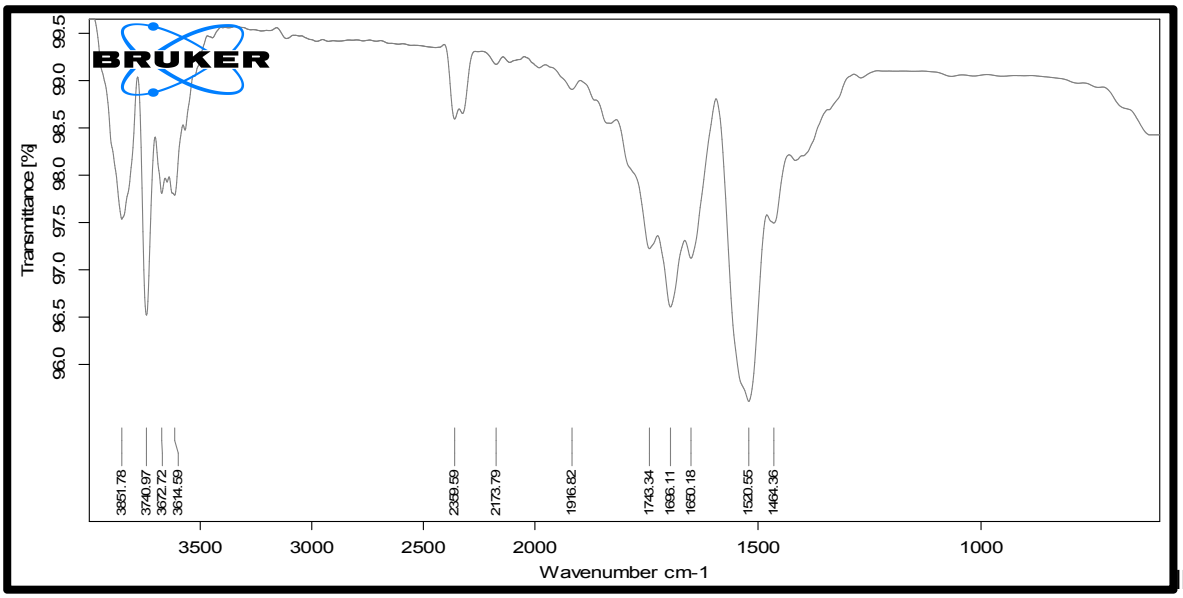

Fig. 1. FT-IR spectrum of ethanolic extract of *T. indica*

Table 1. FT-IR spectral peak values and functional groups obtained from ethanolic extract of *T. indica* leaves

No.	Peak values (cm^{-1})	Bond	Functional group
1.	3851.78	O-H	Alcohol
2.	3740.97	O-H	Alcohol
3.	3672.72	O-H	Alcohol
4.	3614.59	N-H	Aliphatic secondary amine
5.	2359.59	N-H	Amine
6.	2173.79	C≡C	Alkynes
7.	1916.82	C=O	Carbonyl group
8.	1743.34	COOH	Carboxylic acids
9.	1696.11	C=C	Alkene group
10.	1650.16	C=C	Alkene group
11.	1520.55	C=C	Alkene group
12.	1454.36	C=C-C	Aryl (Aromatic ring)

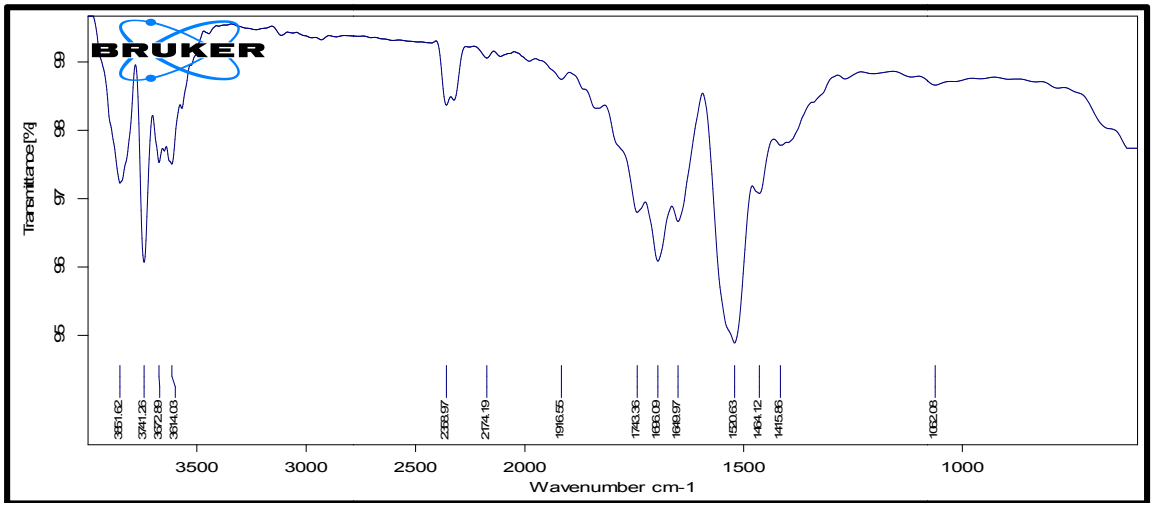

Fig. 2. FT-IR spectrum of aqueous extract of *T. indica*

Table 2. FT-IR spectral peak values and functional groups obtained from aqueous extract of *T. indica* leaves

No.	Peak values (cm^{-1})	Bond	Functional group
1.	3815.62	O-H	Alcohol
2.	3741.26	O-H	Alcohol
3.	33672.89	O-H	Alcohol
4.	3614.03	H-OH	Alcohol
5.	2358.97	N-H	Amine
6.	2174.19	C≡C	Alkynes
7.	1916.55	C=O	Carbonyl group
8.	1743.36	COOH	Carboxylic acids
9.	1696.09	C=C	Alkene group
10.	1649.97	C=C	Alkene group
11.	1520.63	C=C	Alkene group
12.	1464.12	C=C-C	Aryl (Aromatic ring)
13.	1415.86	C-H	Alkene
14.	1062.08	C-O-C	Alkyl-substituted ether

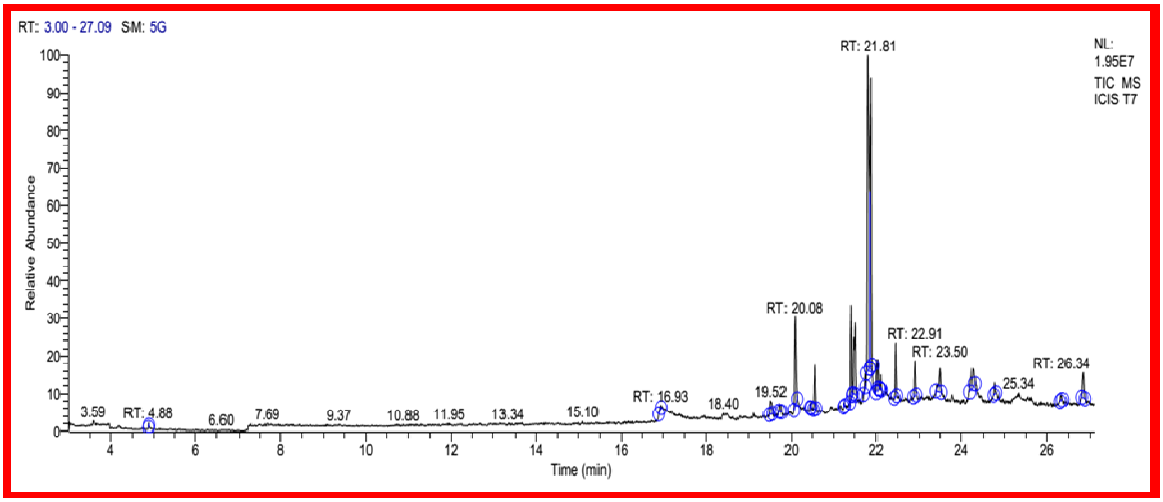

Fig. 3. GC-MS profile of ethanolic extract of *T. indica* leaves

Table 3. Gas chromatography-Mass spectrum analysis of bioactive components of ethanolic extracts of *T. indica* leaves

S No.	RT	Area (%)	Name of the compound	Molecular formula
1.	4.88	0.09	2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$
			2-Hexanol, 2-methyl-	$C_7H_{16}O$
			2-Pentanol, 2,4-dimethyl-	$C_7H_{16}O$
2.	16.93	0.37	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			à-D-Mannofuranoside, 1-O-decyl-	$C_{16}H_{32}O_6$
			L-Glucose	$C_6H_{12}O_6$
3.	19.52	1.65	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$
			Methoxyacetic acid, 2-tridecyl ester	$C_{16}H_{32}O_3$
			Eicosane, 10-methyl-	$C_{21}H_{44}$
4.	19.74	0.57	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]-, methyl ester	$C_{25}H_{42}O_2$
			Cyclopentanetridecanoic acid, methyl ester	$C_{19}H_{36}O_2$
			Tridecanoic acid, 4,8,12-trimethyl-, methyl ester	$C_{17}H_{34}O_2$
5.	20.08	7.4	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
			l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$
			Pentadecanoic acid	$C_{15}H_{30}O_2$
6.	20.45	0.23	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$
			Ethyl 14-methyl-hexadecanoate	$C_{19}H_{38}O_2$
			Ethyl hydrogen dodecanedioate	$C_{14}H_{26}O_4$
7.	20.55	2.9	Eicosane	$C_{20}H_{42}$
			Eicosane, 10-methyl-	$C_{21}H_{44}$
			Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$
8.	21.25	0.18	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3á,5Z,7E)-	$C_{27}H_{44}O_3$
			2-Bromotetradecanoic acid	$C_{14}H_{27}BrO_2$
			Corynan-17-ol, 18,19-didehydro-10-methoxy-,acetate (ester)	$C_{22}H_{28}N_2O_3$
9.	21.41	5.53	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$
			9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$
			Methyl 9-cis,11-trans-octadecadienoate	$C_{19}H_{34}O_2$
10.	21.51	7.34	Eicosane	$C_{20}H_{42}$
			1-Iodo-2-methylundecane	$C_{12}H_{25}I$
			10-Methylnonadecane	$C_{20}H_{42}$
11.	21.74	0.89	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$
			Cyclopropaneoctanoic acid,2-[[2-[(2-ethylcyclopropyl) methyl] cyclopropyl] methyl]-, methyl ester	$C_{22}H_{38}O_2$
			17-Octadecynoic acid	$C_{18}H_{32}O_2$
12.	21.81	35.23	cis-Vaccenic acid	$C_{18}H_{34}O_2$
			trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$
			Oleic Acid	$C_{18}H_{34}O_2$
13.	21.88	20.28	Octadecanoic acid	$C_{18}H_{36}O_2$
			Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	$C_{22}H_{44}O_4$
			Eicosanoic acid	$C_{20}H_{40}O_2$
14.	22.03	2.13	Octadecanoic acid	$C_{18}H_{36}O_2$
			9,12,15-Octadecatrienoic acid,2 [(trimethylsilyl)oxy]-1-[[(trimethylsilyl) oxy]methyl]ethyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$
			i-Propyl 16-methyl-heptadecanoate	$C_{21}H_{42}O_2$

S No.	RT	Area (%)	Name of the compound	Molecular formula
15.	22.09	0.61	6,9,12,15-Docosatetraenoic acid, methyl ester	$C_{23}H_{38}O_2$
			9-Octadecenoic acid (Z)-,2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{21}H_{40}O_4$
			cis-Vaccenic acid	$C_{18}H_{34}O_2$
16.	22.45	3.03	Methoxyacetic acid, 2-tridecyl ester	$C_{16}H_{32}O_3$
			Eicosane	$C_{20}H_{42}$
			Methoxyacetic acid, 3-tetradecyl ester	$C_{17}H_{34}O_3$
17.	22.91	2.57	Benzene, 1,1'-sulfonylbis[4-chloro-	$C_{12}H_8Cl_2O_2S$
			2,4'-Dichlorodiphenylsulfone	$C_{12}H_8Cl_2O_2S$
			di-n-Nonyl sulfide	$C_{18}H_{38}S$
18.	23.5	2.52	Aspidospermidin-17-ol,1-acetyl-19,21-epoxy-15,16-dimethoxy-	$C_{23}H_{30}N_2O_5$
			Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$
			1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$
19.	24.25	2.77	Cholestan-3-ol, 2-methylene-, (3á,5à)-	$C_{28}H_{48}O$
			1-Heptatriacotanol	$C_{37}H_{76}O$
			Isoaromadendrene epoxide	$C_{15}H_{24}O$
20.	24.76	0.64	Corynan-17-ol, 18,19-didehydro-10-methoxy-, acetate (ester)	$C_{22}H_{28}N_2O_3$
			6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$
			9,12,15-Octadecatrienoic acid,2,3-bis [(trimethylsilyl) oxy] propyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4Si_2$
21.	26.34	0.43	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$
			[2-(5-Hydroxypent-2-enyl)-3-oxocyclopentyl] thioac etic acid, S-t-butyl ester	$C_{16}H_{26}O_3S$
			i-Propyl 9-tetradecenoate	$C_{17}H_{32}O_2$
22.	26.84	2.62	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$
			Phthalic acid, di(oct-3-yl) ester	$C_{24}H_{38}O_4$
			Diisooctyl phthalate	$C_{24}H_{38}O_4$

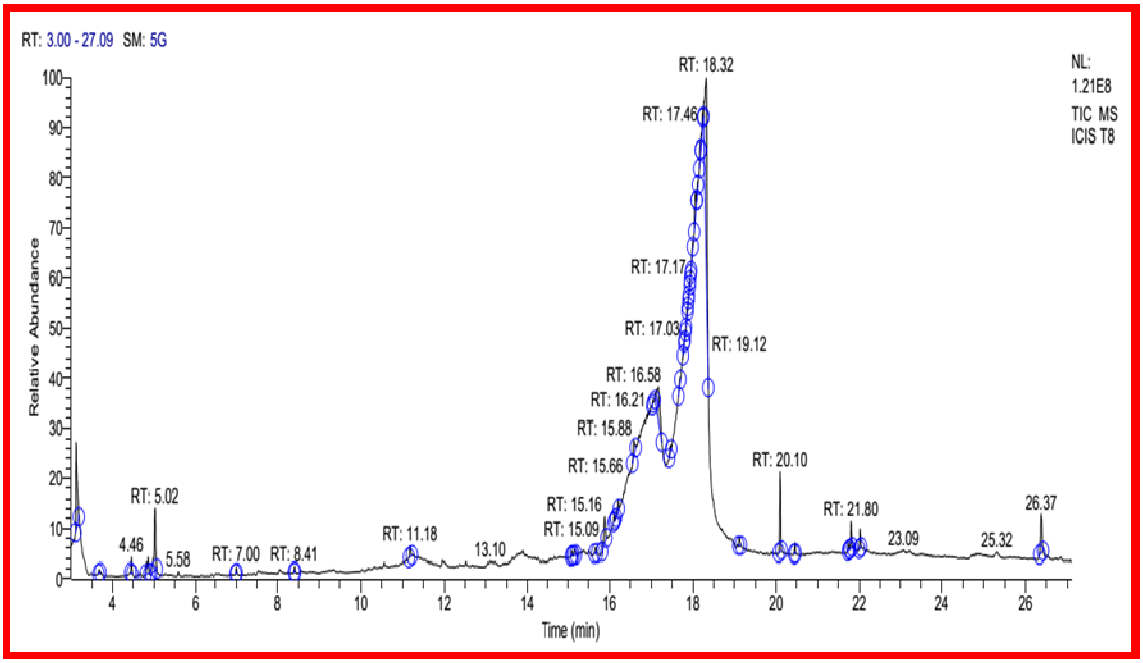

Fig. 4. GC-MS profile of aqueous extract of *T. indica* L. leaves

Table 4. Gas chromatography-mass spectrum analysis of bioactive components of the aqueous extracts of *T. indica* leaves

S.No	RT	Area (%)	Name of the compound	Molecular formula
1.	3.12	**8.93**	Propane, 2,2-dimethoxy-	$C_5H_{12}O_2$
			1,3-Dioxolane	$C_3H_6O_2$
			Ethanol, 2-(1-methylethoxy)-	$C_5H_{12}O_2$
2.	3.67	0.38	Silanediol, dimethyl-	$C_2H_8O_2Si$
			Trimethylsilyl fluoride	C_3H_9FSi
			1,5-Hexadiene, 3,3,4,4-tetrafluoro-	$C_6H_6F_4$
3.	4.46	1.10	3-Penten-2-one, 4-methyl-	$C_6H_{10}O$
			3-Hexen-2-one	$C_6H_{10}O$
			2,4-Azetidinedione, 3,3-diethyl-	$C_7H_{11}NO_2$
4.	4.85	2.24	Furfural	$C_5H_4O_2$
			3-Furaldehyde	$C_5H_4O_2$
			3,5-Dimethylpyrazole	$C_5H_8N_2$
5.	5.02	**7.87**	2-Pentanone, 4-hydroxy-4-methyl-	$C_6H_{12}O_2$
			2-Hexanol, 2-methyl-	$C_7H_{16}O$
			Ethanamine, N-methyl-	C_3H_9N
6.	7.00	0.38	2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$
			Pyrazole-4-carboxaldehyde, 1-methyl-	$C_5H_6N_2O$
			3,4-Furandimethanol	$C_6H_8O_3$
7.	8.41	0.34	Benzeneacetaldehyde	C_8H_8O
			2,4,6-Cycloheptatrien-1-one, 4-methyl-	C_8H_8O
			4,5-Dihydro-2(1H)-pentalenone	C_8H_8O
8.	11.18	0.95	6-Acetyl-á-d-mannose	$C_8H_{14}O_7$
			12,15-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$
			1-Hexen-3-ol, 5-nitro-1-phenyl-, (R*,R*)-	$C_{12}H_{15}NO_3$
9.	15.09	0.22	2H-Indeno[1,2-b]furan-2-one ,3,3a, 4,5, 6,7, 8,8b-octahydro-8,8-dimethyl	$C_{13}H_{18}O_2$
			1,3-Dithiane, 2-[4-(1-ethoxyethoxy)butyl]	$C_{12}H_{24}O_2S_2$
			1-(3-Cyano-4,5,6,7-tetrahydro-2-benzo [b] thienyl)-3-(3,4-dimethoxycinnamoyl)-2-thiourea	$C_{21}H_{21}N_3O_3S_2$
10.	15.16	0.57	Malonodinitrile, 2-(5-dimethylaminopenta-2,4-dienylideno)-	$C_{10}H_{11}N_3$
			1-tert-Butyl-3-(3-methoxyphenyl)-bicyclo [1.1.1] pentan	$C_{16}H_{22}O$
			Oct-3-ene-1,5-diyne, 3-t-butyl-7,7-dimethyl-	$C_{14}H_{20}$
11.	15.66	0.76	1-Fluoro-1-hex-1-ynyl-2,2-dimethyl-cyclopropane	$C_{11}H_{17}F$
			2-Cyclohexen-1-one,2-hydroxy-6-methyl-3-(1-methylethyl)-	$C_{10}H_{16}O_3$
			2-Cyclohexen-1-one,2-hydroxy-6-methyl-3-(1-methylethyl)-	$C_{10}H_{16}O_2$
12.	15.88	3.79	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			2-Acetylamino-3-hydroxy-propionic acid	$C_5H_9NO_4$
			6-Ethoxy-6-methyl-2-cyclohexenone	$C_9H_{14}O_2$
13.	16.11	0.47	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			à-Methyl mannofuranoside	$C_7H_{14}O_6$
			3-Methylmannoside	$C_7H_{14}O_6$
14.	16.21	1.10	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			à-Methyl mannofuranoside	$C_7H_{14}O_6$
			3-Methylmannoside	$C_7H_{14}O_6$
15.	16.58	2.10	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$
			à-d-Mannofuranoside, methyl	$C_7H_{14}O_6$

S.No	RT	Area (%)	Name of the compound	Molecular formula
16.	17.03	0.13	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$
			Myo-Inositol, 2-C-methyl-	$C_7H_{14}O_6$
17.	17.17	**9.54**	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$
			Myo-Inositol, 2-C-methyl-	$C_7H_{14}O_6$
18.	17.46	0.80	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$
			á-d-Mannofuranoside, methyl	$C_7H_{14}O_6$
19.	17.68	0.62	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$
			Myo-Inositol, 2-C-methyl-	$C_7H_{14}O_6$
20.	17.78	0.35	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$
			Myo-Inositol, 2-C-methyl-	$C_7H_{14}O_6$
21.	17.82	0.23	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$
			Myo-Inositol, 2-C-methyl-	$C_7H_{14}O_6$
22.	17.86	0.52	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$
			Myo-Inositol, 2-C-methyl-	$C_7H_{14}O_6$
23.	17.89	0.12	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$
			Myo-Inositol, 2-C-methyl-	$C_7H_{14}O_6$
24.	17.92	0.23	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$
			Myo-Inositol, 2-C-methyl-	$C_7H_{14}O_6$
25.	17.94	0.14	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$
			Myo-Inositol, 2-C-methyl-	$C_7H_{14}O_6$
26.	17.99	0.86	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$
			Myo-Inositol, 2-C-methyl-	$C_7H_{14}O_6$
27.	18.06	2.44	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$
			Myo-Inositol, 2-C-methyl-	$C_7H_{14}O_6$
28.	18.11	0.52	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$
			Myo-Inositol, 2-C-methyl-	$C_7H_{14}O_6$
29.	18.17	1.13	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$
			Myo-Inositol, 2-C-methyl-	$C_7H_{14}O_6$
30.	18.23	1.79	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$
			Myo-Inositol, 2-C-methyl-	$C_7H_{14}O_6$
31.	18.32	**33.55**	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			Myo-Inositol, 4C-methyl-	$C_7H_{14}O_6$
			Myo-Inositol, 2-C-methyl-	$C_7H_{14}O_6$
32.	19.12	0.82	3-O-Methyl-d-glucose	$C_7H_{14}O_6$
			d-Mannose	$C_6H_{12}O_6$
			d-Glycero-l-gluco-heptose	$C_7H_{14}O_7$
33.	20.10	5.84	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
			l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$
			Pentadecanoic acid	$C_{15}H_{30}O_2$

S.No	RT	Area (%)	Name of the compound	Molecular formula
34.	20.45	0.71	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$
			Methyl 3-methyl-pentadecanoate	$C_{17}H_{34}O_2$
			Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$
35.	21.74	0.41	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	$C_{21}H_{38}O_2$
			13,16-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$
			13-Tetradecynoic acid, methyl ester	$C_{15}H_{26}O_2$
36.	21.80	1.95	cis-Vaccenic acid	$C_{18}H_{34}O_2$
			trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$
			Oleic Acid	$C_{18}H_{34}O_2$
37.	22.01	1.38	Octadecanoic acid	$C_{18}H_{36}O_2$
			Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	$C_{22}H_{44}O_4$
			Eicosanoic acid	$C_{20}H_{40}O_2$
38.	26.37	4.72	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$
			Glycerol 1-palmitate	$C_{19}H_{38}O_4$
			Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$

4. DISCUSSION

4.1 Functional Group Analysis by FT-IR of Extracts of *T. indica* Leaves

FT-IR analysis of ethanolic and aqueous extracts of *T. indica* leaves revealed that functional group components of alcohols, amino acids, carboxylic acid, alkanes, alkenes, organic hydrocarbons, aryl (Aromatic ring), thiols group and ethers compounds. Correa et al. [24] analyzed the aqueous leaf extract of *T. indica* by FT-IR and reported that the aromatic ring compounds, alkenes, amines, alcohols and alkyl halides were only present in the extract.

Some aliphatic amines have entered the manufacture of safe drugs, which are used as effective anti-malarial drugs [25]. Also, alkanes and alkenes had antimicrobial effects, and the carboxilic acids are used as antioxidants [26].

4.2 GC-MS Analysis of *T. indica* Leaves Ethanolic Extract

The study on the active principles of *T. indica* leaves flowers by GC-MS analysis exhibited the presence of twenty-two major peaks in the ethanolic extract. The prevailing compounds and present in higher amounts were Pyrene and Fluoranthene in the extract. While the compounds found in smaller amount were 2-Pentanone, 4-hydroxy-4-methyl- and 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3á, 5Z,7E)-. Some of these compounds identified through GC-MS are known to have many important biological and formulation functions, such as 2-Pentanone, 4-hydroxy-4-methyl- is used as antimicrobial [27]. 3-O-Methyl-d-glucose is used as anti-cancer and anti-inflammatory [28]. Tetradecane, 2,6,10-trimethyl- is used as antifungal, antibacterial and nematicidal [29]. n-Hexadecanoic acid is used as antimicrobial and antioxidant [30]. Eicosane has the antibacterial activity as reported by earlier workers [31]. Octadecanoic acid act as antimicrobial, anti-inflammatory, hepatoprotective and nematicide [32]. Cholestan-3-ol, 2-methylene-, (3á,5à)- have activities such as anti-inflammatory and cytotoxic activities [33]. Corynan-17-ol, 18,19-didehydro-10-methoxy-, acetate (ester) is used as anti-diarrhoeal activity [34]. Dasycarpidan-1-methanol, acetate (ester) has pharmacological activity such as antimicrobial, antioxidant and anti-inflammator [28]. Diisooctyl phthalate act as antimicrobial, antifungal, antiviral and antioxidant activities [27].

4.3 GC-MS Analysis of *T. indica* Leaves Aqueous Extract

The GC-MS analysis of a crude aqueous extract of *T. indica* showed the presence of twenty-six (26) different compounds in it. Some of which have never been reported as constituents of *T. indica*. The prevailing compounds and present in higher amounts were Myo-Inositol, 2-C-methyl-, Propane, 2,2-dimethoxy-, 2-Pentanone, 4-hydroxy-4-methyl- present in the extract. While the compounds which found in smaller amount were 1,3-Dithiane, 2-[4-(1-ethoxyethoxy) butyl], Benzeneacetaldehyde. Most of the phytochemicals mentioned in Table 4. had pharmacological action and many important

biologicals such as furfural act as anti-inflammatory activity and analgesic activity [28]. 12,15-Octadecadiynoic acid, methyl ester act as anti-inflammatory [32]. n-Hexadecanoic acid acts as antioxidant and anti-inflammatory [35]. cis-Vaccenic acid act as anti-asthmatic, anti-inflammatory and lowers total cholesterol and triglycerides levels [28,36]. Octadecanoic acid is used hypercholesterolemic, antiarthiritic, anti-inflammatory, hepatoprotective, nematicide and antimicrobial [28]. Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl)ethyl ester act as antioxidant [29].

5. CONCLUSION

The results of this study revealed that extracts of *T. indica* contains pharmacologically active substances with antimicrobial, anti-inflammatory, and antioxidant activity. Therefore, the crude extracts of *T. indica* leaf could be new sources of development of new plant-based therapy for management of several diseases.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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