

Phytochemical Analysis, Identification Of Bioactive Compounds Using FTIR, GC-MS And Evaluation Of Antioxidant Potential From (Hoom) Miliusa Tomentosa

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Abstract:

Plant derived biologically active compounds have gained importance in food and pharmaceutical industries. The present study aimed to explore the bioactive compounds and their antioxidant and antimicrobial properties in the crude extract of *Miliusa tomentosa*. To identify the active components, the extract was deconvoluted using a successive extraction process with hexane, ethyl acetate and chloroform to isolate the active fraction. The chloroform extract demonstrated the highest inhibitory activity and was selected for further phytochemical investigations. Further, the crude extract was subjected to column FTIR and GC-MS to determine the bioactive compounds. γ -Sitosterol, 1-Heptatriacotanol, 4 hydroxyl phenylacetic acid 7-Tetradecenal, n-Hexadecanoic acids were identified as potential compounds. The extracts were also assessed for their antioxidant activity using DPPH, ABTS and FRAP assays, it is concluded that the phytochemicals were revealed that *Miliusa tomentosa* is highly valuable in medicinal usage for the treatment of various human ailments.

INTRODUCTION

Miliusa plants are widely distributed in tropical and subtropical regions, particularly Asia and has been employed in folk medicines. The plants belonging to family Annonaceae are used as antibacterial, consists about 40 species which grows in tropical rainforest of India, Thailand, South China and North Australia etc., The different species of *Miliusa* are invariably small to large trees and are found in a wide range of rainforest communities. The plant is used in folk medicine for different symptom such as gastropathy and glomerulo nephropathy (Son et al., 2019). In Chinese traditional medicine *Miliusa tomentosa* oil has been found to have both antibacterial and analgesic properties. Secondary metabolites from medicinal plants of genus *Miliusa* are renowned for traditional uses and pharmaceutical potentials (Kumar & Shukla 2023). The advances in analytical techniques, including GC-MS and FT-IR that were powerful tools for identification and determination of phytochemicals compounds. Hence, a rising interest has been in developing novel herbal medicines to treat hyperglycemia and oxidative stress (Pereira and Valado, 2023). Herbal medicines are often observed to harness the efficacy of natural compounds with slighter side effects, providing a complementary or an alternative strategy towards oxidative stress and glycaemic control (Vivó-Barrachina et al., 2022). Many herbal plants possess phenolic compounds, flavonoids, terpenoids, glycosides, carotenoids, and alkaloids, known to have hypoglycemic and antioxidant properties (Pang et al., 2019). However, there have not been specific research papers to assess' bioactive compounds from *Miliusa tomentosa* and its therapeutic applications to assess the value of this genus, to the best of our knowledge. Therefore, the present study analysed the phytochemical composition of different solvent extracts of leaf by FTIR and GC-MS and assessed the hypoglycemic and antioxidant potential of the chloroform extracts.

MATERIALS AND METHODS

Collection of Plant Material

Naturally grown healthy leaves and bark of the plant *Miliusa tomentosa* was collected at Umbalebailu, a deciduous forest of Bhadra Wildlife Sanctuary, Shimogga, Karnataka, India in August 2021. A sample specimen of the plant was deposited at the herbarium of the Department of Studies in Botany, University of Mysore, Mysore, India. Plant materials were collected in sterile polythene bags and processed within 12 hours

at the laboratory. The plant was botanically identified and authenticated by Dr. P. Sharanappa, Department of Bioscience, Hemangothry, P. G Centre, Hassan, University of Mysore, Karnataka, India

Preparation of extracts

The leaves of *Miliusa tomentosa* were thoroughly washed with distilled water and subsequently dried under shade at room temperature and coarsely ground to a powder with a functional mechanical grinder. The plant powder was extracted using the maceration procedure using five different solvents: hexane, ethyl acetate, chloroform, ethanol, methanol, and water. 100 g of the plant powder was mixed with 1,000 mL of the solvents separately for 12 h at 600 rpm in a magnetic stirrer. The prepared extracts were then filtered using a Whatman filter paper No. 1 and concentrated at 45°C with a reduced pressure using a rotary evaporator (Tarsons Rockyvac, 300). Further, the sticky concentrate was lyophilized with subsequent storage at 4° C. The following equation was used to calculate the percentage of yield of different solvent extracts of *Miliusa tomentosa* leaves:

Qualitative phytochemical analysis

The presence of carbohydrates, proteins, reducing sugars, saponins, anthraquinones, phenolics, tannins, flavonoids, terpenoids, glycosides, anthocyanins, resins, sterols, alkaloids and anthraquinones in all the extracts were determined through qualitative phytochemical analysis using standard procedures (Oscar et al., 2020).

FTIR analysis

The characteristic peaks and functional groups of the crude extracts (1 mg/mL; dissolved in analytical grade chloroform) were identified through fourier transform infrared spectroscopy (Nicolet iS50, Thermo Scientific, United States) at a resolution of 4 cm⁻¹ and a frequency range of 4,000–500 cm⁻¹. The recorded FTIR peak values were used for analysis (Popescu et al., 2022).

Identification of compounds by GC-MS technique

Gas chromatography-mass spectrometry (GC-MS) analysis of chloroform extracts of the leaves of *Miliusa tomentosa* was carried out to identify the compounds using the GC-MS technique (PerkinElmer Clarus 680/600). A capillary column setup (20 m in length × 0.18 mm in diameter, 1.00 µm in thickness of film) was used to separate the compounds. Helium was employed at a steady rate of 36.3 cm/sec as a carrier gas. The concentrated extract was dissolved in analytical-grade ethyl acetate at 1 mg/mL concentration and filtered using a 0.45 µm syringe filter. 2 µL of the extract was injected via an auto-injector into GC mod coupled with MS mod. The GC oven temperature was transitioned from 200°C to 150°C at 4° C per minute, maintaining the temperature constant for 5 min. The ion source's temperature in the MS and the interface temperature remained constant at 230° C and 280° C, respectively. TurboMass software was used to collect the data, and the National Institute of Standards and Technology (NIST) Library 2005 was used to identify the compounds (Jayaraj et al., 2023).

Evaluation of antihyperglycemic activity

α-amylase assay

Six varying concentrations (100, 200, 300, 400, 500, 600 µg/mL) of the extracts and the standard drug acarbose were prepared in DMSO. 1 mL of the extracts and acarbose was added with 1 mL of 0.5 mg/mL α-amylase dissolved in sodium phosphate buffer of 0.02 M, pH 6.9 with 0.006 M NaCl, and then incubated at 25°C for 10 min. Subsequently, 500 µL of 1% potato starch solution (substrate) was mixed with it, and the mixture was incubated at 25°C for 10 min. The reaction was terminated by mixing with 1 mL of 1% di nitro salicylic acid reagent. After incubating in a water bath at 100°C for 5 min, the mixture was cooled to room temperature. Then, the reaction mixture was diluted to 10 mL with sodium phosphate buffer of 0.02 M, pH 6.9 with 0.006 M NaCl. Absorbance was measured at 540 nm (Roy and Mahalingam, 2017).

α-glucosidase assay

Six varying concentrations (100, 200, 300, 400, 500, 600 µg/mL) of extracts and the standard drug acarbose were prepared in DMSO. 1 mL of the extracts and acarbose was mixed with an equal amount of 0.1 unit α-glucosidase. The reaction was initiated by mixing with 500 µL of 3 mM p-nitrophenyl glucopyranoside (substrate). The reaction proceeded at 37°C for 25 min. The reaction was terminated by mixing with 1 mL of 0.02 M Na₂CO₃, and the mixture was incubated at 25°C for 10 min. Subsequently, the reaction mixture was

diluted to 10 mL with 0.02 M sodium phosphate buffer of pH 6.9 with 0.006 M NaCl. By measuring the release of p-nitrophenol from p-nitrophenyl glucopyranoside, the inhibitory activity of α -glucosidase was assessed at 405 nm (Roy and Mahalingam, 2017).

DPPH radical scavenging assay

DPPH shows absorption at 517 nm in its radical state. However, when it undergoes reduction due to an antioxidant or a radical species, its absorption decreases. Concisely, six different concentrations (100, 200, 300, 400, 500, 600 $\mu\text{g/mL}$) of extracts and the standard (ascorbic acid) were prepared in DMSO. 1 mL of the extracts and ascorbic acid was mixed with 1 mL of the 25 $\mu\text{g/mL}$ DPPH solution in ethanol. The mixture was incubated at 25°C for 40 min in the dark. Then, the absorbance was measured at 517 nm (Fu et al., 2021). Decreased absorbance of the reaction mixture suggests higher free radical scavenging activity. The subsequent equation was used to calculate the DPPH radical scavenging activity:

ABTS radical scavenging assay

In this assay, the antioxidant activity was assessed by measuring the reduction of blue-green ABTS radical. Concisely, ABTS (7 mM/L) with potassium persulfate (2.45 mM/L) was dissolved in 5 mL of distilled water. The solution was incubated at 25° C in dark condition for 12–16 h. Then the ABTS solution was diluted with ethanol (1:89 v/v). 2 mL of the formed ABTS solution was added with 20 μL of extracts and standard (ascorbic acid) of different concentrations (100, 200, 300, 400, 500, 600 $\mu\text{g/mL}$) dissolved in DMSO. The mixture was incubated at 25°C in a dark condition for 30 min. The absorbance was read at 734 nm (Senthilkumar et al., 2013).

Reducing power assay

This assay relies on reducing Fe^{3+} ions in $\text{K}_3[\text{Fe}(\text{CN})_6]$ to Fe^{2+} ions in the presence of antioxidants under acidic conditions. A higher absorbance value indicates a higher reduction potential. Concisely, 1 mL of the extracts and standard (ascorbic acid) of different concentrations (100, 200, 300, 400, 500, 600 $\mu\text{g/mL}$) prepared in DMSO was added with 2.5 mL of phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL of 1% $\text{K}_3[\text{Fe}(\text{CN})_6]$. The mixture was incubated in the water bath at 50°C for 20 min. After the mixture was cooled to room temperature, 2.5 mL of 10% CCl_3COOH was added. Then, the mixture was centrifuged at 6,000 rpm for 10 min at room temperature. 2.5 mL of supernatant was collected and added with distilled water (2.5 mL) and 0.1% FeCl_3 (0.5 mL). The reaction mixture was incubated for 10 min at 25° C. The absorbance was read at 700 nm (Fu et al., 2021).

Statistical analysis

Data was represented as mean $\pm$ SD. One-way analysis of variance and JMP Pro 17 were employed for the statistical analysis. p values less than 0.05 were deemed statistically significant.

RESULTS AND DISCUSSION

Plants are very important source of potentially useful bioactive principles for the development of new chemotherapeutic agents. (Tona et al., 1998). The biological and pharmacological properties of many plants are still unknown. World over, the scientists are exploring the potential of utilizing pharmacologically active compounds from medicinal plants (Karmegam et al., 2012). Herbal medicines are used by 80% of the people worldwide due to its high efficiency, cheap cost, nonnarcotic nature and fewer side effects (Ahmad et al., 2001). In the present study, the exploration of phytochemical screening with different solvent systems (Table-1), However, greater yield was obtained in chloroform extract of *Miliusa tomentosa*, the results showed the presence of carbohydrate, flavonoid, steroid, phenol, alkaloid, tannin, amino acid, terpenoid and glycoside compounds which are known to have remedial activity against diseases producing pathogen (Table 2). Therefore, it can be used pharmacologically to develop new compounds for health benefit. The compounds identified in the crude extracts are mostly belongs to terpenes, alcohols, hydrocarbons, alkaloids and its derivatives. From the literature search, these compounds are found to be known for their therapeutic properties and are previously reported in many different medicinal plants. Some of these compounds are separately isolated in extracts and are used as antimicrobial and radical scavenging agents in medicine formulations (Pakkirisamy et al., 2017). The crude extracts from the *Miliusa tomentosa* leaves were subjected

for GCMS analysis for identifying compounds. The GC-MS chromatogram of the chloroform extract showed 29 peaks representing different phyto-constituents. (Fig-1) Identification of these phyto-constituents was achieved by comparing their retention time (RT), peak area, peak height, and mass fragmentation spectra with known compounds in the National Institute of Standards and Technology (NIST) library (Table-3). GC-MS analysis of compounds with totally, twenty-nine major chemical compounds were identified, such as 2,4-Di-tert-butylphenol, neophytadiene, 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester, n-Hexadecanoic acid, (9E,11E)-Octadecadienoic acid, 7-Tetradecenal, E, E-3,13-Octadecadien-1-ol Octadecanoic acid, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester, 7-Hexadecenal(Z)-2-((2R,4aR,8aS)-4a-Methyl-8-methylenedecahydronaphthalen-2-yl) acryl aldehyde, 1-Heptatriacotanol, gamma sitosterol Stigmasta-5,24(28)-dien-3-ol, (3. beta.,24Z)- Mahanine 5-Thio-D-glucose, 5-Allylsulfanyl-1-(4-methoxy-phenyl)-1H-tetrazole, E)-10-Heptadecen-8-ynoic acid methyl ester and Z-11-Hexadecenoic acid. The compounds identified in *Milium tomentosum* and leaf chloroform extracts are listed according to their column elution time. The FT-IR analysis of the Chloroform extract of *Milium tomentosum* revealed distinctive peaks indicative of various functional groups: C-H stretching of alkane (2925.44/cm), O-H stretching of alcohol (2853.79/cm), C=O stretching of aliphatic ketone (1783.93/cm), C-H bending of alkane-methyl group (1438.40/cm), CO stretching of ester (1172.72/cm), C=C bending of alkene (970.19/cm), and C=C bending of alkene (725.56/cm) in the extract. FTIR spectra are given in (Fig. 2) and the functional groups identified are given in (Table-4). The extract of *Milium tomentosum* leaf shows one characteristic peak at 3351.676 cm⁻¹, representing the O-H group's stretching vibration. This may indicate the presence of phenolics or phytosterols. Stretching vibrations of the C-H group were detected at 2915.842 and 2849.31 cm⁻¹. The C $\equiv$ C group's stretching vibrations were detected at 2359.278 cm⁻¹. The stretching of aldehydes was indicated at 1707.658 cm⁻¹. The stretching of nitro compounds was detected at 1,569.772 cm⁻¹. The peak at 1438.814 cm⁻¹ represented the bending vibrations of amines and amides, suggesting the presence of amino acids or proteins. The peaks at 1,379.818 and 889.0233 cm⁻¹ indicated the bending of alkenes. The peak at 719.3182 cm⁻¹ represented the bending vibrations of aromatic rings. The unique peaks of different functional groups confirmed the presence of phenols, flavonoids, aldehydes, and proteins. The ameliorative properties of antioxidants, antidiabetics, etc., are attributed to the O-H group (Badmus et al., 2020; Azeem et al., 2023).

In vitro antioxidant potential of and inhibitory effects on α -amylase and α -glucosidase in vitro

The effectiveness of plant extracts as antioxidants due to their redox properties and it is attributed by donating a hydrogen atom or by inhibiting peroxide formation. The antioxidant potential of chloroform extracts of *Milium tomentosum* leaf was evaluated using DPPH, ABTS, and FRAP assays, and the results are given in (Table-6,7; Fig 3 and 4). DPPH assay has been commonly employed to assess the antioxidant activity of plant extracts by scavenging free radicals, which results in a color change from purple to yellow (Fu et al., 2021; Globularia et al., 2021). Chloroform extracts of *Milium tomentosum* leaf exhibited effective DPPH scavenging activity in a concentration-dependent manner 86.46% $\pm$ 1.45% at the concentration of 600 μ M. Similarly, ABTS assay is frequently employed to assess the total antioxidant activity of plant extracts (Extracts et al., 2018; Tiji et al., 2021). The total antioxidant capacity of the extracts was assessed by eliminating ABTS free radicals, which resulted in a colour change from green/ blue to yellow. The extracts' concentration and ability to scavenge free radicals in ABTS exhibit a dose-dependent relationship. The ABTS scavenging activity increases from 48.64% $\pm$ 0.99% to 76.73% $\pm$ 1.35% and 41.54% $\pm$ 1.42% to 68.64% $\pm$ 0.99%, respectively, when its concentration ranges from 100–600 μ M. Hence, the ABTS free radical scavenging effects of leaf depends on concentration. The capacity to function as a reductant is a significant factor in assessing antioxidant activity (Fu et al., 2021). The extracts of the leaf showed the ability to reduce the ferric ion complex, tripyridyltriazine (TPTZ), i.e., the reduction of the ferricyanide complex to ferrocyanide, which forms an intense blue Fe²⁺ - TPTZ complex. There is a strong correlation between the extracts' reducing ability and concentration. With the increase of concentration of the chloroform extracts of *Milium tomentosum* (100–600 μ M), the reducing power increases from 0.96 $\pm$ 0.03 to 2.24 $\pm$ 0.02 and 0.28 $\pm$ 0.04 to 1.65 $\pm$ 0.03 respectively. Our results attributed to the presence of fatty acids, 9(E), 11(E)-Conjugated linoleic acid and n-Hexadecanoic acid, Mahanine and its derivatives etc., and were identified as major compounds of the extracts through

GCMS analysis (Saha and Ghosh, 2012; Singh et al., 2022; Ganesan et al., 2024). Reduced postprandial hyperglycemia is accomplished by inhibiting α -amylase and α -glucosidase. Currently, the pharmaceutical sector provides various antidiabetic medications to treat diabetic patients, but their side effects are exceedingly hazardous. To address this issue, herbal therapies that naturally metabolize sugars without causing negative effects (Benayad et al., 2021) The potential antidiabetic efficacy of chloroform extracts of *Milium tomentosum*, was revealed in this study by examining their inhibitory effects on α -amylase and α -glucosidase in vitro (Fig 5 and 6). The inhibitory activities against intestinal α -glucosidase and pancreatic α -amylase, which are rich in phenolic and flavonoids, have significant antidiabetic potential and can manage postprandial hyperglycemia. Furthermore, our research not only confirms the existence of previously known molecules and adds new phytochemicals, but it also sheds light on the wide application of *Milium tomentosum* for possible bio-prospecting for a new chemical entity.

CONCLUSIONS

In summary, for the first time we are reporting in vitro leaf chloroform extract of *Milium tomentosum* showed maximum number of bioactive compounds from FTIR and GCMS analysis. Heptadecanoic acid and dodecanoic acid were identified as potential compounds. The extract also exhibited promising reducing power capability and scavenging activity against DPPH and ABTS free radicals The Chloroform extract of *Milium tomentosum* reduced significantly both α -amylase and α -glucosidase activities and a protective property against protein glycation. The present results concluded that the phytochemicals were observed in chloroform extract which revealed that the *Milium tomentosum*, is potential use in different fields namely medical and pharmaceuticals and highly valuable in medicinal usage for the treatment of various human ailments.

Table 1 Percentage of the yield of various solvent extracts of *Milium tomentosum* leaves.

Solvent system	Leaf extract yield (%)
Hexane	2.53 $\pm$ 0.32
Ethyl acetate	4.25 $\pm$ 0.44
Chloroform	4.84 $\pm$ 0.37
Methanol	1.82 $\pm$ 0.26
Water	7.88 $\pm$ 0.33

Note: The values are represented as the standard deviation of the mean (means $\pm$ SD), n = 3.

Table-2 Phytochemical screening of plant in different solvents

Sl no	Phytochemical tests	Hexane	Chloroform	Ethyle acetate	Methanol
1	Test for Carbohydrates				
	Molisch's Test	+	+	-	+
	Benedicts Test	+	+	-	+
2	Test for Protein				
	Ninhydrin Test	-	-	-	-
	Biuret test	-	-	-	-
3	Tests for Phenols				
	Ferric chloride test	-	+	-	+
	Lead acetate test	-	-	-	+
4	Tests for Tannins				
	Braymer's test	-	+	-	+
	10 % NaOH	-	-	-	+
	Lead acetate test	-	-	-	+
5	Test for flavonoids				

	Alkaline reagent test NaOH	-	-	-	+
	Lead acetate test	-	-	-	+
	Alkaline reagent test NH ₄ OH	-	-	+	+
6	Test for Saponins	-	-	-	+
7	Test for Glycosides				
	Kellarskillani test	+	+	+	+
8	Test for Terpenoids	+	+	+	+
9	Alkaloid test				
	Mayers test	-	-	-	+
	Wagners test	-	-	-	+
	Hagers test	-	-	-	+
10	Test for Polyphenols	-	-	-	+
11	Test for Phytosterols	-	+	-	+
12	Test for Steroids	-	-	-	+
13	Test for Emoids	-	-	-	-
14	Test for Quinones	-	-	-	-
15	Test for Anthocyanins	-	-	-	-

Table-3 Antioxidant activity methanol and chloroform extract of *Miliusa tomentosa*

Concentration (mg/ml)	Radical Scavenging activity (%)		
	α -tocopherol	Methanol extract	Chloroform extract
20	17.16 $\pm$ 0.76	65.56 $\pm$ 0.51*	71.6 $\pm$ 0.72*
40	34.73 $\pm$ 0.25	71.06 $\pm$ 1.0*	72.6 $\pm$ 0.80*
60	46.50 $\pm$ 0.70	71.5 $\pm$ 0.51*	74.8 $\pm$ 0.72*
80	49.30 $\pm$ 1.12	72.6 $\pm$ 0.62*	75.3 $\pm$ 0.30*
100	54.33 $\pm$ 0.61	73.8 $\pm$ 0.76*	76.2 $\pm$ 0.51*
120	58.63 $\pm$ 0.40	76.6 $\pm$ 0.52*	77.4 $\pm$ 0.51*
140	65.80 $\pm$ 0.34	77.0 $\pm$ 0.60*	78.8 $\pm$ 0.36*
160	72.80 $\pm$ 0.72	78.7 $\pm$ 0.25*	81.0 $\pm$ 0.50*
180	73.70 $\pm$ 0.30	79.9 $\pm$ 1.05*	89.6 $\pm$ 0.57*
200	84.10 $\pm$ 0.17	81.4 $\pm$ 0.45*	92.2 $\pm$ 0.64*

*Significant difference at 5 % level; Student t-test analysis of radical scavenging activity in different concentration of standard (α -Tocopherol) and test of *Miliusa tomentosa* (shows statistically different at $p < 0.05$ level)

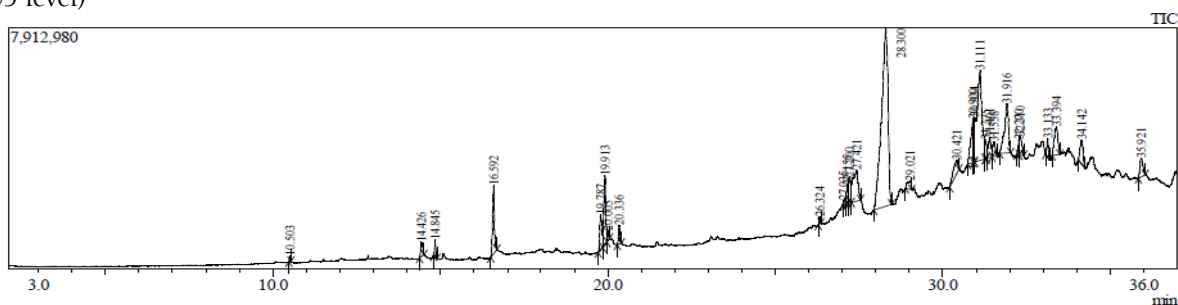


Fig 1: GC-MS chromatogram of the extract of *Miliusa tomentosa*

Table-4. GC-MS analysis revealed the presence of phytochemical components in chloroform leaf extract of *Miliusa tomentosa*

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	10.503	10.475	10.535	301422	0.14	2,4-Di-tert-butylphenol
2	14.426	14.375	14.510	1888374	0.87	Neophytadiene
3	14.845	14.800	14.895	1203919	0.55	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
4	16.592	16.500	16.685	8771123	4.04	n-Hexadecanoic acid
5	19.787	19.710	19.840	4637876	2.14	(9E,11E)-Octadecadienoic acid
6	19.913	19.840	19.970	9169539	4.22	7-Tetradecenal, (Z)-
7	20.005	19.970	20.055	1132000	0.52	E,E-3,13-Octadecadien-1-ol
8	20.336	20.285	20.405	1802241	0.83	Octadecanoic acid
9	26.324	26.285	26.360	549964	0.25	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
10	27.035	27.010	27.105	906669	0.42	7-Hexadecenal, (Z)-
11	27.155	27.105	27.175	2095663	0.97	2-((2R,4aR,8aS)-4a-Methyl-8-methylenedecahydronaphthalen-2-yl)acrylaldehyde
12	27.200	27.175	27.270	4187723	1.93	1-Heptatriacotanol
13	27.421	27.270	27.550	10964673	5.05	1-Heptatriacotanol
14	28.300	27.940	28.470	78267876	36.05	.gamma.-Sitosterol
15	29.021	28.885	29.075	1967682	0.91	Stigmasta-5,24(28)-dien-3-ol, (3.beta.,24Z)-
16	30.421	30.205	30.485	4744576	2.19	Mahanine
17	30.900	30.740	30.915	7499416	3.45	D:A-Friedooleanan-7-one, 3-hydroxy-
18	30.934	30.915	30.955	3428080	1.58	Mahanine
19	31.111	30.955	31.260	30871006	14.22	Mahanine
20	31.275	31.260	31.310	1588060	0.73	(.+/-)-Mahanine
21	31.401	31.310	31.475	4153347	1.91	Murrayamine A
22	31.538	31.475	31.620	2473906	1.14	Murrayamine A
23	31.916	31.700	32.025	13277213	6.11	Glutanol
24	32.270	32.210	32.280	1129595	0.52	(7R,8R)-cis-anti-cis-Tricyclo[7.3.0.0(2,6)]dodec
25	32.310	32.280	32.420	3017373	1.39	(.+/-)-Mahanine
26	33.133	33.075	33.190	1883119	0.87	Koenigine
27	33.394	33.265	33.495	6895860	3.18	Lup-20(29)-en-3-ol, acetate, (3.beta.)-
28	34.142	34.050	34.250	4583179	2.11	Vitamin E
29	35.921	35.840	36.040	3743083	1.72	Retusi

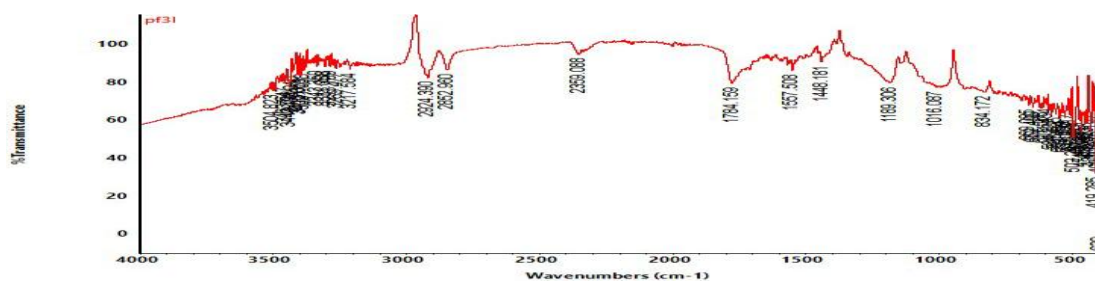


Figure 2. FTIR spectra of the *Miliusa tomentosa* extracts.

Table-5 Observed functional groups identified in *Miliusa tomentosa* using FTIR

Wavenumber (cm ⁻¹)		Band no.	Band interaction	Band assignments	Possible compounds
Band range (literature)(cm ⁻¹)	Band range (experimental)				
1000-650	613	11	stretch	C-Br	halogen compounds
	722, 833	18, 19	bend	C=C	alkene
1400-1000	1025, 1104, 1152, 1167, 1230	12, 20, 13, 21, 22	stretch	C-N/F	amine, fluoro compound
	1104	20	stretch	C-O	secondary alcohol aliphatic ether
	1152, 1167	13, 21	stretch	C-O	tertiary alcohol
	1167	21	stretch	C-O	ester
	1230	22	stretch	C-O	alkyl aryl ether
	1377	23	bend and stretch	O-H and C-F	phenol, alcohol, and fluoro-compound
1600-1300	1461	24	bend	C-H	alkane
1670-1600	1626, 1648	14, 25	stretch	C=C	alkene, conjugated alkene, cyclic alkene
	1626, 1648	14, 25	bend	N-H	amine
2000-1650	1648	25	stretch	N=H	imine/oxime
	1687	26	stretch	C=O	conjugated acid conjugated aldehyde
	1718	15	stretch	C=O	carboxylic acid, aliphatic ketone; α , β -unsaturated ester
	1746	27	stretch	C=O	ester, δ -lactone, cyclopentanone
4000-2500	2854, 2919, 2926	28, 16, 29	stretch	C-H, N-H, and O-H	alkane, amine salt, and alcohol, carboxylic acid
	3008	30	stretch	C-H and O-H	alkene and alcohol, carboxylic acid
	3426, 3445	31, 17	stretch	O-H	alcohol

Table -6 Antioxidant activity of *Miliusa tomentosa* leaves.

Sample	Scavenging activity at 600 µg/ml		Reducing Power
	DPPH	ABTS	
Leaf Extract	74.42±0.56	69.71±0.41	1.641±0.12
Acarbose	82.44±1.4	84.74±0.56	2.53±0.06

Note: The values are represented as the standard deviation of the mean (mean ± SD), n = 3, p < 0.05, Abbreviation: IC50, half maximal inhibitory concentration.

Table-7 Inhibitory potential of chloroform extracts of *Miliusa tomentosa* leaves. on α-amylase and α-glucosidase.

Sample	Inhibition at 600 µg/ml		Reducing Power
	α-amylase	α-glucosidase	
Leaf Extract	74.42±0.56	69.71±0.41	1.641±0.12
Acarbose	82.44±1.4	84.74±0.56	2.53±0.06

Note: The values are represented as the standard deviation of the mean (mean ± SD), n = 3, p < 0.05, Abbreviation: IC50, half maximal inhibitory concentration.

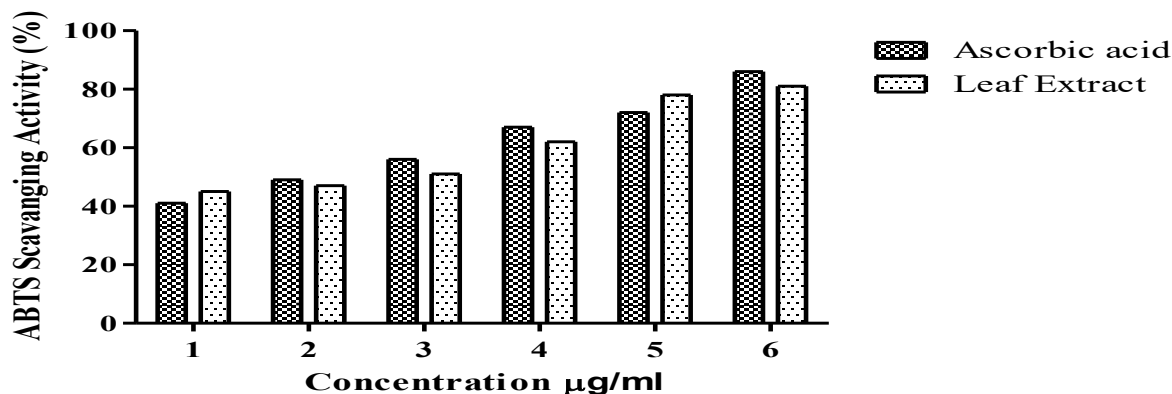


Fig -3 ABTS radical scavenging activity of *Miliusa tomentosa*

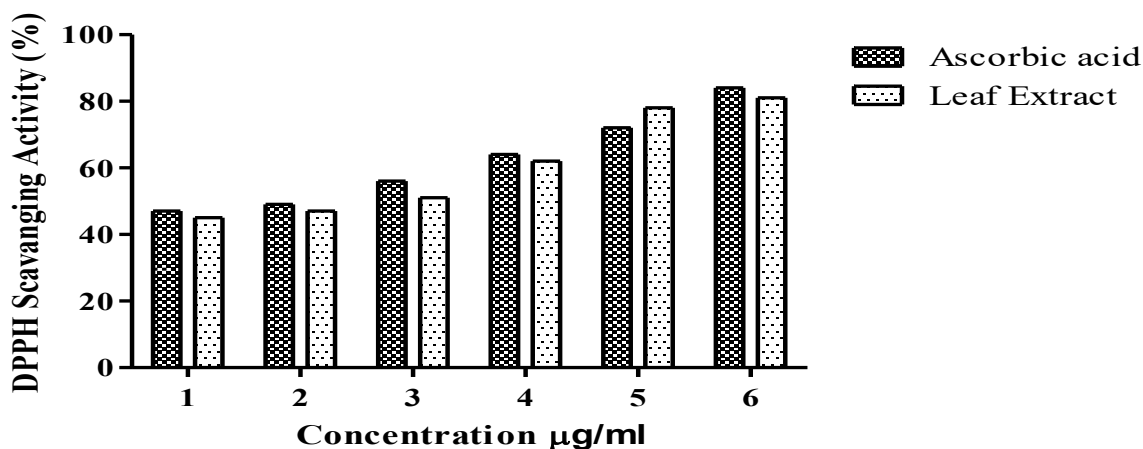


Fig -4 DPPH radical scavenging activity of *Miliusa tomentosa*

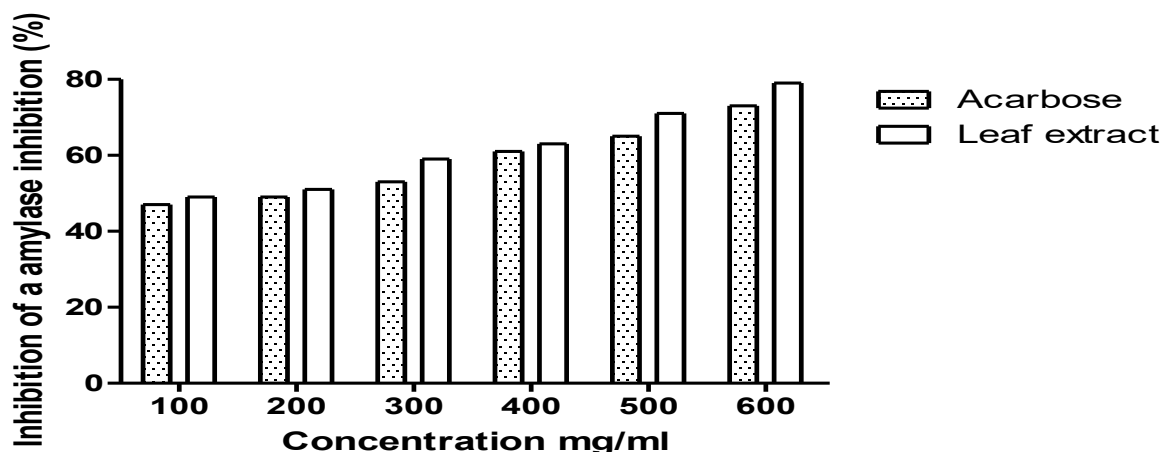


Fig-5 α -amylase inhibition of *Miliusa tomentosa* extract

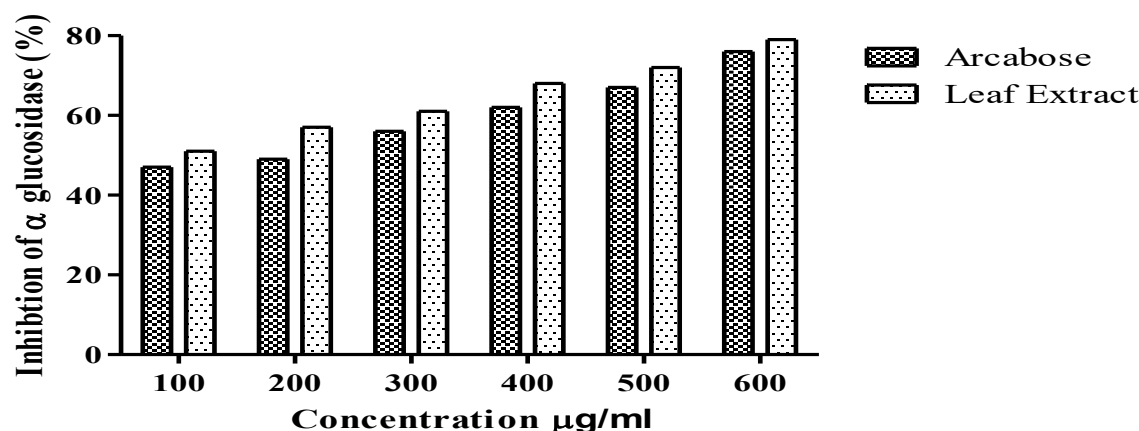


Fig-6 α -glucosidase inhibition of *Miliusa tomentosa* extract

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REFERENCE

- 1) The Son, N. (2019). Genus *Miliusa*: A review of phytochemistry and pharmacology. Evidence-Based Complementary and Alternative Medicine, 2019(1), 8314693.
- 2) Kumar, P., & Shukla, K. (2023). *Saccopetalum tomentosum*: Review of Its Botany, Medicinal Uses, Pharmacological Activities and Phytochemistry. Journal of Drug Delivery and Therapeutics, 13(7), 194-199.
- 3) Tona, L., Kambu, K., Ngimbi, N., Cimanga, K., & Vlietinck, A. J. (1998). Antiamoebic and phytochemical screening of some Congolese medicinal plants. Journal of ethnopharmacology, 61(1), 57-65
- 4) Karmegam, N., Jayakumar, M., & Karuppusamy, S. (2012). Synergistic antibacterial activity of four medicinal plants collected from Dharapuram Taluk of Tiruppur District, south India. Journal of plant sciences, 7(1), 32.
- 5) Ahmad, I., & Beg, A. Z. (2001). Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug-resistant human pathogens. Journal of ethnopharmacology, 74(2), 113-123.
- 6) Pakkirisamy, M., Kalakandan, S. K., & Ravichandran, K. (2017). Phytochemical screening, GC-MS, FT-IR analysis of methanolic extract of *Curcuma caesia* Roxb (Black Turmeric). Pharmacognosy Journal, 9(6).
- 7) El Sayed, A. M., Basam, S. M., El-Naggar, E. M. B. A., Marzouk, H. S., & El-Hawary, S. (2020). LC-MS/MS and GC-MS profiling as well as the antimicrobial effect of leaves of selected *Yucca* species introduced to Egypt. Scientific reports, 10 (1), 17778
- 8) Farag, M. A., Huhman, D. V., Lei, Z. & Sumner, L. W. Metabolic profiling and systematic identification of flavonoids and isoflavonoids in roots and cell suspension cultures of *Medicago truncatula* using HPLC-UV-ESI-MS and GC-MS. Phytochemistry 68, 342-354 (2007).
- 9) Sjögren G, Sletten G, Dahl JE (2000) Cytotoxicity of dental alloys, metals, and ceramics assessed by millipore filter, agar overlay, and MTT tests. J Prosthet Dent 84(2):229-236.

- 10) Benayad, O., Bouhrim, M., Tiji, S., Kharchoufa, L., Addi, M., Drouet, S., ... & Mimouni, M. (2021). Phytochemical profile, α -glucosidase, and α -amylase inhibition potential and toxicity evaluation of extracts from *Citrus aurantium* (L) peel, a valuable by-product from Northeastern Morocco. *Biomolecules*, 11(11), 1555.
- 11) Saha, S., & Ghosh, S. (2012). *Tinospora cordifolia*: One plant, many roles. *Ancient science of life*, 31(4), 151-159.
- 12) Pereira, L., & Valado, A. (2023). Algae-Derived natural products in diabetes and its complications—current advances and future prospects. *Life*, 13(9), 1831.
- 13) Vivó-Barrachina, L., Rojas-Chacón, M. J., Navarro-Salazar, R., Belda-Sanchis, V., Pérez-Murillo, J., Peiró-Puig, A., ... & Pérez-Bermejo, M. (2022). The role of natural products on diabetes mellitus treatment: a systematic review of randomized controlled trials. *Pharmaceutics*, 14(1), 101.
- 14) Pang, G. M., Li, F. X., Yan, Y., Zhang, Y., Kong, L. L., Zhu, P., ... & Lu, C. (2019). Herbal medicine in the treatment of patients with type 2 diabetes mellitus. *Chinese medical journal*, 132(1), 78-85.
- 15) Oscar, S. A., Antonio, C. N., Marina, G. V., Elsa, R. S., & Gabriel, V. A. (2020). Phytochemical screening, antioxidant activity and in vitro biological evaluation of leave extracts of *Hyptis suaveolens* (L.) from south of Mexico. *South African Journal of Botany*, 128, 62-66.
- 16) Azeem, M., Hanif, M., Mahmood, K., Ameer, N., Chughtai, F. R. S., & Abid, U. (2023). An insight into anticancer, antioxidant, antimicrobial, antidiabetic and anti-inflammatory effects of quercetin: A review. *Polymer Bulletin*, 80(1), 241-262.
- 17) Tiji, S., Benayad, O., Berrabah, M., El Mounsi, I., & Mimouni, M. (2021). Phytochemical profile and antioxidant activity of *Nigella sativa* L growing in Morocco. *The Scientific World Journal*, 2021(1), 6623609.