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Larvicidal activity of *Gledistia triacanthos* leaf extract against *Culex quinquefasciatus.*

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Abstract

Mosquitoes spread diseases that kill millions ever year and have acquired resistance to chemical insecticides, adding to higher vectorial capacity. *Culex quinquefasciatus* is a vector of various diseases, including West Nile virus, Japanese encephalitis, and filariasis. Another source of mosquito repellents could be plants. The present study investigated the larvicidal effects of *Gledistia triacanthos* dried leaf extract in methanol and hexane on the *Culex quinquefasciatus* species. The larvicidal effects of the plant extracts were examined in a 100 mL range at various concentrations of 20%, 10%, 5%, and 2.5% for 24 hours using laboratory bioassays. 25 larvae were introduced in five duplicates to prepare vol/vol solutions of plant leaf extract for the larvicidal experiment. Using the mortality data, the lethal concentrations (LC50) needed to kill 50% of the treated larvae of the relevant species was determined. The leaves' phytochemical screening was also evaluated. After 24-hour exposure duration, the results of both extracts showed moderate effects; however, the methane extract had the maximum harmful effect (4.857 mL/100 mL of methanol and 2.73 mL/100 mL of hexane). Numerous plant metabolites were discovered based on phytochemical data, which might have played a role in the larvae's deaths. According to this study the methane extract from *Gledistia triacanthos* leaves may therefore be used as a larvicidal agent,

Keywords: *Gledistia triacanthos*; Mosquitoes; Filariasis; Japanese Encephalitis; Insecticide

1. Introduction

Malaria is thought to be the cause of millions of deaths worldwide each year. Early detection and full treatment of this disease is extremely challenging because of public ignorance. Human health is suffering greatly as a result of the vector mosquito population. Mosquitoes are the direct carriers of diseases like malaria, dengue fever, and filarial. According to Fouad El-Akhal et al. (2021), the *Culex quinquefaciatus* mosquito can cause both local cutaneous reactions and systemic reactions including urticaria and angioedema. *Culex*, one of the most important mosquito genera, is widely recognized for its importance in public health. The blood-drawing *Culex quinquefaciatus* (Diptera: Culicidae) is a major contributor to the spread of human arboviruses, including the West Nile virus (A.E Lalami 2021).

In tropical countries, malaria is the most important and horrible disease, and it is a major public health concern. In Bangladesh, India, Thailand, Indonesia, and Myanmar, malaria outbreaks are common. Infants and young children are the main victims of malaria in regions where the disease is endemic. Babies and young children accounted for 90% of the 241 million cases and 627,000 fatalities that occurred globally in 2020 (WHO, 2010). Dengue is the vector-borne disease with the fastest rate of growth in the globe. Dengue infection has increased 30 times in the last 50 years. Nearly 2.5 billion people are affected in more than 100 endemic nations. According to the WHO, the number of dengue cases has increased more than eight times in the last 20 years, from 505,430 cases in 2000 to over 2.4 million in 2010 and 5.2 million in 2019 (WHO, 2022, 2024).

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Nowadays, synthetic insecticides are the most used method of controlling mosquitoes. In contrast to other control methods, synthetic insecticides have been used in recent decades and have had a negative feedback loop on the environment, affecting non-target organisms and making the majority of mosquito species physiologically resistant to them (VCRC, 1989, Severini, 1993).

However, according to Rao et al. (1995), several mosquito species have become extremely resistant to microbial control treatments. These elements have led to a hunt for mosquito-repelling insecticides that are biodegradable, environmentally friendly, and target-specific. An alternative approach to mosquito control has been the use of active toxic chemicals made from plant extracts since ancient times. These are nontoxic, easily obtainable, affordable, biodegradable, and have broad-spectrum target-specific effects against a variety of vector mosquito species. Plant extracts are an excellent alternative to synthetic insecticides for managing the mosquito population.

Gledistia triacanthos commonly known as honey locust is belongs to family Fabaceae is a deciduous tree. In addition to being used as diuretics and expectorants, it is used to treat carbuncle, scabies, skin conditions, apoplexy, headaches, productive cough, and asthma (Miyase et al., 2010). Studies found that Ehrlich cancer and Sarcoma 180 were moderately inhibited by the alcoholic fruit extract of G. tricanthos. Additionally, the studies discovered that the pigment dihydroxy-4-methoxyisoflavone, which was extracted from the fruit extract of G. triacanthos, had significant cytotoxic and oncostatic properties Sokoloff et al. (1964). The aim of this study was to investigate the larvicidal effects of *Gledistia triacanthos* leaf methanol and hexane extract against *Culex quinquefasciatus* larvae.

2. Materials and Methods

- Collection of plant materials: The leaves of *Gledistia triacanthos* were gathered from the forest area of Telangana state, India's Suryapet district. Identification of the collected plant materials was aided by the Botany department's plant taxonomists. Voucher specimens are stored in the botany department for future use.
- Preparation of leaves extract: After being crushed coarsely, the leaves were rinsed with tap water and allowed to dry in the shade. The dehydrated leaves were crushed into a powder using an electric stainless steel blender. Methanol and hexane were treated separately after the finely powdered plant material was added to a Soxhlet system. The crude extract and solvent were extracted using a revolving vacuum evaporator. The crude residue from this plant is influenced by the solvents used.
- Phytochemical screening and characterization: Following a preliminary phytochemical screening of both extracts, the important phytochemicals were characterized using FITR, GLC, and NMR studies.

2.1. Collection and Rearing of the Insects

The insects were gathered at Osmania University campus. The leading zoologists in the department at Osmania University identified and verified *Culex quinquefasciatus*. In the lab, the insects were raised on a 10% sucrose solution, and the larvae were fed a 3:1 mixture of yeast powder and dog biscuits. The synthesized methanol and hexane leaf extract of *Gledistia triacanthos* was tested for larvicidal activity against the life cycle of *Culex quinquefasciatus*.

2.2. Larvicidal Activity

The larvicidal experiment was performed using the World Health Organization's recommended protocol (Organization, 1996) with minor adjustments taken from Rahuman (Rahuman et al., 2000). By mixing the necessary amount of plant extract, a 100 mL plant extract solution with different concentrations of 20%, 10%, 5%, and 2.5 was created. Hexane and methanol were used to set up the control. After a 24-hour incubation period, some dead larvae were seen among the 25 larvae that were added to the solutions. Five duplicates of the experiment were conducted.

2.3. Statistical Analysis

For every leaf extract, the mean and standard deviation were determined. ANOVA, chi-square, and F-value were then used to determine the lethal concentration 50 (LC50) and the mortality percentage at a 95% confidence interval.

3. Results

- Phytochemical Tests: The phytochemical screening of both methanol and hexane extracts of the leaves of the *Gledistia triacanthos* plant showed the presence of chemical compounds like Alkaloids, Carbohydrates, Flavonoids, Terpenoids and Tannis in methanol extract but Terpenoids not in hexane extract (Table.1).
- Gas chromatography-mass spectrometry (GC-MS) analysis: the GC-MS study of the hexane fraction of *Gledistia triacanthos* leaves revealed 180 compounds in all, each of which displayed distinct phytochemical activity. The

chromatogram as shown in Figure 1 and Table 2 lists the chemical components in the MFHAL together with their retention time (RT), FT, Area and Area% and name of the compound. And the GC-MS study of the methanol fraction of *Gledistia triacanthos* leaves revealed 317 compounds in all, each of which displayed distinct phytochemical activity. The chromatogram as shown in Figure 2 and Table 3 lists the chemical components in the MFHAL together with their retention time (RT), FT, Area and Area% and name of the compound.

- FTIR analysis: FTIR is a rapid, non-destructive, time-saving method that can detect a wide range of functional groups and is sensitive to changes in molecular structure. The physical condition and chemical makeup of the entire sample are the basis for the information provided by FTIR. Chemical components have been identified using FTIR (Figure.3 and Table.4).
- NMR analysis: Figure 4 and Table 5 presents the findings of the NMR analysis.

3.1. Larvicidal effects

Twenty-five larvae were introduced in five duplicates to prepare vol/vol solutions of plant leaf extract for the larvicidal experiment. The mean plus standard deviation (SD) for each of the five replicates is given (Table.6). Due to the presence of bioactive components, *Gledistia triacanthos* extracts in methanol and hexane were found to have larvicidal action. As anticipated, when the content of the leaf extract decreased, a declining trend in larvicidal activity was noted. A p-value of 0.00001 indicated that the ANOVA analysis, which examined different extract concentrations, was significant. Additionally, the lethal concentration 50 (LC50) for the methanolic leaf extract of *Gledistia triacanthos* was determined to be 2.62 mL/100 mL of solvent. The LC50 values for hexane extract were 3.046 mL/100 mL (Table 7). A chi-square test was used to examine the data, and a p-value of less than 0.01 indicated that the results were significant.

Table 1 Preliminary Investigation of Phytochemical Constituents in Methanol and Hexane Extracts of the Elytraria acaulis *Gledistia triacanthos* leaves

Phyto constituents	Types of Tests	Methanol	Hexane
Alkaloids	Mayer's test	+	+
Flavonoids	Salkowski test	+	+
Terpenoids	Knoller test	+	-
Tannins	Ferric chloride test	+	+
Carbohydrates	Molisch's test	+	+

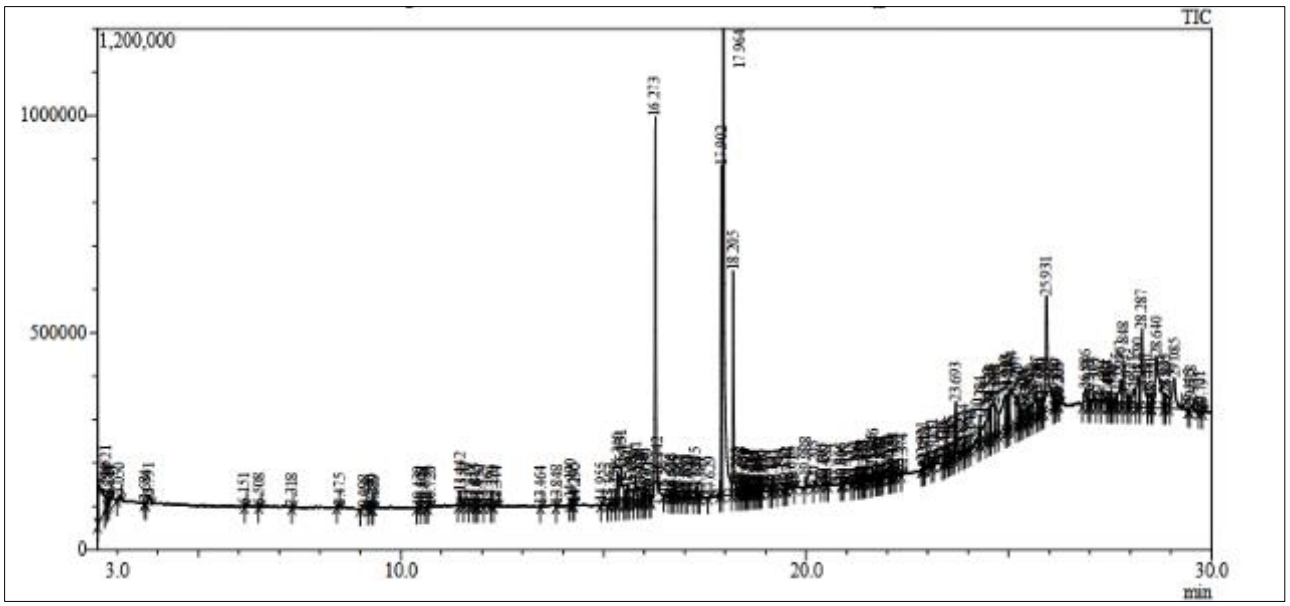

Figure 1 Chromatograms of identified phytochemical constituents' profile in the Hexane extract of the *Gledistia triacanthos* leaves using the GC/MS technique

Table 2 GC-MS analysis of Hexane Extract of the *Gledistia triacanthos* leaves

Peak#	R.Time	F.Time	Area	Area%	Name
1	2.521	2.530	109951	0.44	Hydrazinecarboxamide
2	2.710	2.750	109676	0.43	Benzeneethanamine, N-methyl-
3	2.780	2.795	48779	0.19	Acetic acid, hydrazide
4	2.814	2.850	26855	0.11	1,2-Hydrazinedicarboxamide
5	3.050	3.110	39524	0.16	Eucalyptol
6	3.684	3.710	5908	0.02	4-Methyl-3,4-dihydro-[1,2,3]triazolo[4,5-d]py
7	3.791	3.860	88244	0.35	Undecane
8	6.151	6.205	12411	0.05	Acetic acid, hydrazide
9	6.508	6.550	8113	0.03	4-Hydroxy-1H-pyrazolo[3,4-D]pyrimidine rib
10	7.318	7.345	7403	0.03	Cyacetacide
11	8.475	8.495	13591	0.05	Hydrazinecarboxamide
12	9.098	9.160	35150	0.14	Stearic acid hydrazide
13	9.230	9.275	15979	0.06	Acetylthiosemicarbazide
14	9.289	9.305	8411	0.03	Semioxamazide
15	9.339	9.370	15977	0.06	Hydrazinecarboxamide
16	10.430	10.445	9568	0.04	4-Phenylsemicarbazide
17	10.508	10.520	4856	0.02	Cyacetacide
18	10.624	10.660	11089	0.04	1-Propanol, 2-amino-, (.+/-.)-
19	10.720	10.750	13476	0.05	Propanamide
20	11.442	11.495	96657	0.38	Phenol, 2,4-bis(1,1-dimethylethyl)-
21	11.550	11.595	21160	0.08	Acetic acid, hydrazide
22	11.719	11.740	16629	0.07	Stearic acid hydrazide
23	11.815	11.855	27043	0.11	D-Fructose, 3-O-methyl-
24	11.875	11.895	11287	0.04	4-Hydroxybutyric acid hydrazide
25	11.950	12.035	22203	0.09	1,2-Hydrazinedicarboxamide
26	12.167	12.205	49888	0.20	16-Hexadecanoyl hydrazide
27	12.270	12.290	13252	0.05	Hydrazinecarbothioamide, N-methyl-
28	12.344	12.415	17077	0.07	Cyacetacide
29	13.464	13.495	5498	0.02	4-Methyl-3,4-dihydro-[1,2,3]triazolo[4,5-d]py
30	13.848	13.860	10328	0.04	16-Hexadecanoyl hydrazide
31	14.190	14.230	45510	0.18	Methyl tetradecanoate
32	14.245	14.270	11879	0.05	Hydrazinecarboxamide
33	14.290	14.305	7714	0.03	Stearic acid hydrazide
34	14.955	14.980	15213	0.06	Nonane, 3-methyl-5-propyl-
35	15.162	15.185	15070	0.06	2-Butene ozonide
36	15.259	15.295	19438	0.08	Heptadecanoic acid, 10-methyl-, methyl ester
37	15.340	15.365	145624	0.58	Phytol, acetate
38	15.431	15.510	432496	1.71	2-Pentadecanone, 6,10,14-trimethyl-
39	15.525	15.560	88051	0.35	9-Octadecene, 1,1-dimethoxy-, (Z)-
40	15.601	15.630	146832	0.58	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
41	15.685	15.745	196856	0.78	Phthalic acid, butyl undecyl ester
42	15.791	15.820	148594	0.59	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
43	15.836	15.905	167232	0.66	9-Octadecene, 1,1-dimethoxy-, (Z)-
44	15.920	15.980	105157	0.42	16-Hexadecanoyl hydrazide
45	16.010	16.030	56558	0.22	16-Hexadecanoyl hydrazide
46	16.079	16.120	123044	0.49	9-Hexadecenoic acid, methyl ester, (Z)-
47	16.135	16.170	45044	0.18	Diethylmalonic acid, monochloride, hept-4-yl
48	16.273	17.865	2141016	8.49	Hexadecanoic acid, methyl ester
49	16.342	16.365	10426	0.04	Benzenepropanoic acid, 3,5-bis(1,1-dimethyle
50	16.502	16.605	24854	0.10	Isophytol
51	16.675	16.695	22155	0.09	Phthalic acid, butyl 3-methylbut-2-en-1-yl este
52	16.706	16.730	13524	0.05	4-Methyl-3,4-dihydro-[1,2,3]triazolo[4,5-d]py
53	16.795	16.805	15323	0.06	Pyrimidine-2,4(1H,3H)-dione, 5-amino-6-nitro
54	16.863	16.910	11617	0.05	2-Hexadecenoic acid, methyl ester, (E)-
55	16.996	17.010	15010	0.06	3-Ethyl-3-methylheptane
56	17.053	17.085	25459	0.10	7-Hexadecenoic acid, methyl ester, (Z)-
57	17.100	17.130	7306	0.03	Heptadecanoic acid, 9-methyl-, methyl ester
58	17.275	17.320	52068	0.21	Heptadecanoic acid, methyl ester
59	17.330	17.340	4445	0.02	Ethanamine, 2-(4-cyclohexylphenyl)-
60	17.381	17.425	6413	0.03	Semioxamazide
61	17.629	17.645	10629	0.04	1,2-Hydrazinedicarboxamide
62	17.902	17.930	1246130	4.94	9,12-Octadecadienoic acid (Z,Z)-, methyl este
63	17.964	18.165	4962815	19.67	9,12,15-Octadecatrienoic acid, methyl ester, (Z
64	18.205	18.285	961841	3.81	Methyl stearate
65	18.295	18.350	62716	0.25	Phosphonic acid, (3-methyl-2-oxo-3-pentenyl)
66	18.378	18.425	72810	0.29	Cyclopentanone, 2-(5-oxohexyl)-
67	18.430	18.450	18814	0.07	Acetamide, 2-(2,4-dimethoxybenzylidenehydra
68	18.476	18.505	38425	0.15	Stearic acid hydrazide
69	18.525	18.540	18871	0.07	1,2-Hydrazinedicarboxamide
70	18.555	18.565	12297	0.05	Cyacetacide
71	18.582	18.610	22663	0.09	Ethyl 2-(2-chloroacetamido)-3,3,3-trifluorolac
72	18.675	18.695	35547	0.14	1(2H)-Naphthalenone, 8a.beta.-ethyl-2-furfury
73	18.715	18.735	14480	0.06	1,2-Hydrazinedicarboxamide

73	18.715	18.735	14480	0.06	1,2-Hydrazinedicarboxamide
74	18.749	18.770	13673	0.05	1H-Pyrazole, 1-(3-methylbutyl)-5-(4,4,5,5-tetr
75	18.801	18.830	23668	0.09	1,2-Hydrazinedicarboxamide
76	18.855	18.880	19863	0.08	Octadecane
77	18.890	18.905	7022	0.03	Stearic acid hydrazide
78	18.930	19.075	27083	0.11	3-Phenyl-2H-chromene
79	19.131	19.160	18744	0.07	Nonadecanoic acid, methyl ester
80	19.185	19.195	6105	0.02	Hydrazinecarboxylic acid, ethyl ester
81	19.304	19.335	8973	0.04	4-Phenylsemicarbazide
82	19.450	19.500	14994	0.06	Hydrazinecarboxamide
83	19.512	19.530	8105	0.03	Carbohydrazide
84	19.619	19.650	19285	0.08	1,2-Ethanediamine, N-(2-aminoethyl)-
85	19.722	19.755	8487	0.03	Tridecane, 1-iodo-
86	19.988	20.030	50170	0.20	Eicosanoic acid, methyl ester
87	20.205	20.250	35220	0.14	4,8,12,16-Tetramethylheptadecan-4-olide
88	20.405	20.435	16614	0.07	Nerolidyl acetate
89	20.489	20.505	3832	0.02	Cyacetacide
90	20.554	20.585	10078	0.04	Tridecane, 1-iodo-
91	20.866	20.890	8829	0.03	Hydroxyacetic acid, hydrazide
92	20.906	20.930	6698	0.03	Oxygen
93	20.947	21.080	21495	0.09	1,2-Hydrazinedicarboxamide
94	21.162	21.180	11002	0.04	2H-Pyran-2-methanol, 3,4-dihydro-, methanes
95	21.210	21.230	7484	0.03	Aminoguanidine
96	21.295	21.335	15238	0.06	4-Phenylsemicarbazide
97	21.358	21.390	20179	0.08	2-methylhexacosane
98	21.411	21.435	18273	0.07	9-Octadecenoic acid (Z)-, methyl ester
99	21.446	21.460	5990	0.02	1,2-Hydrazinedicarboxamide
100	21.477	21.510	10403	0.04	2-Methyl-pentanoic acid [4-(2-methyl-pentano
101	21.605	21.625	34866	0.14	Docosanoic acid, methyl ester
102	21.646	21.715	69053	0.27	Bis(2-ethylhexyl) phthalate
103	21.725	21.765	15109	0.06	Cyclohexanamine, N-methyl-n-propyl-
104	21.780	21.810	11013	0.04	1,2-Hydrazinedicarboxamide
105	21.836	21.850	9772	0.04	Semioxamazide
106	21.878	21.895	11940	0.05	Caprolactone oxime, (NB)-O-[(diethylborylox
107	22.033	22.050	40915	0.16	Hydrazine, (1,1-dimethylethyl)-
108	22.060	22.090	6361	0.03	Cyacetacide
109	22.128	22.145	20781	0.08	Triacontane, 1-bromo-
110	22.155	22.185	11780	0.05	p-Cyanophenyl p-(2-propoxyethoxy)benzoate
111	22.262	22.275	14701	0.06	Stearic acid hydrazide
112	22.374	22.390	8857	0.04	Hexacosanoic acid, methyl ester
113	22.871	22.895	28921	0.11	Hexatriacontane

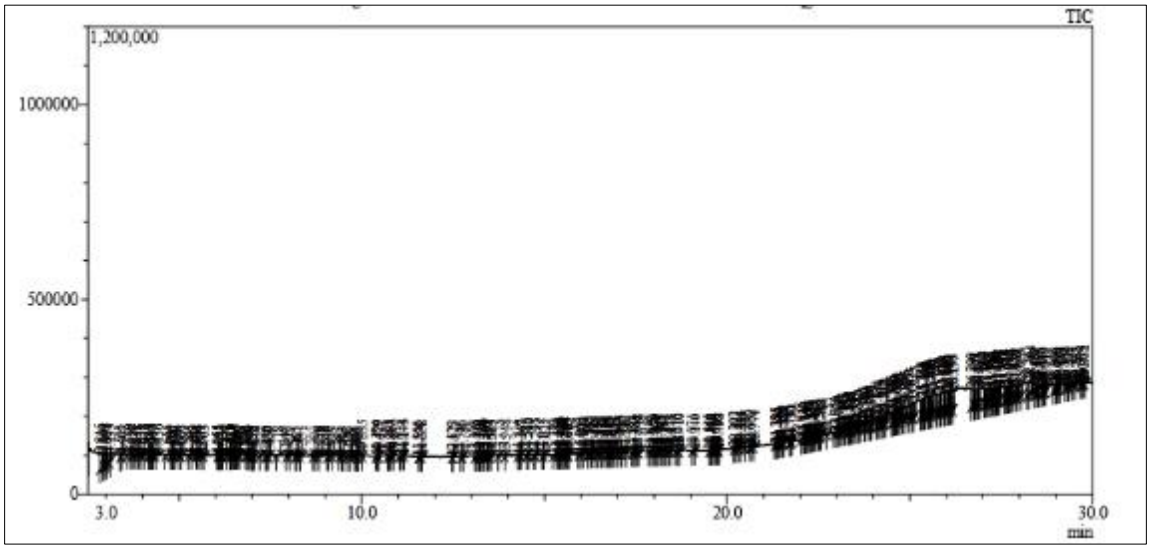

Figure 2 Chromatograms of identified phytochemical constituents' profile in the Methanolic extract of the *Gledistia triacanthos* leaves using the GC/MS technique

Table 3 GC-MS analysis of Methanolic Extract of the *Gledistia triacanthos* leaves

Peak Report TIC

Peak#	R.Time	F.Time	Area	Area%	Name
1	2.845	2.915	189946	1.94	Hydrazine, (1,1-dimethylethyl)-
2	2.930	2.975	118454	1.21	1,2-Hydrazinedicarboxamide
3	2.993	3.015	73060	0.74	Hydrazinecarboxylic acid, ethyl ester
4	3.061	3.105	134974	1.38	L-Cysteine sulfinic acid
5	3.135	3.150	56218	0.57	3-fluoroamphetamine
6	3.405	3.420	13782	0.14	Butyric acid hydrazide
7	3.469	3.480	8900	0.09	2-[4-(1,2-Diphenyl-but-1-enyl)-phenoxy]-ethy
8	3.659	3.690	21252	0.22	Hydrazinecarboxamide
9	3.726	3.740	5530	0.06	Acetic acid, hydrazide
10	3.757	3.810	6866	0.07	Propanoic acid, 3-hydroxy-, hydrazide
11	3.858	3.875	10957	0.11	Propanoic acid, 3-hydroxy-, hydrazide
12	3.935	3.945	10243	0.10	Oxygen
13	4.018	4.030	18166	0.19	Semioxamazide
14	4.075	4.165	25380	0.26	Ergosta-5,22-dien-3-ol, acetate, (3.beta.,22E)-
15	4.183	4.210	7901	0.08	1,2-Hydrazinedicarboxamide
16	4.240	4.255	8141	0.08	Propanoic acid, 3-hydroxy-, hydrazide
17	4.275	4.285	6874	0.07	Acetic acid, hydrazide
18	4.305	4.355	16266	0.17	Butyric acid hydrazide
19	4.436	4.465	26545	0.27	Glycolaldehyde dimer
20	4.655	4.760	11850	0.12	Butanal
21	4.789	4.810	4500	0.05	4-Methyl-3,4-dihydro-[1,2,3]triazolo[4,5-d]py
22	4.822	4.840	3026	0.03	4-Methyl-3,4-dihydro-[1,2,3]triazolo[4,5-d]py
23	4.880	4.890	3238	0.03	1,2-Hydrazinedicarboxamide
24	4.920	5.010	5336	0.05	7-Nitro-9-oxo-9,10-dihydroacridine-4-carboxy
25	5.030	5.045	3384	0.03	Carbohydrazide
26	5.063	5.110	9477	0.10	Hydrazinecarboxamide
27	5.305	5.335	7379	0.08	Semioxamazide

Peak#	R.Time	F.Time	Area	Area%	Name
28	5.359	5.380	2044	0.02	3-Butyn-1-ol
29	5.472	5.485	2384	0.02	Semioxamazide
30	5.504	5.515	1714	0.02	Hydrazinecarboxamide
31	5.530	5.575	7935	0.08	Acetic acid, hydroxy-
32	5.622	5.670	8637	0.09	Hydrazinecarboxylic acid, ethyl ester
33	5.703	5.745	9156	0.09	Hydrazinecarboxamide
34	6.055	6.075	4188	0.04	Propanoic acid, 3-hydroxy-, hydrazide
35	6.095	6.105	4151	0.04	Phenylephrine
36	6.115	6.135	6051	0.06	Hydrazine, 2-butenyl-
37	6.147	6.165	7006	0.07	1-(4-Acetamidoanilino)-3,7-dimethylbenzo[4,
38	6.175	6.195	5428	0.06	Cyacetacide
39	6.236	6.295	16222	0.17	12-Methylaminolauric acid
40	6.326	6.395	20490	0.21	Semioxamazide
41	6.405	6.415	1350	0.01	Hydrazine
42	6.425	6.450	2553	0.03	Semioxamazide
43	6.469	6.495	10219	0.10	1,2-Hydrazinedicarboxamide
44	6.515	6.525	4045	0.04	4-Phenylsemicarbazide
45	6.545	6.560	3430	0.03	Hydrazinecarboxamide
46	6.600	6.615	4989	0.05	1,2-Hydrazinedicarboxamide
47	6.640	6.655	2870	0.03	Calconcarboxylic acid
48	6.667	6.680	2180	0.02	Hydrazinecarboxylic acid, ethyl ester
49	6.830	6.870	5316	0.05	Hydrazine
50	6.885	6.905	5148	0.05	1,5-Pentanediol, 3-methyl-
51	6.920	6.980	14144	0.14	Hydrazinecarboxamide
52	6.990	7.015	2484	0.03	Tricyclo[10.2.2.2(5,8)]octadeca-5,7,12,14,15,
53	7.028	7.050	3407	0.03	Benzeneethanamine, .alpha.-methyl-N-(1-meth
54	7.072	7.110	5070	0.05	1,2-Dideoxy-l-erythro-pentitol
55	7.222	7.240	3100	0.03	Butyric acid hydrazide
56	7.379	7.395	4021	0.04	Methyl cyano(ethoxycarbonyl)methylenamine,
57	7.430	7.445	7437	0.08	Semioxamazide
58	7.460	7.490	4976	0.05	2-(3-Methylaminopropyl)-5-phenylamino-1,3,4
59	7.718	7.785	13304	0.14	Acetamide, 2-pentafluorophenyloxy-
60	7.940	7.970	3615	0.04	Ethanedial, bis(dimethylhydrazone)
61	8.025	8.035	2260	0.02	Propanamide
62	8.195	8.210	4193	0.04	Dithiocarbonic acid, hydrazide, N2-cyclopenty
63	8.261	8.275	9183	0.09	Carbohydrazide
64	8.351	8.365	9442	0.10	2-Pyridineethanol
65	8.707	8.730	14993	0.15	Acetaldehyde, hydroxy-
66	8.798	8.815	14954	0.15	Phosphonoacetic acid
67	8.918	8.935	26001	0.26	16-Hexadecanoyl hydrazide
68	9.070	9.080	3232	0.03	Spiro[2.3]hexan-4-one, 5,5-dichloro-6-methyl-
69	9.095	9.115	3902	0.04	Cyacetacide

70	9.225	9.235	3168	0.03	1,2-Hydrazinedicarboxamide
71	9.385	9.400	2661	0.03	Hydrazinecarboxylic acid, ethyl ester
72	9.464	9.485	7174	0.07	Glycolaldehyde dimer
73	9.495	9.505	2915	0.03	Phenylephrine
74	9.620	9.695	7708	0.08	4-Bromo-N-(piperidinomethyl)phthalimide
75	9.709	9.740	4614	0.05	Hydrazinecarboxylic acid, ethyl ester
76	9.835	9.860	3772	0.04	4-Methyl-3,4-dihydro-[1,2,3]triazolo[4,5-d]py
77	9.880	9.895	3633	0.04	4-methylthioamphetamine
78	9.905	9.930	6766	0.07	Carbamic acid, N-(2,3-dichlorophenyl)-, glycie
79	10.035	10.045	3440	0.04	Ethanedioic acid, dihydrazide
80	10.394	10.410	2909	0.03	Benzeneethanamine, .alpha.-methyl-N-(1-meth
81	10.429	10.465	6878	0.07	Acetamide, 2,2,2-trifluoro-
82	10.479	10.490	2053	0.02	1H-Indole-3-ethanamine, .alpha.-methyl-
83	10.725	10.735	3577	0.04	Cyacetacide
84	10.785	10.795	2318	0.02	Hydrazine
85	10.805	10.860	5861	0.06	N-Ethyl-N-nitroso-N'-nitroguanidine
86	10.875	10.885	5243	0.05	1,2-Hydrazinedicarboxamide
87	11.074	11.085	4309	0.04	1-Methoxy-3,4-dimethyl-1-phenyl-1-germacyc
88	11.144	11.155	5087	0.05	Hydrazinecarboxamide
89	11.175	11.190	3293	0.03	4-Phenylsemicarbazide
90	11.555	11.580	1764	0.02	n-Hexylmethylamine
91	11.590	11.635	3707	0.04	Phosphonoacetic acid
92	11.658	11.715	12512	0.13	Hydrazinecarbothioamide, N-ethyl-
93	12.479	12.490	2135	0.02	1,2-Epoxy-3-(3,3,4,4,5,5,6,6,7,7,8,8,8-trideca
94	12.523	12.535	5313	0.05	Pyridine-2,6-dicarboxylic acid, bis-hydrazinoc
95	12.716	12.740	5524	0.06	Hexanoic acid, 3-hexenyl ester, (Z)-
96	12.780	12.800	5688	0.06	Hydroxyacetic acid, hydrazide
97	13.100	13.120	3755	0.04	Hydrazinecarboxamide
98	13.135	13.195	8837	0.09	2-Acetylpyridine 4-methyl-4-[.beta.-[2-pyridyl
99	13.210	13.225	5477	0.06	Hydrazinecarboxamide
100	13.241	13.255	6994	0.07	Iron, carbonyl[(2,3,4,5-.eta.)-diethyl 2,4-hexac
101	13.310	13.335	18740	0.19	Hydrazinecarboxamide
102	13.350	13.365	7364	0.08	Acetic acid, hydroxy-
103	13.409	13.430	11007	0.11	2,4(1H,3H)-Pyrimidinedione, 6-chloro-5-nitro
104	13.455	13.465	6318	0.06	Hydrazinecarboxamide
105	13.494	13.535	9019	0.09	Aminoacethydrazide
106	13.565	13.580	4114	0.04	Propanethioamide, 3-hydroxy-2-[2-t-butoxyca
107	13.775	13.790	9534	0.10	Butyric acid hydrazide
108	13.948	13.960	7023	0.07	Tetraethyl 4,4'-(1,3-phenylene)bis(1,4-dihydro
109	14.276	14.310	3120	0.03	Cyacetacide
110	14.345	14.355	1265	0.01	Methyl Alcohol
111	14.367	14.385	2032	0.02	4-Imidazolidinecarboxylic acid, 4-hydroxy-2,5
112	14.540	14.555	4379	0.04	Methyl Alcohol
113	14.565	14.590	5151	0.05	1,2-Hydrazinedicarboxamide

Peak#	R.Time	F.Time	Area	Area%	Name
114	14.735	14.750	4204	0.04	cis-Aconitic anhydride
115	14.795	14.815	9717	0.10	Adenosine, 4'-dehydroxymethyl-4'-[N-ethylam
116	14.955	14.980	3448	0.04	16-Hexadecanoyl hydrazide
117	15.025	15.070	8090	0.08	Carbonic acid, decyl methyl ester
118	15.250	15.325	8227	0.08	N-2,4-Dnp-L-arginine
119	15.354	15.385	8185	0.08	Acetaldehyde, hydroxy-
120	15.400	15.425	5935	0.06	Methyl formate
121	15.446	15.470	7161	0.07	Methyl Alcohol
122	15.505	15.515	8018	0.08	4-Methyl-3,4-dihydro-[1,2,3]triazolo[4,5-d]py
123	15.532	15.570	10748	0.11	Acetic acid, hydrazide
124	15.580	15.595	2537	0.03	Hydrazinecarboxamide
125	15.625	15.635	4626	0.05	(+)-2-Aminoheptane
126	15.645	15.660	2008	0.02	Acetic acid, hydrazide
127	15.702	15.720	6702	0.07	1,2-Hydrazinedicarboxamide
128	15.950	15.980	8642	0.09	1,2,4-Benzenetricarboxylic acid, 4-dodecyl dis
129	16.005	16.060	7166	0.07	Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6
130	16.099	16.115	7473	0.08	Hydrazinecarboxamide
131	16.190	16.205	3297	0.03	N-Cyano-N-[4,6-bis(dimethylamino)-1,3,5-tria
132	16.260	16.290	14596	0.15	Semioxamazide
133	16.300	16.310	3735	0.04	Ethanedial, bis(dimethylhydrazone)
134	16.329	16.345	7172	0.07	(1R)-Propanol, (2S)-[(tert.butyloxycarbonyl)a
135	16.450	16.460	9074	0.09	1,2-Hydrazinedicarboxaldehyde
136	16.502	16.520	10935	0.11	Trichloroacetic acid, hex-4-yn-3-yl ester
137	16.575	16.590	5767	0.06	Aminocyanoacetic acid
138	16.610	16.690	15993	0.16	Acetic acid, hydrazide
139	16.706	16.730	7319	0.07	Butylsemithiocarbazide
140	16.745	16.755	3620	0.04	Magnesium, bis(2,4-pentanedionato-O,O')-, (T
141	16.765	16.775	2553	0.03	1,2-Hydrazinedicarboxamide
142	16.805	16.835	7428	0.08	Phosphonic acid, (3-methyl-2-oxo-3-pentenyl)
143	16.852	16.875	5197	0.05	Hydrazinecarboxamide
144	16.925	16.965	4874	0.05	1,2-Hydrazinedicarboxamide
145	16.975	16.985	3123	0.03	Hydrazinecarboxamide
146	17.055	17.075	19266	0.20	Benzonitrile, 2,6-dichloro-
147	17.095	17.120	7210	0.07	Hydrazine
148	17.132	17.230	16474	0.17	5-Iodohistidine
149	17.240	17.260	1718	0.02	Hydrazinecarboxamide
150	17.395	17.430	3150	0.03	Carbohydrazide
151	17.455	17.470	4757	0.05	Methyl Alcohol
152	17.481	17.520	9495	0.10	p-Mentha-6,8-dien-2-one, semicarbazone
153	17.548	17.575	10389	0.11	1,2-Hydrazinedicarboxamide
154	17.618	17.640	11124	0.11	Propanoic acid, 3-hydroxy-, hydrazide
155	17.870	17.880	4032	0.04	Folic Acid
156	17.959	17.990	9708	0.10	Malonic acid, dihydrazide
157	18.020	18.035	8920	0.09	4-Methyl-3,4-dihydro-[1,2,3]triazolo[4,5-d]py
158	18.070	18.090	10369	0.11	Acetonitrile, bromo-

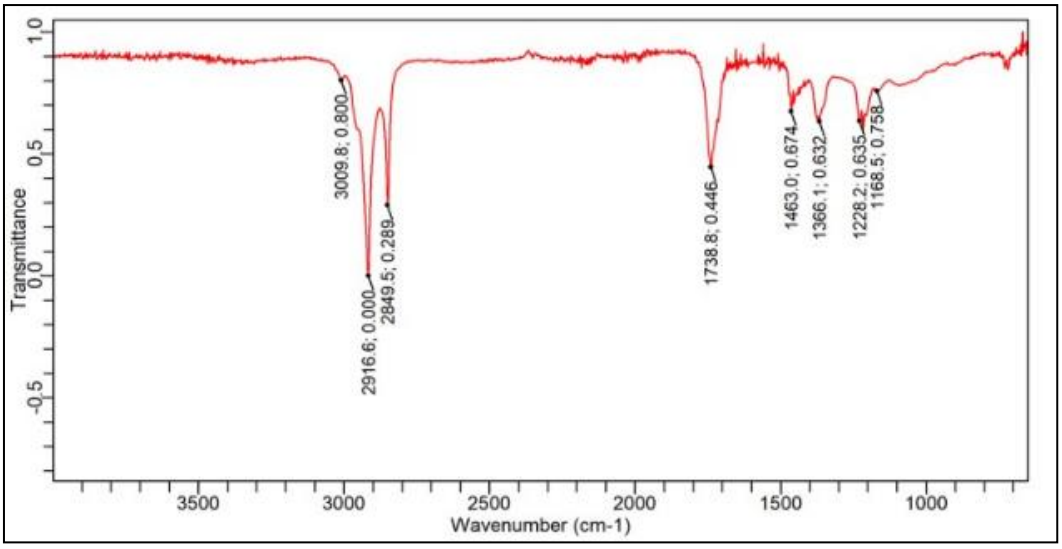

Figure 3 FTIR spectrum representing potential bands in the methanolic extract of the *Gledistia triacanthos* leaves

Table 4 All Potential Bands, Corresponding Functional Groups, and Possible Compounds Identified in the methanolic extract of the *Gledistia triacanthos* leaves using FT-IR Spectroscopy

- 3009 CH stretch Alkene (Aliphatic alkene)
- 2916 CH stretch Alkane (Aliphatic alkane)
- 2849 CH stretch Alkane (Aliphatic alkane (or) alcohol)
- 1738 c = o stretch (Carbonyl alpha alkyl)
- 1463 CH bend CH2 (Aliphatic ether (or) sulfonate salt)
- 1366 CH bend CH3 (Aryl strained alkane(or) activated carbonyl)
- 1228 CO stretch Alkoxy substistment
- 1168 CO stretch Alkoxy substistment

Peak numbers and band types of FTIR spectra of methanolic extract of the *Gledistia triacanthos* leaves

Peak Number	Wavenumber (cm^{-1})	Intensity
1	1168.52041	0.75758
2	1228.15782	0.63536
3	1366.06932	0.63234
4	1462.98010	0.67426
5	1738.80310	0.44604
6	2849.54978	0.28927
7	2916.64186	0.00000
8	3009.82530	0.80031

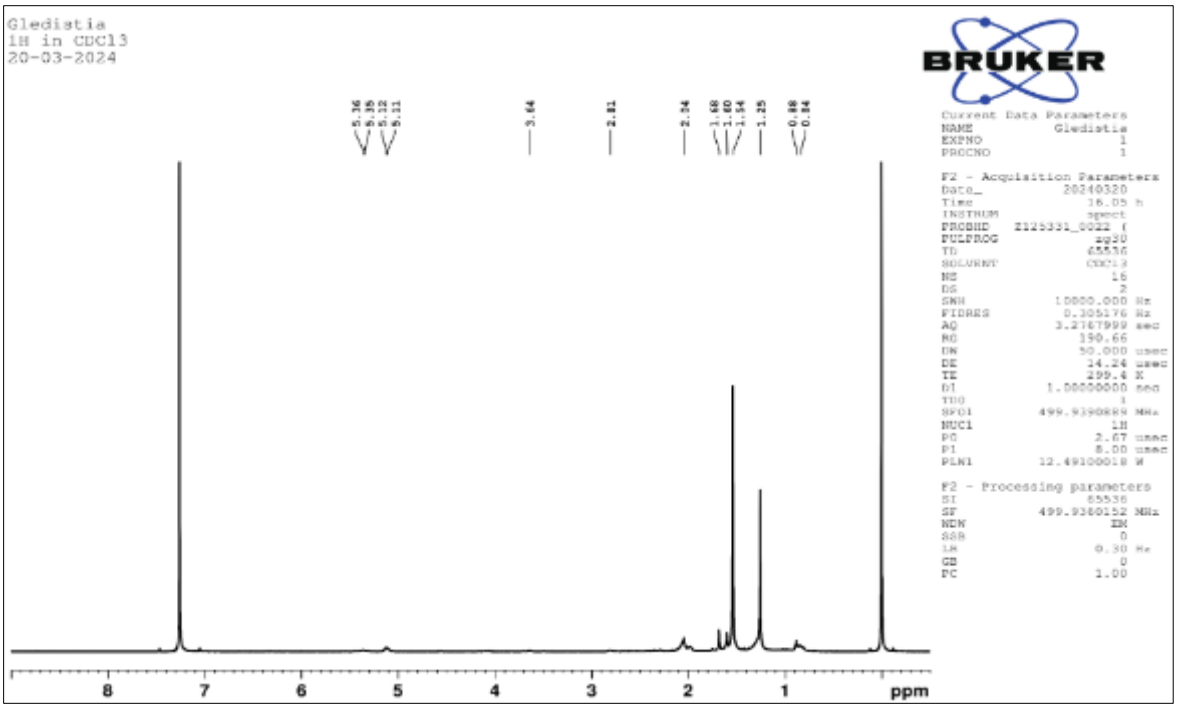

Figure 4 NMR spectrum of methanolic extract of the *Gledistia triacanthos* leaves

Table 5 NMR spectrum of methanolic extract of the *Gledistia triacanthos* leaves

- 1.25 ppm (chemical shift) C-H Hydrogen(alkane) at aliphatic region.
- 1.54 ppm 9- C-H hydrogen at region.
- 2.40 C-H2 hydrogen at aliphatic region.
- 7.3 C-H hydrogen at aromatic region

Table 6 Larvicidal activity of Methanolic and Hexane Leaf extracts of *Gleditsia tricanthos* against *Culex quinquefasciatus* expressed in mean and standard deviation

Concentration of Leaf Extract (Vol/Vol)	***Gleditsia tricanthos***
	(Mean + SD)
Methanolic Extract	
20	25 + 0
10	23.2 + 1.3038
5	19 + 1.5811
2.5	13.4 + 1.1402
Hexane Extract	
20	25 + 0
10	23.8 + 0.8367
5	17.8 + 1.7889
2.5	14 + 1.2247

Table 7 ANOVA of within treatment of various concentrations of methanol and hexane extract

Concentration of Leaf Extract (Vol/Vol)	% of Larvicidal activity	LC50	95% Confidence Interval		R2	p-value
			Upper	Lower		
Hexane Extract of Gledistia tricanthos						
20	100					
10	95.2					
5	71.2					
2.5	56	2.73	1.37	5.75	8.695	0.0032
Methanol Extract of Gledistia tricanthos						
20	100					
10	92.8					
5	76					
2.5	53.6	4.857	1.72	8.69	8.743	0.0031

4. Discussion

Gleditsia tricanthos have been used in folk medicine and the leaves of this species are primarily researched for their therapeutic qualities. Triacanthine alkaloid was extracted from *G. triacanthos* leaves by Panova et al. in 1971. Some of the other studies are reported vitexin, luteolin, isovitexin, and quercetin in *G. triacanthos* leaves (Panova et al., 1972, Leibovici et al, 1986). Mohammed et al. 2014 investigated their cytotoxic and antioxidant properties and also flavonoid content in leaves. Characterizing the composition of leaves and testing their harmful effect on *Culex quinquefasciatus* larvae are the goals of the current study.

The world of plants has a wealth of undiscovered phytochemicals that might be widely used in place of industrial pesticides in the fight against mosquitoes. The goal is to find newer insecticides that are affordable, safe, and easily accessible. Certain plant extracts have insecticidal actions that kill mosquitoes in addition to their repellant qualities. Pyrethrum's efficacy against Aedes mosquitoes was shown in a study by Govindarajan et al. (2016) (1), highlighting its potential as an environmentally friendly substitute for synthetic insecticides. Numerous plants have been studied in relation to mosquitoes, including *Melia azedarach* (Chinaberry) (Govindarajan et al., 2016), *Corymbia citriodora* (Lemon Eucalyptus) (Lee et al., 2018), *Cinnamomum spp*. (Cinnamon) (Mahran et al., 2023), *Azadirachta indica* (Neem) and *Chrysanthemum cinerariifolium* (Pyrethrum) (Govindarajan et al., 2016).

In relation to *Culex quinquefasciatus*, *G. triacanthos* showed potential larvicidal action. With an LC50 value of 2.73 mL/100 mL for hexane leaf extract and 4.85 mg/100 mL for methanolic leaf extract. The results of similar study from our lab with *Elytraria acaulis* hexane leaf extract have shown LC50 value as 3.046 mL/100 mL. These results are consistent with Rajan Maheswaran and Soorya Sukumaran's (2020) research. Similarly, powdered versions of Nerium oleander, Calotropis procera, and Ricinus communis were tested for persistent toxicity against *Culex quinquefasciatus* (Kehail et al., 2017). A phytochemical investigation found that the methanolic and hexane extracts included substantial amounts of flavonoids, alkaloids, carbohydrates, and terpenoids, but not tannins, among other components in hexane extract. According to Kiruthika (Kiruthika et al., 2012), the larvicidal effects of E. acaulis may be due to these compounds.

Numerous studies have effectively established the importance of phytochemicals in mosquito control (Chowdhury et al., 2007; Singha et al., 2011). One such study examined the larvicidal activity of plants such as Annona squamosa L., Chrysanthemum indicum L., and Tridax procumbens L against Anopheles subpictus Grassi and the Japanese encephalitis vector, *Culex tritaeniorhynchus* Giles. *Annona squamosa* leaf extract was discovered to be more powerful than other plants (Kamaraj et al., 2011). *Otanthus maritimus and Ammi visnaga* extracts showed the highest larvicidal effect, followed by *Acer pseudoplatanus*, *Humulus japonicus*, *Acer platanoides*, *Satureja hortensis, Ocimum basilicum* and *Thymus vulgaris*, respectively, according to Roman Pavela's comparison of nearly 56 plant species for their larvicidal

activity against the mosquito *Culex quinquefasciatus* (Roman Pavela, 2008). Additionally, *Euphorbiaceae tirucalli* has been shown in a study by Abdul Rahuman et al. (2008) to be a promising larvicide against *Culex quinquefasciatus*. According to Anjali Rawani et al. (2009), three plants—*Carica papaya, Murraya paniculata,* and *Cleistanthus collinus*—have larvicidal effects on the target species, *Culex quinquefasciatus*.

Nonetheless, the current investigation assessed the specific toxicity of *G. triacanthos* leaf extracts in methanol and hexane to *Culex quinquefasciatus* larvae. GCMS investigations of the methanol extract from G. triacanthos leaves revealed 317 chemicals with distinct phytochemical activity. Additionally, 180 distinct compounds were discovered from the same leaves using the hexane fraction of *G. triacanthos*, each of which had distinct properties.

From GC–MS analysis few major compounds have been identified and of them 4- phenyl semicarbazide, cyacetacide, 1-propanol, 2 –amino, 1, 2 -Hydrazine di carboxamide, 16 - Hexadecanoyl hydrazine, 4-methyl -3, 4 -dihydro - [1, 2, 3] triazolo have been previously reported for their mosquito larvicidal activity. Flavonoids and alkaloids belong to the class of compounds that are known to be harmful to *C. quinquefasciatus* (Muthu et al., 2012). FTIR analysis of *G. triacanthos* (Hexane extract of leaf) displayed 1168, 1228, 1366, 1462, 1738, 2849, 2916 and 3009 cm−1 characteristic absorptions corresponding to CO stretch Alkoxy substistment , CO stretch Alkoxy substistment, CH bend CH3 (Aryl strained alkane (or) activated carbonyl), CH bend CH2 (Aliphatic ether (or) sulfonate salt), c = o stretch (Carbonyl alpha alkyl, CH stretch Alkane (Aliphatic alkane (or) alcohol), CH stretch Alkane (Aliphatic alkane) and CH stretch Alkene (Aliphatic alkene) stretching showing the presence of aliphatic acids. The molecular structure of the cyclohex--enyl-propionic acid, phenyl semicarbazide, and cyacetacide compounds was validated by the NMR-analysis. The leaves of these two extracts showed overall bioefficacy (acute toxicity), and this is because of the chemicals found in the leaves of *Gleditsia triacanthos*.

In this study it has been illustrated the larvicidal potential of hexane extract of *Gleditsia triacanthos* against larva of *C. quinquefasciatus*. This study reports that the hexane extract of Gleditsia *triacanthos* was more effective than ethanol extract to *C. quinquefasciatus* larvae.. Previous research on bark extract from *Gleditsia triacanthos* has shown 100% larval mortality (Umar et al., 2020).

5. Conclusion

The study highlights the significant larvicidal potential of *Gleditsia triacanthos* leaf extracts, particularly the hexane extract, against *Culex quinquefasciatus* larvae, suggesting that these extracts could serve as effective and environmentally friendly alternatives to synthetic insecticides in mosquito control. The findings underscore the importance of exploring plant-based phytochemicals for their insecticidal properties, contributing to sustainable pest management strategies.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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