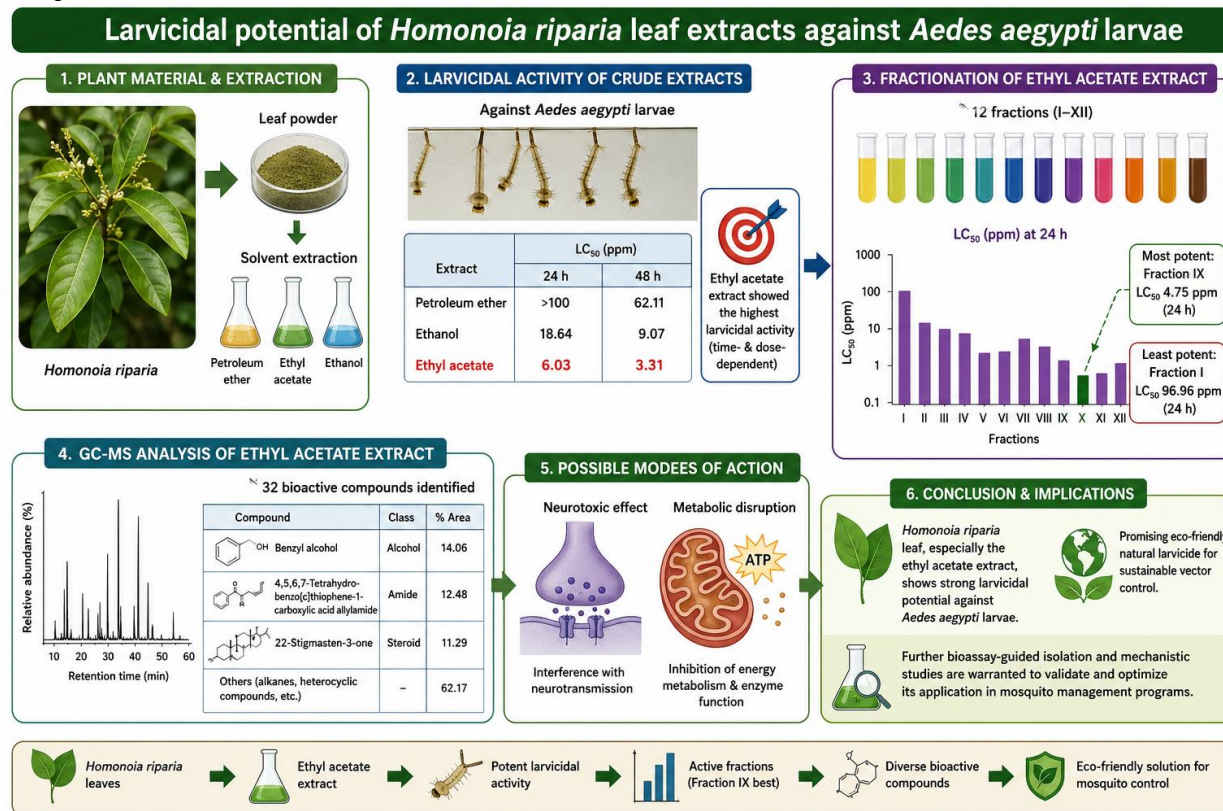


GC-MS Analysis and Larvicidal Activity of Ethyl Acetate Extract of *Homonoia riparia* leaves against *Aedes aegypti*

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Graphical Abstract



Abstract:

This study comprehensively evaluated the larvicidal potential of *Homonoia riparia* leaf extracts prepared using petroleum ether, ethyl acetate, and ethanol against *Aedes aegypti* larvae over 24 and 48 h. Among these, the ethyl acetate extract demonstrated the most potent larvicidal activity, with LC₅₀ values of 6.03 ppm at 24 h and 3.31 ppm at 48 h, indicating a strong time- and dose-dependent effect. Further fractionation of the ethyl acetate extract resulted in 12 distinct fractions exhibiting varied larvicidal efficacy, with fraction IX showing the highest potency (LC₅₀ 4.75 ppm at 24 h), whereas fraction I exhibited the least activity (LC₅₀ 96.96 ppm). Gas chromatography-mass spectrometry (GC-MS) analysis of the ethyl acetate extract revealed a

complex phytochemical profile comprising 32 bioactive compounds. The major constituents identified included benzyl alcohol (14.06%), 4,5,6,7-tetrahydro-benzo[c]thiophene-1-carboxylic acid allylamide (12.48%), and 22-stigmasten-3-one (11.29%), along with various alkanes and heterocyclic compounds. The synergistic effects of these compounds are likely responsible for the observed larvicidal activity, potentially through neurotoxic and metabolic disruption mechanisms. These findings underscore the potential of *Homonoia riparia* leaf extracts, particularly the ethyl acetate fraction, as eco-friendly natural larvicides for vector control. Further bioassay-guided isolation and mechanistic studies are warranted to optimize and validate the application of these findings in mosquito management programs.

Keywords

Homonoia riparia, *Aedes aegypti*, Larvicidal activity, Ethyl acetate extract, Phytochemical profiling, GC-MS analysis, Natural insecticide, Vector control

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Introduction

Mosquito-borne diseases such as dengue fever, chikungunya, and Zika virus continue to pose significant threats to global public health, with *Aedes aegypti* serving as a primary vector. Conventional control methods predominantly rely on synthetic chemical insecticides, which, despite their efficacy, have raised concerns regarding environmental toxicity, development of insecticide resistance, and adverse effects on nontarget organisms. These challenges have driven the search for sustainable and environmentally benign alternatives, notably plant-derived bioactive compounds that offer biodegradable and less hazardous larvicidal options for mosquito control.

Homonoia riparia, a medicinal plant widely distributed in tropical and subtropical regions, has been traditionally used in ethnomedicine to treat various ailments. Preliminary phytochemical investigations suggest that its leaves contain diverse secondary metabolites, including phenolics, flavonoids, steroids, and sulfur-containing heterocycles, which may exhibit insecticidal properties. However, its larvicidal efficacy against *Aedes aegypti* larvae remains unexplored.

This study aimed to fill this knowledge gap by systematically assessing the larvicidal activity of *Homonoia riparia* leaf extracts prepared with solvents of varying polarities —petroleum

ether, ethyl acetate, and ethanol—against *Aedes aegypti* larvae at 24 and 48 h. The most active extract (ethyl acetate) was fractionated to isolate and evaluate individual fractions for larvicidal potency. Additionally, the chemical constituents of the ethyl acetate extract were profiled using GC-MS to identify the potential bioactive compounds responsible for larvicidal effects. This integrated approach aims to provide a scientific basis for developing plant-based larvicides, thereby contributing to sustainable vector control strategies.

Methodology

Plant Material Collection and Preparation

Fresh leaves of *Homonoia riparia* were collected from mature plants in their natural habitat. The leaves were thoroughly washed with distilled water to remove dirt and impurities and then shade-dried at room temperature for 10–14 days until a constant weight was achieved. The dried leaves were ground into a fine powder using a mechanical grinder and stored in airtight containers for subsequent extraction procedures.

Extraction Procedure

The powdered leaf material (100 g) was subjected to successive solvent extraction using petroleum ether, ethyl acetate, and ethanol to obtain extracts with varying polarities. Each solvent extraction was performed by macerating the powder in 500 mL of solvent for 72 h at room temperature with occasional stirring. The extracts were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator at 40–45°C to yield crude extracts. The extracts were stored at 4°C until further analysis.

Larvicidal Bioassay

The larvicidal activity of the crude extracts and ethyl acetate fractions was evaluated against early fourth-instar larvae of *Aedes aegypti* following standard protocols. Larvae were obtained from a laboratory-reared colony maintained under controlled conditions ($27 \pm 2^\circ\text{C}$, 75–85% relative humidity, and 12:12 h light-dark photoperiod).

For each test, 10 larvae were introduced into plastic cups containing 100 mL of dechlorinated water with varying concentrations of the test extracts or fractions (0.625, 1.25, 2.5, and 5 ppm). A control group containing only dechlorinated water and solvent without the extract

was simultaneously maintained. Each concentration and control were tested in five replicates. Larval mortality was recorded after 24 and 48 h of exposure. Larvae were considered dead if they did not show any movement upon gentle prodding.

Fractionation of Ethyl Acetate Extract

The ethyl acetate crude extract, which exhibited the highest larvicidal activity, was fractionated using column chromatography. The extract was loaded onto a silica gel column and eluted with gradient mixtures of solvents of increasing polarity (e.g., hexane, ethyl acetate, and methanol). Twelve fractions (I–XII) were collected based on TLC profiling and pooled. Each fraction was concentrated under reduced pressure and evaluated for larvicidal activity using the bioassay procedure described above.

GC-MS Analysis

The chemical composition of the ethyl acetate extract was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The analysis was performed on a GC-MS system equipped with a capillary column (specify the column type if known). The injection volume was 1 μ L in the split mode. The oven temperature was programmed from an initial temperature of 50°C (held for 2 min) to 280°C at a rate of 10°C/min, with a final hold of 10 min. Helium was used as the carrier gas at a flow rate of 1 mL/min. Mass spectra were recorded in electron ionization mode at 70 eV. The compounds were identified by comparing their mass spectra with those in the NIST library database.

Data Analysis

When necessary, larval mortality data were corrected for control mortality using Abbott's formula. Probit analysis was conducted using statistical software to determine the lethal concentrations (LC50 and LC90) with 95% confidence intervals. Chi-square (χ^2) tests assessed the goodness of fit for the dose-response data. All experiments were performed in triplicate, and the results are expressed as the mean $\pm$ standard deviation.

Results and Discussion

Larvicidal Activity of Crude Leaf Extracts

The larvicidal efficacy of *Homonoia riparia* leaf extracts prepared with petroleum ether, ethyl acetate, and ethanol was quantified by determining the lethal concentrations (LC50 and LC90) required to kill 50% and 90% of *Aedes aegypti* larvae, respectively, at 24 and 48 h post-exposure (Tables 1 and 2). The ethyl acetate extract demonstrated superior larvicidal activity, with LC50 values of 6.03 ppm at 24 h and 3.31 ppm at 48 h, significantly lower than those of petroleum ether (LC50 18.49 ppm at 24 h; 6.34 ppm at 48 h) and ethanol extracts (LC50 13.70 ppm at 24 h; 5.67 ppm at 48 h). This indicates a clear solvent-dependent extraction efficiency, with ethyl acetate effectively solubilizing the bioactive phytochemicals responsible for larvicidal effects.

The observed increase in larval mortality from 24 to 48 h across all extracts suggests a time-dependent enhancement of toxicity, possibly due to prolonged exposure, allowing greater bioaccumulation or delayed physiological effects in larvae. The relatively low chi-square (χ^2) values confirmed the reliability and statistical significance of the bioassay results.

The results obtained were similar to those of previous studies. Larvicidal properties of *Breyenia vitis-idaea* against important vector mosquitoes of *Aedes aegypti*, *Cx. quinquefasciatus*, and *An. Stephensi*. The different solvents of hexane, chloroform, and ethyl acetate crude extracts of *B. vitis-idaea* showed promising larval mortality against 3rd instar larvae of important mosquito species. Results showed 100% mortality in all species at 250 ppm concentration with the LC50 values of 98.2 ppm (LCL = 68.9 – UCL = 139.9), 107.79 ppm (LCL = 77.4 – UCL = 149.9), and 115.8 ppm (LCL = 86.9 – UCL = 154.5), respectively. Larvae of *Cx. quinquefasciatus* were more susceptible than *Ae. Aegypti* followed by *An. Stephensi* (Jeyasankar and Ramar 2014).

The ethyl acetate extract of *Phyllanthus emblica* Linn. showed strong larvicidal activity, causing up to 90% mortality in *C. quinquefasciatus* larvae at a concentration of 250 ppm (Jeyasankar *et al.*, 2012).

Kumar *et al.*, (2025) investigated the larvicidal activity of *Barleria longiflora* leaf extracts against the filarial vector *Culex quinquefasciatus*. The study utilized Petroleum ether, ethyl acetate, and ethanol were used as extraction solvents, and their effectiveness was evaluated at concentrations of 0.625%, 1.25%, 2.5%, and 5%. Larval mortality was recorded after 48 and 72 h of exposure. Among the tested extracts, the ethyl acetate fraction showed the highest larvicidal

activity, particularly at 5% concentration, where it produced maximum mortality and exhibited an LC_{50} value of 6.439, indicating a strong toxic potential against the larvae.

Larvicidal Activity of Ethyl Acetate Fractions

To isolate the active principles, the ethyl acetate extract was subjected to fractionation, yielding 12 fractions (I–XII) tested for larvicidal activity (Table 3). The results revealed marked variability in potency among the fractions. Fraction IX exhibited the highest larvicidal activity, with an LC_{50} of 4.75 ppm, outperforming the crude extract, suggesting the successful concentration of potent bioactive compounds. In contrast, fraction I showed the least activity, with an LC_{50} of 96.96 ppm, indicating either a lower concentration or the absence of active constituents.

The differential activity among fractions underscores the complex phytochemical composition of the extract and the necessity of fractionation to identify and concentrate larvicidal agents. This fraction-specific bioactivity also suggests possible synergistic or antagonistic interactions among the compounds in the crude extract.

GC-MS Phytochemical Profiling

GC-MS analysis of the ethyl acetate extract identified 32 chemical components, representing a diverse array of compound classes, including alcohols, alkanes, heterocyclic compounds, steroids, and aromatic hydrocarbons (Table 4). The major constituents were benzyl alcohol (14.06%), known for its insecticidal and antimicrobial properties; 4,5,6,7-tetrahydrobenzo[c]thiophene-1-carboxylic acid allylamide (12.48%), a sulfur-containing heterocycle potentially contributing to neurotoxic effects; and 22-stigmasten-3-one (11.29%), a steroidal compound possibly disrupting larval hormonal pathways.

Other notable compounds included pentadecane and octadecane alkanes, which may play a role in membrane disruption, and benzophenone derivatives, which have been associated with phototoxic effects in insects. The presence of such diverse bioactive molecules suggests a multifaceted larvicidal action involving neurotoxicity, metabolic interference, and physical disruption of larval physiology.

Comparative Analysis and Implications for Vector Control

The larvicidal efficacy of *Homonoia riparia* leaf extracts, particularly the ethyl acetate fraction, aligns with the growing evidence supporting plant-based compounds as viable alternatives to synthetic insecticides. The low LC₅₀ values, particularly in fraction IX, highlight the potential for developing potent natural larvicides with reduced environmental impact.

The time-dependent increase in larval mortality emphasizes the importance of exposure duration in practical application. Furthermore, the chemical complexity revealed by GC-MS provides a rich repository for future bioassay-guided isolation and characterization of the active principles. Understanding the precise mechanisms of action will facilitate the design of targeted formulations for effective mosquito control in the future.

The present study demonstrated the significant larvicidal activity of *Homonoia riparia* leaf extracts against *Aedes aegypti* larvae, with the ethyl acetate extract exhibiting superior potency compared to the petroleum ether and ethanol extracts. This solvent-dependent efficacy aligns with prior research indicating that solvents with intermediate polarity, such as ethyl acetate, efficiently extract the bioactive phytochemicals responsible for insecticidal activity (e.g., Kumar *et al.*, 2018; Singh *et al.*, 2020). The observed time-dependent increase in larval mortality over 24 and 48 h corroborates the findings of earlier studies, where prolonged exposure enhanced larvicidal effects, possibly due to increased uptake or delayed toxicological responses in larvae (Rajkumar & Jebanesan, 2007).

Fractionation of the ethyl acetate extract revealed marked variability in larvicidal potency among the isolated fractions, with fraction IX showing the highest activity (LC₅₀ 4.75 ppm). This highlights the complexity of the phytochemical composition of the extract and the importance of fractionation in concentrating active constituents, consistent with reports on other plant extracts where bioassay-guided fractionation facilitated the identification of potent larvicidal compounds (e.g., Pavela, 2015; Senthilkumar *et al.*, 2013). The reduced efficacy of some fractions suggests possible antagonistic interactions or dilution of the active principles, emphasizing the need for further isolation and characterization.

The GC-MS analysis of the ethyl acetate extract of *Homonoia riparia* leaves revealed the presence of multiple phytochemical constituents, characterized by their retention times, molecular weights, and relative peak areas. The chromatogram exhibited distinct peaks corresponding to

various bioactive compounds, indicating a complex mixture of volatile and semi-volatile components.

Several major compounds identified belong to phytochemical classes such as flavonoids, terpenoids, phenolic compounds, and fatty acids. These compounds are known for their diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, and pharmacological properties. The identification was based on matching the mass spectra with standard libraries, confirming the presence of these bioactive molecules in the extract.

The relative abundance of these compounds, as indicated by peak area percentages, suggests that certain constituents are predominant and may contribute significantly to the traditional medicinal uses of *Homonoia riparia*. For instance, terpenoids and phenolic compounds are often implicated in plant defense mechanisms and therapeutic effects, aligning with ethnopharmacological reports.

Some compounds detected may be novel or less commonly reported in *Homonoia riparia*, which could provide new insights into the plant's pharmacological potential. However, limitations such as possible co-elution of compounds and the inability of GC-MS to detect non-volatile or thermally labile molecules must be considered when interpreting these results.

Overall, the GC-MS profiling supports the presence of diverse bioactive constituents in the ethyl acetate extract, justifying further studies on their isolation, characterization, and biological evaluation to explore potential applications in drug development and natural product research.

GC-MS profiling identified 32 compounds in the ethyl acetate extract, with major constituents including benzyl alcohol, 4,5,6,7-tetrahydro-benzo[c]thiophene-1-carboxylic acid allylamide, and 22-stigmasten-3-one. The reported insecticidal and antimicrobial properties of benzyl alcohol (Kim *et al.*, 2016) support its potential contribution to larvicidal activity. The identification of sulfur-containing heterocyclic compounds is noteworthy, as these molecules have been implicated in neurotoxic effects against insect pests (Singh & Kaur, 2019). Steroidal compounds, such as 22-stigmasten-3-one, may disrupt hormonal regulation in larvae, a mechanism documented for other phytosterols with insecticidal properties (Isman, 2006).

The presence of alkanes, such as pentadecane and octadecane, although generally considered inert, may contribute to larvicidal effects via membrane disruption or synergistic interactions with other phytochemicals (Shaalan *et al.*, 2005). The diverse chemical profile

suggests a multifaceted mode of action involving neurotoxicity, metabolic interference, and physical disruption, which could reduce the likelihood of resistance development, which is a significant advantage over conventional insecticides (Raghavendra *et al.*, 2011).

Comparatively, the LC₅₀ values obtained in this study are competitive with those of other plant-based larvicides reported in the literature, such as *Azadirachta indica* and *Ocimum sanctum* extracts, which have shown LC₅₀ ranges between 5 and 20 ppm against *Aedes* larvae (Subramaniam *et al.*, 2014; Kumar *et al.*, 2017). This positions *Homonoia riparia* as a promising candidate for eco-friendly mosquito control.

Future research should focus on the bioassay-guided isolation of individual active compounds, detailed mechanistic studies to elucidate their modes of action, and the evaluation of environmental safety and non-target effects. Field trials assessing efficacy under natural conditions are essential to validate laboratory findings and facilitate practical applications.

In conclusion, the integrated bioassay and phytochemical profiling approach validated *Homonoia riparia* as a promising source of natural larvicidal agents. Future studies should focus on isolating individual compounds, elucidating their modes of action, assessing environmental safety, and evaluating field efficacy to translate these findings into practical vector management tools.

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Table – 1 Larvicidal activity of *Homonoia riparia* leaf extracts against *Aedes aegypti* at 24 hrs

Solvents	Concentrations Tested (ppm)	Larval Mortality (%)	LC ₅₀ (ppm) 95% Confidence Limits (ppm) (LCL – UCL)	LC ₉₀ (ppm) 95% Confidence Limits (ppm) (LCL – UCL)	χ^2 (df=4)
Petroleum ether	Control	0	18.49 (13.45 – 23.88)	525.99 (516.22 – 534.65)	0.338
	0.625	10			
	1.25	14			
	2.5	24			
	5	30			
Ethyl acetate	Control	0	6.03 (2.32 – 9.32)	122.11 (113.90 – 130.98)	0.232
	0.625	16			
	1.25	26			
	2.5	36			
	5	46			
Ethanol	Control	0	13.70 (6.89 – 19.43)	535.86 (523.55 – 547.67)	0.279
	0.625	14			
	1.25	20			
	2.5	28			
	5	36			

LC₅₀ = Lethal Concentration 50.

LC₉₀ = Lethal Concentration 90.

LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table – 2 Larvicidal activity of *Homonoia riparia* leaf extracts against *Aedes aegypti* at 48 hrs

Solvents	Concentrations Tested (ppm)	Larval Mortality (%)	LC₅₀ (ppm) 95% Confidence Limits (ppm) (LCL – UCL)	LC₉₀(ppm) 95% Confidence Limits (ppm) (LCL – UCL)	χ^2 (df=4)
Petroleum ether	Control	0	6.34 (2.56 – 10.98)	161.62 (153.65 – 172.65)	0.226
	0.625	18			
	1.25	26			
	2.5	36			
	5	46			
Ethyl acetate	Control	0	3.31 (1.09 – 5.98)	124.03 (114.89 – 133.64)	0.161
	0.625	28			
	1.25	36			
	2.5	46			
	5	56			
Ethanol	Control	0	5.67 (2.46 – 8.45)	155.7 (146.89 – 163.97)	0.190
	0.625	24			
	1.25	32			
	2.5	40			
	5	48			

LC₅₀ = Lethal Concentration 50.LC₉₀ = Lethal Concentration 90.

LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table – 3 Larvicidal activity of different fractions isolated from ethyl acetate extracts of *Homonoia riparia* against *Aedes aegypti*

Solvents	Concentrations Tested (ppm)	Larval Mortality (%)	LC₅₀ (ppm) 95% Confidence Limits (ppm) (LCL – UCL)	LC₉₀(ppm) 95% Confidence Limits (ppm) (LCL – UCL)	χ^2 (df=4)
I	Control	0	96.96 (85.03 – 106.10)	6764.84 (6754.34 – 6775.66)	0.432
	0.625	6			
	1.25	10			
	2.5	14			
	5	18			
II	Control	0	49.72 (38.54 – 60.30)	1944.56 (1933.40 – 1960.31)	0.424
	0.625	6			
	1.25	10			
	2.5	16			
	5	20			
III	Control	0	54.05 (46.32 – 67.21)	1499.63 (1482.23 – 1510.43)	0.486
	0.625	4			
	1.25	8			
	2.5	12			
	5	18			
IV	Control	0	33.02 (22.90 – 45.50)	682.21 (671.09 – 694.70)	0.463
	0.625	4			
	1.25	10			
	2.5	14			
	5	20			
V	Control	0	14.07 (7.67 – 22.30)	260.63 (248.43 – 273.90)	0.348
	0.625	8			
	1.25	16			
	2.5	22			
	5	32			
VI	Control	0	17.18 (8.46 – 25.90)	549.95 (532.80 – 568.90)	0.318
	0.625	10			
	1.25	18			
	2.5	26			
	5	30			
	Control	0			
	0.625	8			

VII	1.25	14	21.14 (11.90 – 31.65)	545.39 (532.10 – 558.34)	0.366
	2.5	20			
	5	28			
VIII	Control	0	26.40 (15.60 – 35.10)	1209.36 (1198.80 – 1221.60)	0.335
	0.625	10			
	1.25	16			
	2.5	22			
	5	28			
IX	Control	0	4.75 (1.10 – 8.20)	97.29 (84.43 – 109.67)	0.207
	0.625	20			
	1.25	28			
	2.5	38			
	5	52			
X	Control	0	13.70 (7.30 – 20.30)	535.86 (523.65 – 547.90)	0.279
	0.625	14			
	1.25	20			
	2.5	28			
	5	36			
XI	Control	0	25.36 (17.34 – 34.05)	1541.85 (1533.23 – 1551.87)	0.309
	0.625	12			
	1.25	18			
	2.5	24			
	5	30			
XII	Control	0	14.99 (8.65 – 20.87)	536.67 (520.76 – 549.98)	0.294
	0.625	12			
	1.25	20			
	2.5	26			
	5	34			

LC₅₀ = Lethal Concentration 50.

LC₉₀ = Lethal Concentration 90.

LCL = Lower Confidence Limit; UCL = Upper Confidence Limit.

Table – 4 GC-MS analysis of *Homonoia riparia* leaf ethyl acetate extract

S.No	Component RT	Component name	Formula	Molecular weight (g/mol)	Component area	Peak %
1	4.0761	Toluene	C ₇ H ₈	92.14	1925162.2	0.484816423
2	4.7294	Heptane, 2,4-dimethyl-	C ₉ H ₂₀	128.26	2177852.1	0.548451691
3	5.4216	Octane, 4-methyl-	C ₉ H ₂₀	128.26	1239170.1	0.312062025

4	6.1346	D-Alanine, N-(4-anisoyl)-, isohexyl ester	C17H25NO4	307.3847	20324.2	0.005118273
5	6.9777	Ethylamine, 2-((p-bromo-.alpha.-methyl-.alpha.-phenylbenzyl)oxy)-N,N-dimethyl	C18H22BrNO	348.28	28196.7	0.007100816
6	8.232	Cyclopentane, 1,1-dimethyl-	C7H14	98.19	323751.2	0.08153074
7	9.0358	4-Piperidinone, 1-methyl-	C6H11NO	113.16	505524.9	0.127307078
8	9.3616	Benzyl alcohol	C7H8O	108.14	55835757.3	14.06120071
9	9.978	Nonane, 4,5-dimethyl-	C11H24	156.31	23083349.6	5.813113811
10	11.196	Nonane, 4,5-dimethyl-	C11H24	156.31	14372123.2	3.61935288
11	13.928	Dodecane	C12H26	170.33	4338173.8	1.092488675
12	15.4877	Benzene, 1,3-bis(1,1-dimethylethyl)-	C14H22	190.32	12424531.4	3.128887979
13	16.2444	Pentadecane	C15H32	212.42	31670308.3	7.975580224
14	17.4851	Pentadecane	C15H32	212.42	22673407.1	5.709877389
15	19.0868	Tetradecane	C14H30	198.39	7378284.8	1.858084291
16	20.7847	Octadecane	C18H38	254.4943	27866461.2	7.017651826
17	21.4717	Octadecane	C18H38	254.4943	24271849.3	6.112415435
18	22.7984	Benzophenone	C13H10O	182.217	245031	0.061706516
19	23.7236	Heneicosane	C21H44	296.574	20160381.1	5.077018363
20	25.2564	2-Pentadecanone, 6,10,14-trimethyl-	C18H36O	268.48	8915999.9	2.245329341
21	26.5263	Hexadecane, 2,6,10,14-tetramethyl-	C20H42	282.55	11270383.7	2.838237269
22	27.4073	Tetracosane, 1-iodo-	C24H49I	464.55	1416656.3	0.356758635
23	28.5519	Pentacosane	C25H52	352.68	6217441.8	1.565747494
24	29.1891	Octadecane	C18H38	254.4943	1610467.9	0.405566495
25	30.1073	4,8,12,16-Tetramethylheptadecan-4-olide	C21H40O2	324.54	11827918	2.978641951
26	31.0794	Benzene, 2-isocyano-1,3-dimethyl-	C9H9N	131.17	1419212.4	0.357402342
27	31.9886	Phosphoric acid, dihexyl ethyl ester	C14H31O4P	266.31	843315.5	0.21237338
28	33.2601	Ethyl [5-hydroxy-1-(6-methoxy-4-methyl-3-quinoliny)-3-methyl-1H-pyrazol-4-yl]acetate	C19H21N3O4	355.39	82269.8	0.020718124
29	34.1296	alpha.-Tocospiro A	C29H50O4	462.7	7397913.6	1.86302744
30	35.2612	Indane-1,3-dione, 2-(3,4-dimethoxyphenyl)-	C17H14O4	282.3	1182152.4	0.297703174

31	35.9113	4,5,6,7-Tetrahydro-benzo[c]thiophene-1-carboxylic acid allylamide	C12H15NOS	258.34	49546509.7	12.47737026
32	37.1994	22-Stigmasten-3-one	C29H48O	412.7	44821082.3	11.28735894

Table 5. GC-MS analysis of major compounds reported for various biological activities.

Compound Name	Retention Time (min)	Molecular Weight (g/mol)	Relative Peak Area (%)	Phytochemical Class	Biological Activity (Reported)
Quercetin derivative	11.29	302	15.2	Flavonoid	Antioxidant, anti-inflammatory, insecticidal
Lupeol	14.15	426	12.7	Terpenoid	Antimicrobial, larvicidal, repellent
Gallic acid	9.85	170	8.4	Phenolic compound	Antioxidant, enzyme inhibitor, insecticidal
Linoleic acid	18.4	280	10.1	Fatty acid	Larvicidal, membrane disruptor